

RESEARCH ARTICLE

# Sexual Preferences in Nutrient Utilization Regulate Oxygen Consumption and Reactive Oxygen Species Generation in *Schistosoma mansoni*: Potential Implications for Parasite Redox Biology

Matheus P. Oliveira, Juliana B. R. Correa Soares, Marcus F. Oliveira\*

Laboratório de Bioquímica de Resposta ao Estresse, Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro, Cidade Universitária, Rio de Janeiro, Brazil

\* [maroli@bioqmed.ufrj.br](mailto:maroli@bioqmed.ufrj.br)



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**Citation:** Oliveira MP, Correa Soares JBR, Oliveira MF (2016) Sexual Preferences in Nutrient Utilization Regulate Oxygen Consumption and Reactive Oxygen Species Generation in *Schistosoma mansoni*: Potential Implications for Parasite Redox Biology. PLoS ONE 11(7): e0158429. doi:10.1371/journal.pone.0158429

**Editor:** Marc Liesa, University of California Los Angeles, UNITED STATES

**Received:** March 31, 2016

**Accepted:** June 15, 2016

**Published:** July 5, 2016

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**Data Availability Statement:** All relevant data are within the paper and its Supporting Information files.

**Funding:** This work was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, <http://www.cnpq.br/>) grant 402409/2012-4 and the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ, <http://www.faperj.br/>) grants E-26/102.333/2013 and E-26/111.169/2011. MFO was a research scholar from CNPq (grant 307636/2012-7), JBRCS was a PAPD-FAPERJ fellow (grant E26/

## Abstract

*Schistosoma mansoni*, one of the causative agents of human schistosomiasis, has a unique antioxidant network that is key to parasite survival and a valuable chemotherapeutic target. The ability to detoxify and tolerate reactive oxygen species increases along *S. mansoni* development in the vertebrate host, suggesting that adult parasites are more exposed to redox challenges than young stages. Indeed, adult parasites are exposed to multiple redox insults generated from blood digestion, activated immune cells, and, potentially, from their own parasitic aerobic metabolism. However, it remains unknown how reactive oxygen species are produced by *S. mansoni* metabolism, as well as their biological effects on adult worms. Here, we assessed the contribution of nutrients and parasite gender to oxygen utilization pathways, and reactive oxygen species generation in whole unpaired adult *S. mansoni* worms. We also determined the susceptibilities of both parasite sexes to a pro-oxidant challenge. We observed that glutamine and serum importantly contribute to both respiratory and non-respiratory oxygen utilization in adult worms, but with different proportions among parasite sexes. Analyses of oxygen utilization pathways revealed that respiratory rates were high in male worms, which contrast with high non-respiratory rates in females, regardless nutritional sources. Interestingly, mitochondrial complex I-III activity was higher than complex IV specifically in females. We also observed sexual preferences in substrate utilization to sustain hydrogen peroxide production towards glucose in females, and glutamine in male worms. Despite strikingly high oxidant levels and hydrogen peroxide production rates, female worms were more resistant to a pro-oxidant challenge than male parasites. The data presented here indicate that sexual preferences in nutrient metabolism in adult *S. mansoni* worms regulate oxygen utilization and reactive oxygen species production, which may differently contribute to redox biology among parasite sexes.

102.752/11), and MPO is a CNPq fellow (grant 160357/2012-8). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

**Abbreviations:** NTD, neglected tropical diseases; O<sub>2</sub>, molecular oxygen; ROS, reactive oxygen species; H<sub>2</sub>O<sub>2</sub>, hydrogen peroxide; O<sub>2</sub><sup>•-</sup>, superoxide; CS, citrate synthase; ΔΨ<sub>m</sub>, mitochondrial membrane potential; OXPHOS, oxidative phosphorylation; TCA, tricarboxylic acid; Prx, peroxiredoxin; GSH, reduced glutathione; Trx, thioredoxin; TGR, thioredoxin glutathione reductase; Glc, glucose; Gln, glutamine; OCR, oxygen consumption rate; R, respiration; NR, non-respiration; OS, other substrates; DTNB, 5,5-dithiobis(2-nitrobenzoic acid); DHE, dihydroethidium; NAC, *n*-acetyl cysteine; SEM, standard error of mean; AA, antimycin a; αKGDHC, α-ketoglutarate dehydrogenase complex; CPT1, carnitine palmitoyltransferase I; ETS, electron transport system.

## Introduction

The trematode *Schistosoma mansoni* is a long-living intravascular parasite and a major causative agent of human schistosomiasis, a chronic disease afflicting more than 240 million people worldwide [1]. Clinically, this illness is manifested in two distinct phases: an early acute one, when infected individuals exhibit mild symptoms such as fever, diarrhea and cough [2], and a late chronic phase, when severe clinical features including liver fibrosis, portal hypertension and hepatosplenomegaly, manifest [3]. These events are consequence of an intense inflammatory granulomatous reaction elicited by egg antigens deposited in intestine and liver [4–6]. Indeed, adult *S. mansoni* worms living in the mesenteric blood vessels are directly targeted by the host immune response [7,8]. Despite this, adult worms can live for decades within humans, implying the existence of efficient evasive mechanisms to counteract host immune response, which include *i*) reduced expression of antigenic proteins and the sequestering of host erythrocyte glycolipids at tegument surface [9,10], *ii*) the ability to mediate complement C3 degradation [8], *iii*) and the build-up of parasite antioxidant defenses along development [11–14].

