Research Article

Effect of a Hydrogen Sulfide Donor on Skeletal Muscle Contraction and Fatigue in Adult Male Albino Rats

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Abstract

Background: In skeletal muscles, the role of hydrogen sulfide (H_2S) in contraction has not been fully investigated although impaired formation may be involved in chronic fatigue syndrome; therefore we aimed to study this role. **Methods:** ٢٤ adult male albino rats were classified into ٤ groups; injected intraperitoneally (i.p.) with a single dose of the following: **control**; injected with saline (vehicle), **L-NAME treated**; injected with ٢٥ mg/Kg body weight of N^G – nitro-L- arginine methyl ester (L-NAME) as nitric oxide synthase (NOS) inhibitor***;*** **Sodium hydrosulfide (NaHS) treated;** injected with NaHS as H_2S donor, at a dose of ١٠٠ µmol/kg body weight; **NaHS + L-NAME treated;** rats were coadministered both chemicals in the same previous doses. The right intact gastrocnemius was stimulated by low frequency (LF) electric current ٣٠ min. after injection and subtetanic contractions were recorded till the force declined to ٥٠٪ of the peak force, while the left gastrocnemius was resting (control). Both gastrocnemius muscles were then excised and assayed biochemically and mechanically. **Results:** NaHS significantly increased the peak force, and delayed the time till ٥٠٪ fatigue with improving biochemical conditions; decreased lipid peroxides, increased nitric oxide, and creatine kinase activity. The reverse occurred with L-NAME and was associated with increased muscle lactate. Given together, NaHS could significantly, but partially antagonize the fatiguing effects of L-NAME. **Conclusion:** NaHS improves muscle fatigue even when nitric oxide synthesis is blocked indicating that the effect is not NO dependent. It could be promising in fatigue syndromes especially those associated with NO deficiency as myopathies.

Key Words: Hydrogen Sulfide donor, skeletal muscle contraction, fatigue, NO, L-NAME, creatine kinase.

Introduction

"Muscle fatigue" is defined as a drop in physical work capacity *i.e.* reduced force or power production in response to contractile activity(١). Fatigue is a common symptom of numerous acute and chronic diseases, including myopathies, neurodegenerative diseases, metabolic syndrome and chronic debilitating diseases like cancer(٢).

Skeletal muscle fatigue is multifactorial. Defective neuromuscular transmission, failure of excitation-contraction coupling (EC) mechanism, failure of mitochondrial oxidative mechanism with development of oxidative stress and depletion of energy stores could be reasons(٣).

Nitric oxide (NO); is the endothelium derived relaxation factor that plays an important role in vascular smooth muscle relaxation and regulation of muscle blood flow during activity. In physiological concentrations, it increases muscle blood flow during activity, acts as an antioxidant, and postpones muscle fatigue. However, its production in excessive concentrations under abnormal conditions by inducible nitric oxide synthase (iNOS) increases the formation of the most toxic free radical peroxynitrite and accelerates muscle fatigue. On the other hand, reduced nitric oxide synthesis in skeletal muscles is a pathophysiological mechanism contributing to chronic fatigue syndrome(٤).

Hydrogen sulfide (H_2S) was thought for hundreds of years to be a toxic gas that smelled like rotten eggs, but the gas is now believed to be a molecule involved in intensive physiological and pathological processes(٥). Cystathionine gamma lyase, ٣-

mercaptopyruvate sulfurtransferase (٣-MST) and cysteine aminotransferase (CAT) can produce H_2S in different tissues [٦]. H_2S has been found to be involved in protecting the heart against acute myocardial infarction[٧] and ischemia/reperfusion injury, regulating blood pressure[٨], mediating smooth-muscle relaxation[٩] and inhibiting insulin release and renin activity[١٠,١١]

In skeletal muscles, the role of H_2S in contraction has not been fully investigated although it was hypothesized that impaired formation may be involved in chronic fatigue syndrome[٢,١٢,١٣], Therefore, this work was designed to study the effect of an H_2S donor; sodium hydrosulfide (NaHS) on skeletal muscle contraction and fatigue in adult male albino rat' gastrocnemius muscle and the possible interaction with nitric oxide.