Reactive oxygen species (ROS) are naturally produced in distinct cell compartments, such as in peroxisomes [15], in mitochondria [16], and at plasma membrane [17], playing key signaling roles in determining cell fate, growth and survival [18,19]. ROS have also been directly implicated as part of the immune arsenal in sterile inflammation, and during infections, mediating pathogen killing [20]. Interestingly, the observed increase in hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) detoxification capacity and resistance to multiple sources of ROS along *S. mansoni* development in the vertebrate host results from improved parasite antioxidant defenses [11–14]. This suggests that adult parasites are more exposed to redox challenges than young stages [21,22], which might include pro-oxidant products derived from physiological blood digestion [23–26], and ROS generated by activated immune cells [21,22] and, potentially, from parasite aerobic energy metabolism. In fact, adult *S. mansoni* are exposed to ROS produced by circulating immune cells, which play an essential role in parasite killing [21,22,27]. Thus, compelling evidence indicate that increased ability to detoxify ROS in *S. mansoni* represent an adaptive mechanism to avoid redox imbalance and parasite cell death triggered by host immune system.

Despite a clear relationship between energy and redox metabolism was established over the years for many organisms [16,28–35], knowledge on this metabolic intersection, and the potential consequences to basic aspects of trematode parasite biology were scarcely investigated in the literature [36–42]. Indeed, adult *S. mansoni* worms, living in the vertebrate bloodstream, are continuously exposed to molecular oxygen (O<sub>2</sub>), which is known to be physiologically utilized by the parasites to sustain egg production [43,44] and energy demand through the oxidative phosphorylation (OXPHOS) [42,45,46–55]. Regarding the nutrient requirements, it is known that carbohydrates [55], and particularly glutamine metabolism, play a key role to maintain parasite energy homeostasis, motility, tegumental resting membrane potential, muscle tension and viability in adult worms [56–59]. Although adult *S. mansoni* cannot synthesize *de novo* long chain fatty acids, they are able to produce complex lipids from host lipid sources [60,61]. Recently, fatty acid oxidation was demonstrated to play a central energetic role in adult female worms to support egg production, but not their viability, since genetic or pharmacological interference on lipolysis/β-oxidation drastically reduced parasite respiration and oogenesis [42,62].

Schistosomiasis control and treatment programs have relied for decades on a single therapeutic agent (praziquantel) [63]. However, growing concerns on the potential development of resistance to this drug [64] have prompted research on novel targets against *S. mansoni* [25,65–70]. Results from these efforts revealed the complexity, importance and uniqueness of antioxidant defense mechanism to parasite survival [65–72]. Noteworthy, beyond the presence

of classical antioxidant enzymes [11,13,72,73] and the complete lack of catalase, *S. mansoni* also express specific 2-cys peroxiredoxins (Prx) 2 and 3, which catalyses the decomposition of  $H_2O_2$  and organic hydroperoxides using both reduced glutathione (GSH) and thioredoxin (Trx) as electron donors [67,71,73]. Also, a unique thioredoxin glutathione reductase (TGR), which inter-convert reduced and oxidized forms of both GSH and Trx, plays a key antioxidant role [66,68–70]. Indeed, a number of strategies targeting specifically *S. mansoni* redox metabolism were designed and validated as new tools to treat schistosomiasis [25,65,68–70,74,75]. However, despite the knowledge on *Schistosoma* redox biology, the physiological sources of ROS, and their biological effects to adult *S. mansoni* worms remain largely unknown.

*Schistosoma* parasites are dioecious, with two genetically defined and dimorphic sexes [76–78], and these differences can be extended to worm energy and redox metabolism [47,52,57]. For example, glutamate, aspartate, and alanine uptake were reportedly higher in males [52,57], while no apparent gender trend was observed in glucose uptake [47,52,79–81]. Also, the rates of glucose, glutamate, and alanine oxidation were higher in males of both *S. mansoni* and *S. japonicum*, compared to females [52,57,82], suggesting increased OXPHOS in males. Beyond these mechanisms, two interesting metabolic features were also observed in adult worms: *i*) that copula regulates glucose metabolism [82–84] in a way that paired parasites exhibit higher glucose uptake than separated ones [83]; *ii*) males were able to transfer glycogen-derived glucose to females [83,84]. Regarding redox metabolism, evidence indicate that females contain higher levels of antioxidant mechanisms than males [67,78,85–87], including higher expression of ferredoxin NADP(H) oxidoreductase [85], peroxiredoxins 1,2, and 3 [67], Trx [78,86], TGR [78], and superoxide dismutases [78,87]. Indeed, the trend is that female worms are more dependent on antioxidant defenses than males, since depletion of GSH by oltipraz resulted in lower worm burden, particularly females [65], while simultaneous treatment with  $H_2O_2$  and distinct antioxidant enzyme inhibitors more intensely affected female than male worms [12].

Despite redox metabolism having been validated as a key chemotherapeutic target against schistosomiasis, and that parasites use distinct nutrients and  $O_2$  to meet their energy requirements [42–59], it remains elusive how endogenous ROS are produced by the parasite's own metabolism and their potential biological consequences. Here, we investigated how nutrients and gender regulate  $O_2$  utilization by distinct pathways and ROS generation in whole unpaired adult *S. mansoni* worms. We also assessed the susceptibilities to a pro-oxidant challenge among parasite sexes. The data shown here indicate that unpaired adult *S. mansoni* worms exhibit sexual preferences in substrate utilization that modulate  $O_2$  consumption and ROS generation pathways which might provide differential tolerance to oxidative stress among parasite sexes.

## Methods

### Ethics statement

All animal experiments were overseen and approved by the Commission for Evaluation of Animal Use for Research from the Federal University of Rio de Janeiro (CAUAP-UFRJ), protocol number IBQM#050), under strict accordance to the Guide for the Care and Use of Laboratory Animals [88]. Female Swiss mice at four weeks of age were obtained from Fundawer Instituto Oswaldo Cruz breeding colony (Fiocruz-RJ, Brazil) and housed at Institute of Medical Biochemistry Leopoldo de Meis (IBqMLdM-UFRJ) animal care facility. Mice were kept in a 12/12-hr light/dark cycle, fed with regular chow diet and water *ad libitum* and care was taken to minimize pain and suffering of the animals. Technicians dedicated to the IBqMLdM-UFRJ animal care facility carried out all aspects related to mice husbandry under strict guidelines to ensure careful and consistent handling of the animals.

## Parasites

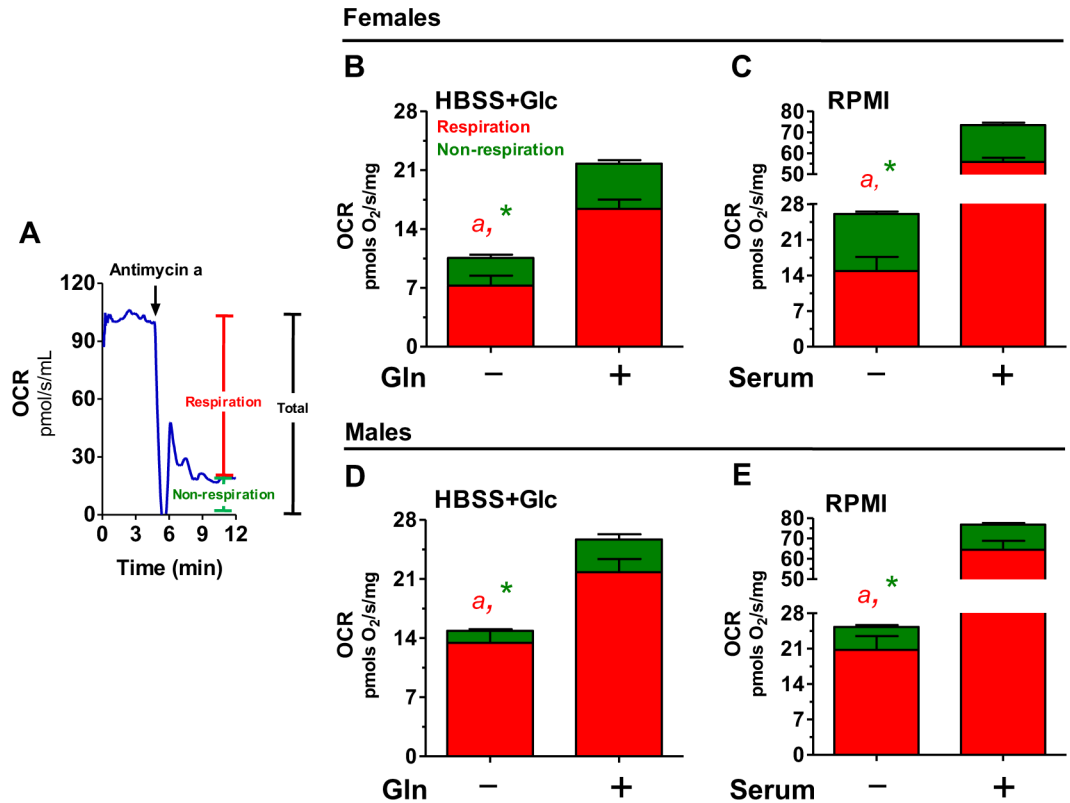
*S. mansoni* cercariae (LE strain) were obtained from infected *Biomphalaria glabrata* snails at Department of Malacology (Fiocruz-RJ, Brazil), as previously described [89]. Mice were infected by subcutaneous injection of 1 mL of water containing about 150 cercariae using an insulin syringe and then kept at IBqMLdM-UFRJ animal care facility for about forty-two days. Then, mice were humanely euthanized by CO<sub>2</sub> asphyxiation, and all efforts were made to minimize animal suffering. Adult worm pairs were manually collected from mice mesenteries and then pre-incubated for 1h at 37°C and 5% CO<sub>2</sub> in one of the following "pre-incubation media": *i*) Hank's Balanced Salt Solution (HBSS) containing 5.5 mM Glucose (hereafter named Glc); *ii*) HBSS containing 5.5 mM Glucose and 5.5 mM Glutamine (hereafter named Glc+Gln); *iii*) RPMI 1640 (LCG Biotecnologia, Brazil) containing 11 mM Glucose, 2.05 mM Glutamine, several aminoacids and vitamins (hereafter named RPMI); *iv*) RPMI containing 11 mM Glucose, 2.05 mM Glutamine, several aminoacids, vitamins and supplemented with 10% fetal bovine serum (hereafter named RPMI+Serum). During pre-incubation, worm pairs spontaneously separated from each other and sex-specific sets of worms (male or female) were utilized for all the subsequent experiments.

## Respirometry analyses

In order to assess O<sub>2</sub> utilization, 30 male or female worms were recovered from pre-incubation, as described in "parasites" section, and placed separately in 2 mL of the same "pre-incubation media" per chamber of a two-channel titration injection respirometer (Oxygraph-2k, Oroboros Instruments, Innsbruck, Austria). Data were recorded by using DatLab 5.0 software and measurements of O<sub>2</sub> consumption were carried out at 37°C with sustained stirring at 750 rpm. In these experiments, O<sub>2</sub> consumption by whole worms was recorded in basal conditions for 10 minutes (total OCR), followed by inhibition of ETS complex III activity by injecting 6 µg/mL antimycin A (AA) in each respirometer chamber. Then, respiration was left to proceed for 5–6 minutes. A typical O<sub>2</sub> flux trace is depicted in Fig 1A. In order to assess the contribution of β-oxidation to worm respiration, 200 µM etomoxir (previously prepared in water) was added in basal conditions and then O<sub>2</sub> fluxes recorded for about 10 minutes. Subsequently, 6 µg/mL AA was added to each respirometer chamber. In order to determine the mechanisms involved in O<sub>2</sub> utilization in whole worms, we assumed the O<sub>2</sub> consumption rate (OCR) sensitive to AA as respiration (R), the OCR sensitive to etomoxir as β-oxidation (β-ox) and finally, the OCR insensitive to AA as non-respiration (NR). O<sub>2</sub> consumption rates resistant to etomoxir and sensitive to AA were considered as the contribution of oxidation of other substrates (OS) to respiration. To assess the contribution of oxidative phosphorylation (OXPHOS) and proton leak (LEAK) to basal respiration, we titrated oligomycin (5–45 µg/mL) in whole worms kept in HBSS containing 5.5 mM glucose and then O<sub>2</sub> fluxes were recorded for about 10 minutes followed by addition of 6 µg/mL AA. We assumed the OCR sensitive to oligomycin as OXPHOS, while the LEAK OCR the one resistant to oligomycin and sensitive to AA.