Material and Methods

I. Animals:

Adult male albino rats from the local strain, weighing ١٨٠-٢٠٠ grams were used in this study. Rats were housed at room temperature with natural light/dark cycles for one week acclimatization to lab conditions. Rats were fed a standard diet of commercial rat chow and tap water *ad libitum* until the time of the experiment. All the experimental procedures were approved by the local animal care committee in Minia Faculty of Medicine, Minia University, Egypt.

Rats were divided into the following equal groups (٦ rats each):

١- **Control group;** where rats were injected with saline solution (٠.٩% sodium chloride; NaCl), intraperitoneally (i.p.),

٢- **L-NAME treated group;** in which each rat was injected with a single dose of ٢٥ mg/Kg body weight i.p. of L-NAME as a nitric oxide synthase (NOS) inhibitor[١٤].

٣- **NaHS treated group;** in which each rat was administered NaHS as an H_2S donor, i.p. at a dose of ١٠٠ µmol/kg body weight [١٥].

٤- **L-NAME+ NaSH treated group;** in which each rat was administered both L-NAME and NaHS at the same dose and route as in group ٢- and ٣- .

Both L-NAME and NaHS were purchased from Sigma Co. and were dissolved in saline just before injection. The different injections were given ٣٠ min. prior to electrical stimulation of the gastrocnemius muscle and the dosing volume was kept at ١ ml/١٠٠ g body weight.

II. Experimental protocol:

- Electric stimulation of the right gastrocnemius muscle:

This was done in each rat according to the method of ***MacIntosh and Gardiner***[١٦]. Under urethane anesthesia (١.٥ g/kg; i.p.), rats were fixed to a dissection board, the skin of both hind limbs was incised longitudinally from the calcaneus to the back of the knee, the gastrocnemius was exposed, the right tendoachillis was tied with silk, cut and fixed to a force transducer **(Bioscience, Holliston, MA, USA).** The manipulation avoided severing the vascular supply of the muscle, aligned the muscle with the transducer, and optimum muscle length that gives the maximal force was adjusted by moving the transducer and when reached it was measured with a tape (L cm). Silver electrodes were directly applied to the right gastrocnemius muscle and connected to a stimulator **(Bioscience, Holliston, MA, USA).** The voltage used was ٠.٥ V higher than the lowest voltage that produced maximal twitch amplitude (maximal voltage), and the pulse width was set at ٠.٥ ms. We used low frequency (LF) stimulus (١٠ Hz) to allow incomplete tetanus with incomplete relaxation allowing for ischemia/reperfusion, and to simulate natural contractions inside the body. The muscle was kept moist all the time with continuous dripping of saline at ٣٧°C. While the right gastrocnemius muscle was the experimental muscle, the left was left unstimulated as control. Previous studies have shown no significant biochemical differences between both sides in the tested parameters at rest[١٧]. The force of contraction was recorded on a calibrated chart of the oscillograph **(Bioscoence, USA).** The force of contraction increased gradually to a maximum level, and then decreased gradually thereafter. The experiment was terminated by cessation of electric stimulation when the force of contraction declined to ٥٠٪ of the maximal

force reached (٥٠٪ fatigue). Immediately, the gastrocnemius muscles were excised, weighed and placed in liquid nitrogen until biochemically assayed. The following parameters were determined:

- **The maximal force (F)** reached was determined from the calibrated chart.
- **The time till ٥٠٪ fatigue**: was determined from the start of stimulation till ٥٠٪ fatigue using the speed of the chart recording.
- **The cross sectional area of the muscle**: This was done considering the muscle as a cylinder of measured length and an average density of ١.٠٦ g/cm^{٣} , the cross sectional area is calculated according to ***Eu et al.*** [١٨]**;**
  Cross sectional area (A; cm^{٢}) = muscle weight (g) / [density x length (cm)]
- The force/ unit surface area of the muscle was calculated by dividing F/A.