## Mitochondrial enzyme activities

To determine mitochondrial enzyme activities, about 30 male or female worms were recovered from pre-incubation, as described in "parasites" section, and placed separately in 0.5 mL of hypotonic buffer (25 mM potassium phosphate and 5 mM MgCl<sub>2</sub>, pH 7.2) and then gently homogenized in a Potter-Elvehjem tissue grinder using a glass pestle. After homogenization, all samples were subjected to three liquid nitrogen-warm water bath freeze-thawing cycles. The preparation was maintained at 4°C throughout the procedure. CS activity was determined by measuring the reduction of 5,5-dithiobis (2-nitrobenzoic acid)(DTNB) according to the



**Fig 1. Nutrients regulate respiratory and non-respiratory pathways in adult *S. mansoni* worms:**  $O_2$  consumption rates (OCR) of intact adult *S. mansoni* worms were determined by high resolution respirometry in media containing four different nutrient compositions as following: HBSS containing 5.5 mM glucose (Glc), HBSS containing 5.5 mM glucose plus 5.5 mM glutamine (+Gln), RPMI 1640 (RPMI), RPMI 1640 containing 10% fetal bovine serum (+Serum). (A) Representative OCR trace obtained from 30 intact *S. mansoni* males in RPMI 1640. To determine the contribution of different pathways of  $O_2$  utilization, total OCR was measured followed by addition of 6  $\mu\text{g}/\text{mL}$  antimycin a (AA) to the respirometer chamber. The AA-sensitive component of the OCR was considered as "respiration", while the AA-insensitive OCR, as "non-respiration". In Fig B, C, D and E red bars represent respiration while green bars, non-respiration OCR determined in four media composition. Data are expressed as mean  $\pm$  SEM of at least four different experiments. Comparisons between groups were done by Student's *t* test or Mann-Whitney's test. Red letters and green asterisks represent statistical differences between different media in respiration and non-respiration, respectively. Fig (B): <sup>a</sup> $p = 0.0009$  (Student's *t* test) relative to +Gln,  $*p < 0.01$  (Student's *t* test) relative to +Gln. Fig (C): <sup>a</sup> $p = 0.0008$  (Mann-Whitney's test) relative to +Serum,  $*p = 0.03$  (Mann-Whitney's test) relative to +Serum. Fig (D): <sup>a</sup> $p = 0.0074$  (Mann-Whitney's test) relative to +Gln,  $*p = 0.0017$  (Mann-Whitney's test) relative to +Gln. Fig (E): <sup>a</sup> $p = 0.001$  (Mann-Whitney's test) relative to +Serum,  $*p = 0.0008$  (Mann-Whitney's test) relative to +Serum.

doi:10.1371/journal.pone.0158429.g001

literature [90] with slight modifications. DTNB reduction was followed in a coupled reaction with coenzyme A and oxaloacetate. The reaction mixture (1 mL) containing, 0.25 mM DTNB, adult *S. mansoni* homogenate (12  $\mu\text{g}$  protein), 0.3 mM acetyl CoA and 75 mM Tris-HCl, pH 8.0, was placed in a plastic cuvette and light absorption at 412 nm was assessed during 2 min (basal) at room temperature using a Shimadzu spectrophotometer UV/VIS 2450 (Shimadzu Scientific Instruments, Japan). Subsequently, CS reaction was started by adding 0.5 mM oxaloacetate to the cuvette and the rates of DTNB reduction were registered at 412 nm. CS activity was calculated by subtracting the rates of DTNB reduction induced by oxaloacetate from the basal using the molar extinction coefficient ( $\epsilon = 13,600 \text{ M}^{-1} \cdot \text{cm}^{-1}$ ) and expressed as nmol of reduced DTNB/min/mg protein. NADH:Cyt<sub>c</sub> oxido-reductase activity of the electron transport system (ETS) was measured by following the increase in absorbance at 550 nm due to

cytochrome *c* reduction [91]. The reaction containing 1 mL hypotonic buffer, 50  $\mu\text{M}$  oxidized cytochrome *c*, 100  $\mu\text{g}$  of protein from freeze-thawed worm homogenate samples and 1 mM KCN was initiated by adding 200  $\mu\text{M}$  NADH and the increase in absorbance at 550 nm was monitored at room temperature in a Shimadzu spectrophotometer UV/VIS 2450 (Shimadzu Scientific Instruments, Japan) for about 5 minutes. Rotenone (5  $\mu\text{M}$ ) was added to inhibit complex I activity, which was considered as the rotenone-sensitive rate of cytochrome *c* reduction ( $\epsilon = 18.5 \text{ mM}^{-1} \cdot \text{cm}^{-1}$ ). Cytochrome *c* oxidase activity was measured by following the decrease in absorbance due to the oxidation of ferrocytochrome *c*. The reaction containing 1 mL hypotonic buffer, 50  $\mu\text{M}$  reduced cytochrome *c* was initiated by the addition of 100  $\mu\text{g}$  of protein from freeze-thawed worm homogenate samples and the reduction in absorbance was measured at 550 nm. KCN (1 mM) was added to inhibit cytochrome *c* oxidase activity, which was considered as the cyanide-sensitive rate of cytochrome *c* oxidation.

### Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) release

$\text{H}_2\text{O}_2$  production was assessed in whole intact worms by recovering individuals from pre-incubation in HBSS containing 5.5 mM Glucose or HBSS 5.5 mM Glucose + 5.5 mM Glutamine, as described in "parasites" section, and placing 30 male or female worms separately in 2 mL of the same pre-incubation media in a spectrofluorimeter cuvette. Parasite  $\text{H}_2\text{O}_2$  production rates were determined by monitoring resorufin fluorescence due to the oxidation of 5  $\mu\text{M}$  amplex red (Invitrogen, USA) in the presence of 200  $\mu\text{g}/\text{mL}$  horseradish peroxidase (Sigma, USA) at room temperature using a Cary Eclipse spectrofluorimeter (Varian, USA) adapted with a continuous stirring device. Fluorescence was assessed using excitation and emission wavelengths of 530 nm and 590 nm, respectively. After each measurement, a standard curve of reagent grade  $\text{H}_2\text{O}_2$  (Merck, Germany) was performed.

### Determination of total reduced thiol content

Total reduced thiol content was determined based on a previous method [92]. In this assay, 10 male or female worms were recovered from pre-incubation, as described in "parasites" section, and transferred separately to 24-well plates containing 500  $\mu\text{L}$  RPMI 1640 without serum and phenol red (control), or 500  $\mu\text{L}$  RPMI 1640 without serum and phenol red plus 10  $\mu\text{M}$  menadione. The plates were incubated for 1 h at 37°C and 5%  $\text{CO}_2$ . After that, all the well content (media + worms) was collected and homogenized in a Potter-Elvehjem tissue grinder using a glass pestle at 4°C. Aliquots of 200  $\mu\text{L}$  of homogenates were immediately mixed with 150  $\mu\text{L}$  of 200 mM Tris buffer, pH 8.2, 10  $\mu\text{L}$  of 10 mM DTNB (5, 5'-dithiobis-(2-nitrobenzoic acid) and 640  $\mu\text{L}$  of 100% methanol. Sample blanks consisting of RPMI 1640 without serum, phenol red and DTNB were also prepared accordingly. All samples were incubated at room temperature (25°C) for 15 minutes, subsequently centrifuged at 3,000  $\times g$  for 15 minutes at 25°C, and supernatant light absorption was measured at 412 nm in a SpectraMax M5 plate reader (Molecular Devices, US). The content of total reduced thiols in homogenates was determined by subtracting the values found in the experimental group homogenates minus the values found in the sample blanks using the molar extinction coefficient ( $\epsilon = 13,600 \text{ M}^{-1} \cdot \text{cm}^{-1}$ ) to calculate the amount of reduced DTNB in each sample. Data for each sex was expressed as % total reduced thiols content in menadione treated worms relative to their respective controls.

### Quantification of intracellular oxidant levels by fluorescence microscopy

Worms recovered from pre-incubation in RPMI 1640 without serum and phenol red, as described in "parasites" section, were transferred separately to 96 wells plates and then incubated with 50  $\mu\text{M}$  dihydroethidium (DHE) prepared in RPMI 1640 without serum and phenol

red, at a density of 1 worm/well, in darkness for 20 minutes at 37°C and 5% CO<sub>2</sub>. Parasites were subsequently washed twice with 1 x PBS pH 7.4, re-suspended in 200 µL of 1 x PBS and whole body fluorescence images were captured in a stereo microscope (Olympus model MVX10) using excitation at 546 nm and emission at 590 nm. Images were analyzed by ImageJ/Fiji software [93], split into red, green and blue channels, but only the red channel images were used for quantification purposes. Masks were created on every image to define the worm body as the regions of interest, thus eliminating background signals. Average fluorescence intensity of masked images were registered and expressed as arbitrary fluorescence units.

## Viability assay

Worms recovered from pre-incubation in RPMI 1640 without serum and phenol red, as described in "parasites" section, were transferred separately to 48-well plates, at a density of 3 worms/well. Then, parasites were challenged with different concentrations of the pro-oxidant menadione (0–50 µM) in 500 µL RPMI 1640 without serum and phenol red for 2h at 37°C and 5% CO<sub>2</sub>. Antioxidant control experiments of worms exposed to 50 µM menadione and 1 mM *n*-acetyl cysteine (NAC) were also performed. Viability of worms was then assessed under a light microscope using the motility scale previously described [94]. The distinct parasite motile phenotypes were scored on a scale of 0–4 (4 = normally active; 3 = slow activity; 2 = minimal activity, occasional movement of head and tail; 1 = absence of motility apart from gut movements; 0 = total absence of mobility). Data were expressed as mean ± SEM of at least three different experiments

## Data and statistics

Data were presented as mean ± SEM values for all conditions. D'Agostino and Pearson normality test was performed to assess Gaussian distribution. Comparisons between groups were done by one-way ANOVA and *a posteriori* Tukey's test for pair-wise comparisons. When appropriate, two-way ANOVA or unpaired Student's *t*-tests or Mann-Whitney's test were employed. Outlier values were excluded from data only when Gaussian distribution was achieved, using the online tool available at <http://graphpad.com/quickcalcs/Grubbs1.cfm>. Differences of  $p < 0.05$  were considered to be significant. Linear regression slopes comparisons between groups were conducted by using analysis of covariance (ANCOVA). All graphs and analyses were carried out by using the GraphPad Prism software version 5.00 for Windows (GraphPad Software, USA).