- Biochemical analysis:

For NO and lipid peroxide determination, known weight of gastrocnemius muscle was homogenized in cold saline, centrifuged and the supernatant was used for:

- **Determination of muscle nitric oxide:**

Muscle NO was determined using a nitrite assay kit (Biodiagnostic, Egypt) for the colorimetric determination of NO based on the enzymatic conversion of nitrate to nitrite; the stable degradation products of NO by nitrate reductase. The reaction is followed by addition of Griess reagent to the muscle homogenate to convert nitrite into a deep purple azo compound; the absorbance of which is read at ٥٢٠ nm using a spectrophotometer **(spectronic ٢٠٠٠, Baush and Lomb, Rochester, NY, USA).** Concentrations were calculated from a standard curve made using sodium nitrite solution of different concentrations[١٩]**.**

- **Determination of total lipid peroxides:**

We used the thiobarbituric acid method described by **Ohkawa et al.** [٢٠]**.** In brief; malondialdehyde (MDA) breakdown products of lipid peroxides in muscle homogenates form colored complexes with thiobarbituric acid that are extracted using n-butanol/pyridine, and the absorbance was read at ٥٣٢ nm using the spectrophotometer. Concentrations were calculated from a standard curve made using ١,١,٣,٣-tetramethoxypropane (TMP).

- **Determination of muscle creatine kinase (CK) activity:**

Known weights of gastrocnemius muscle samples were rinsed in phosphate-buffered saline, pH ٧.٤, to remove blood, homogenized in potassium phosphate, pH ٧.٥, buffer, centrifuged for ١٥ minutes at ٤°C using a cooling centrifuge, and the cleared supernatant was used for the assay. We used Sigma Diagnostic (St. Louis, MO) assay kit. The principle according to kit's reference [٢١] that total CK activity was determined by using a hexokinase/glucose-٦-phosphate dehydrogenase – coupled enzyme system which ultimately yields reduced NADP (NADPH) proportionate to CK activity. The rate of increased absorbance at ٣٤٠ nm due to production of NADPH by the CK activity was determined. One unit of CK is the amount of enzyme that will transfer ١.٠ µmole of phosphate from phosphocreatine to ADP per minute at pH ٦.٠.

- **Determination of muscle lactate:**

Known weights of gastrocnemius muscle were excised and homogenized in ٢ mL of distilled water. Then, ٤ mL of ١٠٪ trichloroacetic acid was added and, after mixing, tubes were incubated ٥ min at ٤°C. Immediately afterwards, they were centrifuged at ٢٠٠٠ × g for ١٠ min and the supernatants were collected and assayed for lactic acid using the Sigma lactate colorimetric assay kit[٢٢].

III. Statistical Analysis

The results were expressed as the mean ± standard error of the mean (SEM). Statistical analysis of the mean differences between groups was performed using one way ANOVA followed by Tukey-Kramer multiple comparisons tests using InStat, GraphPad Software (version ٤, San Diego, USA). Values of P<٠.٠٥ were considered significant.

Results

١- Mechanical changes of the right (stimulated)* gastrocnemius *muscle of the different groups (table ١).

- Low Frequency (LF) stimulation of the right gastrocnemius muscle of rats of the control group produced peak force of contraction of ٢٩±٢ g/cm^{٢} cross sectional

area CSA, then gradual progressive decline in muscle power achieving ٥٠٪ fatigue after ٨٥±٦ sec.

- While L-NAME produced a significant shortening (acceleration) of the time till ٥٠٪ fatigue as compared to the control group, administration of the H_2S donor; NaHS produced the opposite effect and significantly delayed the onset of fatigue. When combined, NaHS opposed the rapid onset of fatigue produced by L-NAME and significantly delayed its onset. The time reached was insignificant from that of the control group.
- Regarding the peak force of contraction, the lowest value was recorded in L-NAME group, while, the highest value was achieved in NaHS group. Both were significantly different from control level. When co-administered, NaHS partially improved the L-NAME inhibition, but the level reached was still significantly lower than the control group.

٢- Effect of L-NAME and NaHS treatments alone or combined on tested biochemical parameters of the right (stimulated) and left (resting) gastrocnemius muscles of the different groups.