## Results and Discussion

### a) Nutritional requirements to sustain respiratory and non-respiratory O<sub>2</sub> utilization in adult worms

Adult stages of *S. mansoni* live in the vertebrate bloodstream, where proteins, carbohydrates, lipids, and O<sub>2</sub> are readily available and utilized by the parasites to sustain egg production [42–44] and energy demand through OXPHOS [45,46,50,51,56–59]. Since cells utilize O<sub>2</sub> not only to couple nutrient oxidation to respiration, but also to allow reactions mediated by oxygenases [95–98], and reactive oxygen species (ROS) generation [15–17], in Fig 1 we assessed O<sub>2</sub> utilization by whole intact adult unpaired females and males in media with four different nutrient compositions. Fig 1A shows a representative trace of O<sub>2</sub> consumption rate (OCR) obtained from 30 intact *S. mansoni* males in RPMI 1640 media, where total OCR were registered at basal levels to define the total parasite O<sub>2</sub> utilization. Then, 6 µg/mL antimycin A (AA) was injected to the respirometer chamber, and the resulting AA-sensitive component of total OCR was

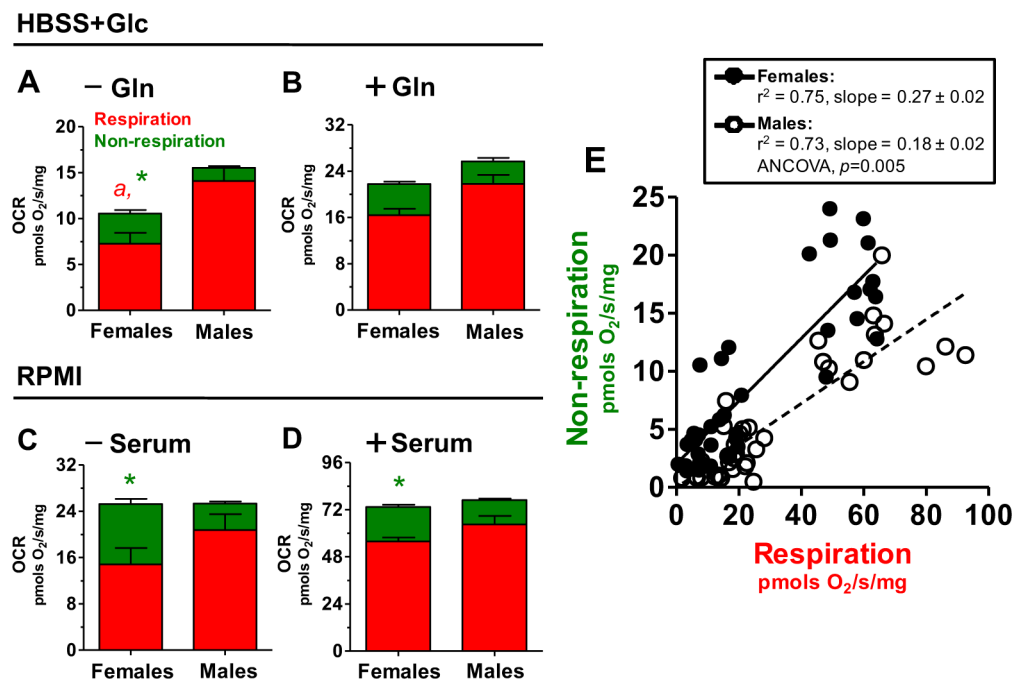
considered as "respiration", while the AA-insensitive, as "non-respiration" (Fig 1A). We observed that total OCR (S1A–S1D Fig), as well as the respiratory and non-respiratory components (Fig 1B–1E), were strikingly distinct depending on the nutrient availability. The lowest OCR values were obtained when 5.5 mM glucose was the only carbon source available for both parasite sexes (S1A, S1C, S1B and S1D Fig), while the highest ones were observed in the presence of RPMI+Serum (S1B, S1D, S1C and S1E Fig). Given that glutamine has previously reported to play a key energetic role in adult *S. mansoni* [56–59], our next step was to determine the contribution of this amino acid to distinct O<sub>2</sub> utilization pathways. Glutamine supplementation to HBSS + 5.5mM glucose, significantly increased total (S1A Fig), including the respiratory and non-respiratory OCR components in female worms (Fig 1B). Indeed, the presence of glutamine increased by 106% total OCR, 126% respiratory, and only 68% non-respiratory OCR when compared to glucose, indicating the preferential use of this amino acid to sustain respiration in female worms. Supporting evidence from the literature demonstrate that glutamine metabolism does not affect glucose oxidation in adult worms [59]. In male parasites, glutamine significantly increased total (S1C Fig), as well as respiration and non-respiration OCR components relative to glucose (Fig 1D). Interestingly, the relative increases in OCR by glutamine supplementation were 73% in total, 62% in respiration, and 171% in non-respiration, indicating the remarkable contribution of glutamine metabolism to non-respiratory O<sub>2</sub> utilization in male *S. mansoni*. Given that glutamine contributes to tricarboxylic acid cycle through  $\alpha$ -ketoglutarate dehydrogenase complex ( $\alpha$ KGDHC) activity, which represents an important site of mitochondrial hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) generation [99–101], it is possible that higher non-respiratory OCR induced by glutamine in male worms might result from increased ROS production through  $\alpha$ KGDHC. Indeed, higher glutamate oxidation observed in male worms relative to females [57], provide supporting evidence for this proposal. In order to determine the contribution of other nutrients and vitamins to parasite O<sub>2</sub> utilization, respirometry was also carried out in worms maintained in RPMI 1640 media in the absence or the presence of 10% fetal bovine serum (S1B and S1D Fig and Fig 1C and 1E). In general, absolute respiratory OCR values obtained in HBSS+Glc+Gln were quite similar as those from RPMI, suggesting that RPMI components other than glucose and glutamine have a negligible contribution to respiration in both parasite sexes. However, non-respiratory OCR in females were higher in RPMI relative to HBSS+Glc+Gln. Given that RPMI contains tyrosine, female tyrosinase activity might explain high non-respiratory OCR provided by this media, as this enzyme uses O<sub>2</sub> to produce melanin allowing the formation and hardening of *Schistosoma* eggshell [88,102]. The highest parasite OCR values were achieved when serum was supplemented to RPMI 1640 media (S1 Fig), which might be due to increased supply of co-factors, nutrients and/or hormones present in serum that ultimately activate worm metabolism and O<sub>2</sub> utilization [54,103,104]. Serum supplementation caused a relative increase by 190–195% in total OCR, 210–276% in respiration, and 56–173% in non-respiration in adult worms, indicating the remarkable contribution of serum to non-respiratory O<sub>2</sub> utilization in male *S. mansoni*. However, since serum has a complex and undefined composition, we focused the next experiments in defined media without serum supplementation. As a result of respirometry analyses conducted in Fig 1 and S1 Fig, we observed that respiration represent the dominant mechanism of O<sub>2</sub> utilization in *S. mansoni*, regardless the nutrient source available and parasite sex (S2 Fig). In female worms, the percent contribution of respiratory rates to total parasite O<sub>2</sub> utilization were from 57% to 76%, while for non-respiration varied between 23% and 42%. In male parasites, the percent contribution of respiration to total OCR was higher than in females, ranging from 81% to 87%, while the opposite was observed for non-respiration, ranging from 12% to 18% (S1 Fig). Therefore, we conclude that glutamine and serum importantly contribute



to respiratory and non-respiratory OCR in both parasites sexes, and that glutamine metabolism has a prominent contribution towards respiration in females and to non-respiration in males.

**b) Opposite sexual trends in O<sub>2</sub> metabolism: high respiratory rates in male worms contrast with high non-respiratory rates in females independent of nutritional sources**

Based on reported sexual differences in *Schistosoma* energy metabolism [46,51,56,76–83], and the distinct effects of nutrients on parasite O<sub>2</sub> consumption shown in the present work (Fig 1 and S1 Fig), we then compared O<sub>2</sub> utilization pathways between unpaired female and male parasites in different media compositions (Fig 2 and S2 and S3 Figs). We observed that males exhibited slightly higher OCR values in glucose (Fig 2B) and RPMI media (Fig 2C) compared to females. However, the general trend observed was that respiration in male worms was slightly higher than females (Fig 2A, 2B, 2C and 2D), reaching significance only when parasites were maintained in 5.5 mM glucose as the only nutritional source ( $p < 0.005$ , Fig 2A). These observations are consistent with higher expression and activity of glycolytic pathway enzymes in male worms relative to females [78,105–107]. Also, these data agree with similar total O<sub>2</sub> consumption rates observed among sexes [47], despite CO<sub>2</sub> production from glucose, glutamate and other aminoacids were higher in male worms compared to females in two



**Fig 2. Respiratory rates are higher in male worms, while non-respiratory O<sub>2</sub> utilization rates are higher in females:** Comparative analyses of respiratory (red bars) and non-respiratory (green bars) OCR in adult female and male worms was determined by high resolution respirometry in media containing four different nutrient compositions as following: HBSS containing 5.5 mM glucose (HBSS+Glc) (A), HBSS containing 5.5 mM glucose plus 5.5 mM glutamine (+Gln) (B), RPMI 1640 (RPMI) (C), RPMI 1640 containing 10% fetal bovine serum (+Serum) (D). (E) Correlation analysis of non-respiratory and respiratory OCR in adult *S. mansoni* females (closed black circles) and males (open circles) determined in four media compositions, as described in Fig 1 and S1–S3 and S6 Figs. Data are expressed as mean  $\pm$  SEM of at least four different experiments. Comparisons between groups were done by Student’s t-test, Mann-Whitney’s test or linear regression followed by ANCOVA analysis. Fig (A): <sup>a</sup> $p = 0.0013$  (Student’s t test) and \*  $p = 0.0023$  (Mann-Whitney’s test) relative to males. Fig (C): \*  $p = 0.03$  (Mann-Whitney’s test) relative to males. Fig (D): \*  $p = 0.0025$  (Mann-Whitney’s test) relative to males. Fig (E): ANCOVA  $p = 0.005$ .

doi:10.1371/journal.pone.0158429.g002

*Schistosoma* species [52,57]. In this sense, the activity of tricarboxylic acid (TCA) cycle enzymes aconitase, malate dehydrogenase and isocitrate dehydrogenase were reportedly higher in male *Schistosoma* compared to females [54,105–107]. This strongly indicates that male *S. mansoni* worms have improved capacity to mediate nutrient oxidation and respiration, which is substantiated by the data shown in Fig 2A–2D. Indeed, mitochondria isolated from males exhibit higher respiratory rates using glutamate, malate and succinate as substrates compared to females (S1 Methods and S4 Fig), despite the relative increases in OCR provided by ADP were quite similar in both sexes (140% and 127% for females and males, respectively). Also, the contribution of oxidative phosphorylation (OXPHOS) to basal respiration do not differ among sexes, while the proton leak OCR have a trend to be higher in males (S5 Fig). Interestingly, assessment of non-respiratory O<sub>2</sub> utilization rates reveals an opposite sexual trend, where females exhibited significantly higher absolute OCR values than males, in most experimental conditions (Fig 2A, 2C and 2D). Indeed, the relative contribution of non-respiratory pathways to total O<sub>2</sub> utilization in females was significantly higher (23–42% of total OCR) than in males (12–18% of total OCR), regardless the nutritional sources available (S3A, S3B, S3C and S3D Fig). Fig 2E shows a correlation analysis of absolute rates of O<sub>2</sub> utilization through respiratory and non-respiratory pathways for female and male worms in all nutritional conditions tested in Fig 2. We observed that the slope of the curve generated for female worms was significantly steeper than that for males ( $p = 0.006$ ), indicating that O<sub>2</sub> utilization through non-respiratory mechanisms is higher in females, while respiratory rates were higher in male worms. Conceivably, increased activity of distinct oxygenases reportedly to be functional in *Schistosoma* [88,102,108–110], or cellular ROS production by the own parasite metabolism might explain high non-respiration OCR values observed in females. In conclusion, an opposite sexual trend in O<sub>2</sub> utilization was observed in adult *Schistosoma* parasites, in a way that high respiratory rates in male worms contrast with high non-respiratory rates in females independent of nutritional sources.