Table (١) shows that:

- Compared with the control group, NO levels in resting limbs were significantly lower in L-NAME treated groups (either alone or combined with NaHS). Administration of the H_2S donor; NaHS did not produce any significant change in muscle NO content as compared to the control level.
- Regarding muscle lipid peroxides (LP) content in resting limbs, L-NAME alone failed to produce any significant change from the control, while NaHS treatment significantly lowered muscle LP than that of the control group either alone or combined with L-NAME.
- Low frequency (LF) stimulation produced significant higher muscle NO contents in all groups as compared to their corresponding resting limbs. The gastrocnemius of the stimulated limb of L-NAME group showed the lowest NO level, while the highest NO level was achieved in NaHS group. In the stimulated limb of the combined group, NaHS significantly attenuated the L-NAME induced inhibition on NO synthesis, but partially since NO level was significantly higher than that of the corresponding L-NAME, but it was still significantly lower than that of the corresponding control.
- Muscle LP contents in the stimulated limbs were significantly higher than their corresponding resting limbs in all groups. Comparing the differential effect of treatments used, L-NAME significantly increased while NaHS significantly decreased LP. NaHS partially attenuated the increased level produced by L-NAME and all measures were significantly different from the control group.

Table (٢) shows that:

- In resting limbs, muscle lactate was significantly higher than that of control group only in the L-NAME group; **figure (١).** While, creatine kinase (CK) activity was significantly lower in L-NAME treated group than that of the control group, NaHS treatment had no significant effect. Combined administration of NaHS with L-NAME partially reversed the inhibitory effect of L-NAME on the enzyme activity and caused a significant higher level than that of L-NAME alone, but it was still significantly lower than that of the control; **figure (٢).**
- Muscle lactate concentrations of the stimulated limbs of all groups also showed significantly higher lactate levels than their corresponding resting limbs. NaHS did not significantly change the level as compared to that of stimulated limb of control group while L-NAME significantly increased it. NaHS could partially prevent the increase in muscle lactate produced by L-NAME, however, the level remained significantly higher than that of the control group; **figure (١).**

Table (١): Mechanical and biochemical parameters of the right (stimulated) and left (resting) gastrocnemius muscles of the different studied groups.

Parameters \ Groups		Control		L-NAME		NaHS		NaHS + L-NAME	
		Rest. limb	Stim. limb	Rest. limb	Stim. limb	Rest. limb	Stim. limb	Rest. limb	Stim. limb
Time until ٥٠٪ fatigue (s)			٨٥±٦		٦٦±٥.٤		١٤٥±٧.٣		١٠٠±٦.٢
% change from control					-٢٢°		+٧١°		+١٨
% change From L-NAME					-		+١٢٠*		+٥٢*
% change From NaHS					-		-		-٣١#
Peak force of contraction (g/cm٢ CSA)			٢٩±٢		١٤±١.٨		٤٣±٢.٨		٢١±١.٩
% change from control					-٥٢°		+٤٨°		- ٢٨°
% change From L-NAME					-		+٢٠٧*		+٥٠*
% change From NaHS					-		-		-٥١#
At ٥٠٪ Fatigue	**NO (µg/g wet weight muscle)**	٢٠±١.٥	٣٣±٣.٦	٥.٦±٠.٨	٩.٣±٠.٧	١٨.٤±٢.١	٤٥.٦±٣.٩	٦.٥±٠.٧	١٤.٣±١.٨
	% change From:								
	-Resting limb		+٦٥•		+٦٦•		+١٤٨•		+١٢٠•
	-Correspond. control			-٧٢°	-٧١.٨°	-٨	+٣٨°	-٦٨°	-٥٧°
	-Correspond. L-NAME				-	+٢٢٩*	+٣٩٠*	+١٦	+٥٤*
	-Correspond. NaHS				-	-	-	-٦٥#	-٦٩#
	Lipid peroxides (µmol/g wet weight muscle)	١١±٠.٢	٢٤±١.٩	١٠.٢±٠.٨	٤٧.٦±٣.٨	٥.٤±٠.٦	١٣±٠.١١	٦.٢±٠.٤	٣٣±٢.٧
	% change From:								
	-Resting limb		+١١٨•		+٣٦٧•		+١٤١•		+٤٣٢•
	-Correspond. control			-٧	+٩٨°	-٥١°	-٤٦°	-٤٤°	+٣٨°
	-Correspond. L-NAME				-	-٤٧*	-٧٣*	-٣٩*	-٣١*
	-Correspond. NaHS				-	-	-	+١٥	+١٥٤#

Data are expressed as mean ± SEM of ٦ rats in each group. (Rest.:Resting, Stim.:Stimulated)
●: Significant from resting limb. ○: Significant from corresponding limb of control group.
*: Significant from corresponding limb of L-NAME group. #: Significant from corresponding limb of NaHS group.

Finally, LF stimulation produced significant higher creatine kinase activity in the stimulated limbs of all groups when compared with their corresponding non stimulated limbs. NaHS treatment did not produce any significant change in creatine kinase (CK) activity when compared to the control group. On the other hand, L-NAME treatment significantly lowered it. When NaHS was combined with L-NAME, NaHS could partially reverse the inhibitory effect of L-NAME on CK activity which became significantly higher than L-NAME alone, but was still significantly lower than the level of the control group; **figure (٢).**

Table (٢): Lactate concentration and creatine kinase activity changes in the right (stimulated) and left (control) gastrocnemius muscles of the different studied groups.

Parameters \ Groups		Control		L-NAME		NaHS		NaHS + L-NAME	
		Rest. limb	Stim. limb	Rest. limb	Stim. limb	Rest. limb	Stim. limb	Rest. limb	Stim. limb
At ٥٠٪ Fatigue	**Muscle lactate (µmol/g wet weight muscle)**	٠.٦٥±٠.٠٢	٠.٩١±٠.٠٥	٠.٨٣±٠.٠٧	١.٧±٠.٠٨	٠.٦١±٠.٠٣	٠.٨٧±٠.٠٦	٠.٧٤±٠.٠٦	١.٣±٠.٠٨
	% change from:								
	Resting limb		+٤٠•	-	+١٠٥•	-	+٤٣•	-	+٧٦•
	Correspond. control		-	+٢٨°	+٨٧°	-٦	-٤	+١٤	+٤٣°
	Correspond. L-NAME		-	-	-	-٢٧*	-٤٩*	-١١	-٢٤*
	Correspond. NaHS		-	-	-	-	-	+٢١	+٤٩#
	Creatine kinase (U/١٠ mg wet weight muscle)	٣٦±١.٢	٤٨±٢.٩	١٢±٠.٨	١٦.٤±١.٨	٣٧±٢.١	٥٥±٣.٥	١٩.٢±١.٤١	٣٨±٢.٩
	% change from:								
	Resting limb		+٣٣•	-	+٣٧•	-	+٤٩•	-	+٩٨•
	Correspond. control		-	-٦٧°	-٦٦°	+٣	+١٥	-٤٧°	-٢١°
	Correspond. L-NAME		-	-	-	+٢٠٨*	+٢٣٥*	+٦٠*	+١٣٢*
	Correspond. NaHS		-	-	-	-	-	-٤٨#	-٣١#

Data are expressed as mean ± SEM of ٦ rats in each group.
●: Significant from resting limb. ○: Significant from corresponding limb of control group.
*: Significant from corresponding limb of L-NAME group. #: Significant from corresponding limb of NaHS group.

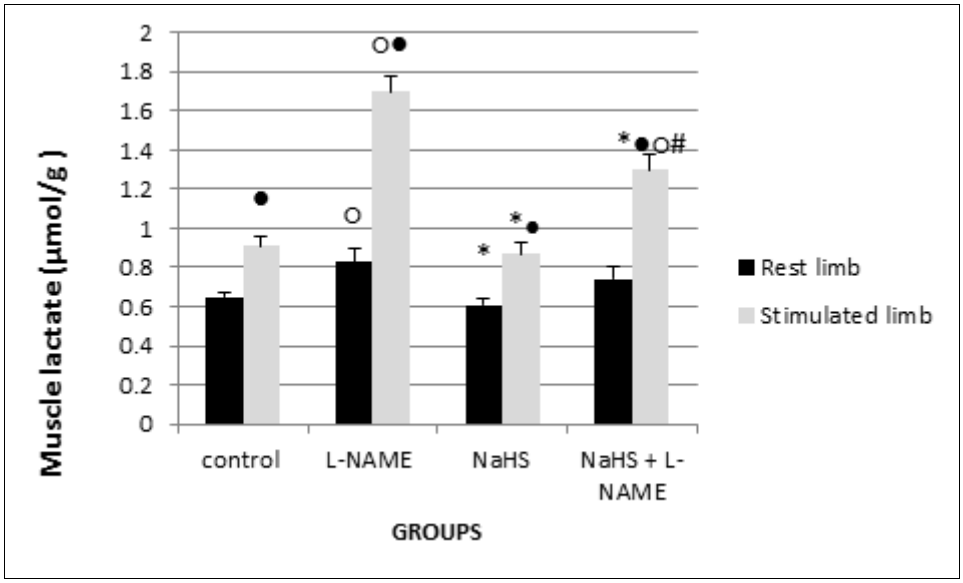

Figure (١): Changes in muscle lactate content (µmol/g wet weight muscle) in different studied groups.

Data are expressed as mean ± SEM of ٦ rats in each group.
●: Significant from resting limb. ○: Significant from corresponding limb of control group.
*: Significant from corresponding limb of L-NAME group. #: Significant from corresponding limb of NaHS group.

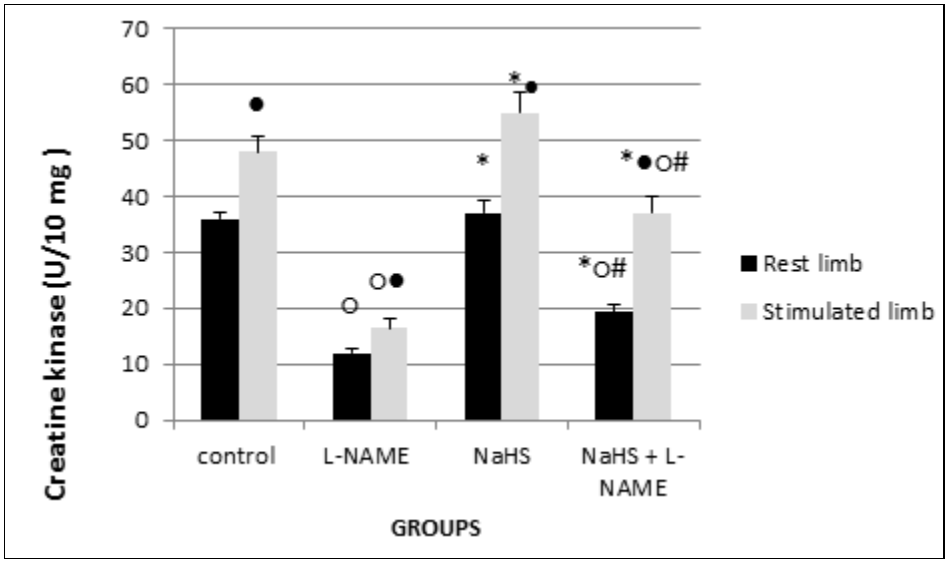

Figure (٢): Changes in muscle CK activity (U/١٠ mg wet weight muscle) in different studied groups.

Data are expressed as mean ± SEM of ٦ rats in each group.
●: Significant from resting limb. ○: Significant from corresponding limb of control group.
*: Significant from corresponding limb of L-NAME group. #: Significant from corresponding limb of NaHS group.

Discussion

In the present study, an attempt was made to study some of the factors contributing to skeletal muscle fatigue in the right gastrocnemius muscle of intact male albino rats under different treatment conditions as compared to the left resting gastrocnemius. LF electric current stimulation produced subtetanic contraction allowing ischemia/ reperfusion that generates high concentrations of free radicals. A significant increase in NO, lipid peroxides, muscle lactate and CK activities were present at ٥٠٪ fatigue in the stimulated gastrocnemius muscle as compared to the resting one of the control group.

Nitric oxide (NO), is formed from the amino acid L-arginine *via* nitric oxide synthases (NOS); neuronal (nNOS), and endothelial (eNOS) linked to dystrophin protein of the muscle cytoskeleton close to muscle blood vessels, where it regulates muscle blood flow during rest and contraction[٢٢]. Stimulation of e-NOS and n-NOS occurs during muscle contraction probably by increasing cytosolic $Ca^{٢+}$ since they are $Ca^{٢+}$ dependent[٢٤]. NO not only increases muscle blood flow during contraction, but also through stimulation of peroxisome proliferator activator receptor-γ cofactors can induce mitochondrial proliferation (mitochondrial biogenesis), oxidative enzymes of the citric acid cycle and β-oxidation of fatty acids which provide the energy sources during contraction[٢٥]. NO also through nitrosylation of glucose transporter proteins can enhance glucose extraction by contracting skeletal muscle independent of insulin[٢٦].

Basal levels of peroxides as markers of reactive oxygen species (ROS) were detected in the resting gastrocnemius muscle of this work and could be produced by the respiratory chain complex of the mitochondria or in the cytosol *via* xanthine oxidase, NADPH oxidases, and phosphorlipase $A_٢$ that generate superoxide radicals[٢٧]. Physiological increase in ROS generation during muscle contraction is essential for excitation-contraction (EC) coupling through oxidation of the thiol (-SH) groups of calcium sensor channels and other ion channels maintaining excitability[٢٨]., facilitates glucose uptake through an AMP-activated glucose transporter, and activates nuclear peroxisome proliferator activator receptor alpha (PPARα) to enhance fatty acid uptake and β-oxidation. In addition, free radicals maintain Redox homeostasis in the muscle

by feedback stimulation of antioxidant defense mechanisms[٢٩-٣١]. However, excess ROS production produces the reverse, especially excessive oxidation of myosin heads with failure of EC coupling, resulting in fatigue[٣٢]. NO is a double faced coin. In physiological concentrations, it is a scavenger of ROS, but in greater concentrations it forms the cytotoxic peroxinitrite radical and can contribute to fatigue[٣٣].

Creatine kinase (CK) catalyzes the reaction; Creatine phosphate + ADP + H^+ ↔ Creatine + ATP, in either direction, thus ensures proper channeling of high energy phosphate between the creatine/ phosphocreatine and the ADP/ATP systems to ensure adequate energy supply for the skeletal muscle at rest and during activity. In the mitochondria, Mitochondrial CK couples oxidative phosphorylation of the respiratory chain to creatine phosphate formation i.e. shift to the left of the above reaction, while Cytosolic CK is confined to the sites where it is in close proximity to ATPase as in myosin M line near the heads of myosin, the sarcoplasmic reticulum and the sarcolemma and shifts the reaction to the right [٣٤,٣٥]. The significant high CK level in the gastrocnemius of the stimulated limb of the control group in this study ensures a maintained ATP supply at the expense of CP and is compatible with ***McMullen et al.*** [٣٦]**.** However, CK activity can also contribute to muscle fatigue especially under oxidative stress conditions when the breakdown of CP results in excessive accumulation of inorganic phosphate (P_i). P_i interferes with the cross bridge cycle, and could diffuse to sarcoplasmic reticulum and precipitates $Ca^{٢+}$ according to previous researches [٣٧, ٣٨].

Muscle lactate increased in the muscle of the stimulated limb as compared with the contralateral relaxing limb. Muscle lactate system' contribution to ATP production is minimal, but it is essential for reoxidation of $NADH^+$ to NAD^+ ensuring continuous oxidation. It is a marker of anaerobic glycolysis. Because this system generates lactate and H^+ in equimolar concentrations and it was difficult to measure intracellular H^+, lactate was therefore incriminated as the cause of fatigue; however this concept is a subject of greater debate. Researches, in the past few years, proved that lactic acid accumulation during contraction of skeletal muscle keeps muscle excitability, EC coupling, and force production by decreasing chloride permeability through inhibiting voltage gated chloride channels in the T tubules thus preventing hyperpolarization of overactivity[٣٩,٤٠]. Previous concepts ascribed fatigue to increased H+ and not lactate that suppress the contractile machinery including, $Ca^{٢+}$ release and binding to troponin C and cross bridge cycling in addition to altering enzyme activity particularly phosphorylases of glycogenolysis and phosphofructokinase of glycolysis and hence block metabolic oxidative pathways[٣٩, ٤١].

In the present work, blocking NO synthesis by L-NAME injection significantly reduced the peak force, shortened the time till ٥٠ % fatigue, lowered NO, and CK activity, but increased lipid peroxides and muscle lactate. These results agree with ***Tidball and Henricks,*** [٢٤] who found that patients who lack NO as in Duchene myopathy suffer from muscle weakness and atrophy due to low muscle blood flow rate. The absent physiological role of NO in muscle contraction and the loss of its ROS scavenging function was associated with increased lipid peroxidation and accelerating fatigue according to previous research [٤٢].The highest levels of lactate obtained in this L-NAME group is a proof of the extreme hypoxic conditions due to lack of the vasorelaxant effect of NO. Furthermore, under such hypoxic or oxidative stress conditions, ATP consumption should be switched off to prevent its depletion to critical levels. This is mediated by inhibiting CK activity by a phosphorylating protein enzyme (AMP-activated protein kinase; AMPK) or by increased leakage from the muscle membrane according to previous researches[٣٤, ٣٥].

In the present study, i.p. injection of sodium hydrosulfide; as an exogenous donor of $H_٢S$ not only increased the peak force of contraction, but also delayed the onset of ٥٠% fatigue in the stimulated gastrocnemius muscle. This could be ascribed to decreased ROS as evidenced by a

significant lower lipid peroxide level. Muscle lactate and CK activity were not significantly different from the control group. H_2S is endogenously produced in different body tissues and plays a physiological role most probably as antioxidant. It is also produced in skeletal muscle; however its role in contraction and fatigue was not fully investigated although it was hypothesized to be incriminated in chronic fatigue syndrome[٣]. Therefore this study aimed to study this effect. The increased peak force and delayed fatigue found in the present study could be ascribed to the vasorelaxant effect of H_2S which increases blood flow according to previous results[٤,٤٣]. Furthermore, NAHS/H_2S could modify calcium channels by sulfhydration of channel proteins, an effect that protects channel proteins against oxidative damage by ROS according to ***Zhang et al*** [٤٤]. This could keep adequate calcium ions for excitation/contraction coupling. These results are consistent with previous researches[٤٥,٤٦] who found that in a rat model of ischemia/reperfusion of the hind limb skeletal muscles, preischemic delivery of hydrogen sulfide limits ischemia-reperfusion injury-induced cellular damage and reduced the apoptotic index in *in vitro* myotubes and *in vivo* skeletal muscles. This suggests that, when given in the appropriate dose, this molecule may have significant therapeutic applications in multiple clinical fatigue syndromes.

NaHS, in this study partially but significantly improved the fatiguing effect of L-NAME and improved the mechanical and metabolic conditions of the muscle. This may be ascribed to the role of NaHS/H_2S in scavenging ROS, minimizing oxidative stress, and improving blood flow. A vasorelaxant effect of H_2S through activation of voltage-sensitive potassium channels and not NO dependent was found by ***Kohn et al.*** [٤٧] and is compatible with the improved conditions in the combined group. Improved oxidative stress state is the cause of decreased muscle lactate and increased CK activity to generate more ATP.

Conclusion

NaHS administration alone improved rat gastrocnemius response to electric stimulation by increasing peak force production and delaying the onset of ٥٠٪ fatigue. This could result from ameliorating the oxidative stress response as evidenced by reduced lipid peroxides with consequent improvement of high energy phosphate regeneration. Increased CK activity and decreased lactate concentration is a good proof. Blocking nitric oxide generation by L-NAME produced opposite effects reflecting the importance of NO in EC coupling. Coadministration of NaHS with L-NAME significantly but incompletely corrected the fatiguing effects of L-NAME. This indicated that the antioxidant effects of H_2S are not NO dependent and there are physiological effects of NO that could not be completely substituted for by H_2S. The results also reflect the clinical significance of H_2S donors as a future promising treatment for chronic fatigue syndrome including different myopathies that lack NOS activity.

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