### c) Similar contribution of $\beta$ -oxidation to respiration among parasite sexes

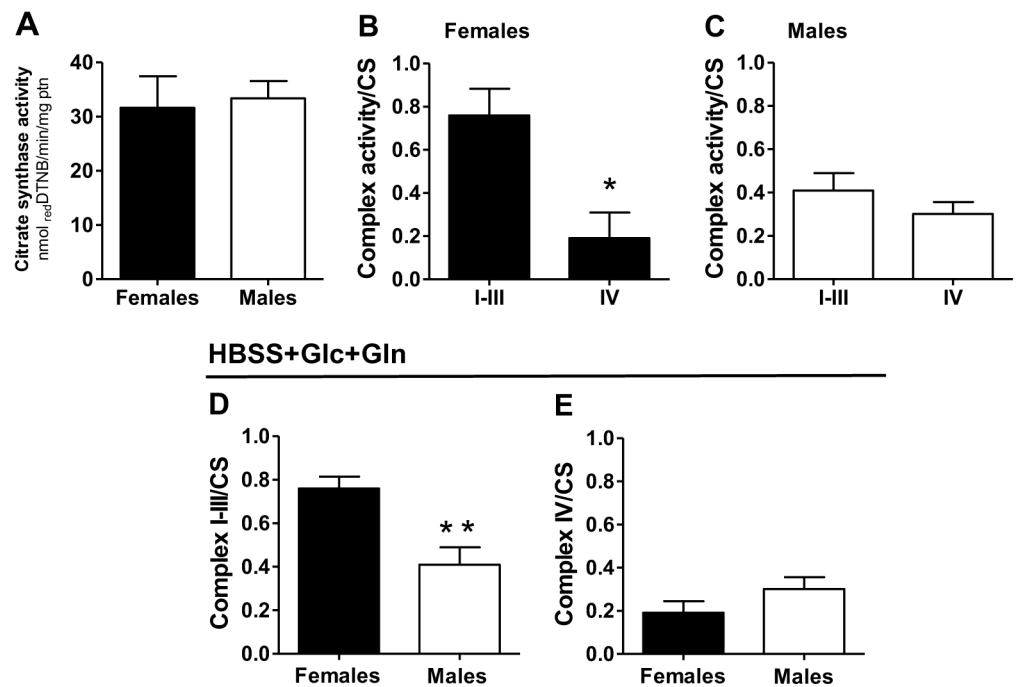
Although morphology and motility of adult worms hardly change in anoxic conditions, egg production and viability are drastically affected [43]. Interestingly, fatty acids provided *in vitro* were able to support egg laying and development [111], and recent evidence demonstrated that lipolysis and  $\beta$ -oxidation are absolutely required to allow oogenesis [42]. Given the distinct sexual trends in nutrient utilization observed in the present work (Figs 1 and 2, S1–S3 Figs), and the importance of fatty acid oxidation to *S. mansoni* physiology [42], we then investigated the contribution of  $\beta$ -oxidation to respiration in both parasite sexes kept in RPMI+serum, by means of pharmacological intervention of carnitine palmitoyltransferase I (CPT1) provided by etomoxir [112]. We observed that blockage of fatty acid transport to mitochondria by etomoxir had a consistent reduction in respiration in both parasite sexes. Quantification of the etomoxir-sensitive OCR demonstrated that the extent by which  $\beta$ -oxidation contributes to respiratory rates is roughly similar among parasite sexes (31% and 29% in female and male parasites, respectively) (S6 Fig). Interestingly, these data agree with quite similar CD36 expression levels among parasite sexes [78], which mediates cholesteryl ester uptake and might participate in  $\beta$ -oxidation in adult worms [113]. Although the data shown in S6 Fig are consistent with the key role of lipolysis and  $\beta$ -oxidation to O<sub>2</sub> utilization and egg production in female worms [42], the biological significance of mitochondrial fatty acid oxidation in male worms remain elusive. Two possibilities should be considered in this context: *i) motor activity*: given the abundance of muscle fibers in adult male worms, the energy demand to sustain muscle contraction might be partially achieved through  $\beta$ -oxidation, similar to vertebrate muscle fibers

[114,115]; ii) reproduction: although the contribution of fatty acid oxidation to spermatozoa motility is quite limited in other models [116], this process may play an energetic role in male *S. mansoni* spermatozoa. Finally, no gender differences were observed in the capacity to oxidize fatty acids, suggesting that mitochondrial content and/or the enzyme machinery directly involved in this process are also comparable among parasite sexes.

**d) Increased complex I-III, but not complex IV activity in female worms, favors electron delivery to mitochondria**

Given that male parasites exhibit higher activity of some TCA cycle enzymes [54,105–107], and higher respiratory rates compared to females (Fig 2 and S3 Fig), we then investigated whether mitochondrial content and electron transport system (ETS) complex activities might explain these sexual differences. S7 Fig and Fig 3A show that citrate synthase (CS) activity was quite similar among tested media and parasite sexes, indicating that mitochondrial content per protein mass was similar in female and male worms independent of nutritional sources available. Indeed, CS activities measured in our experimental conditions were quite close in absolute values to those reported earlier in the literature [105]. Interestingly, we observed that complex I-III activity was higher than complex IV specifically in females (Fig 3B and 3C), regardless the

**HBSS+Glc+Gln**



**Fig 3. Higher electron transport chain complex I-III activity in adult *S. mansoni* females despite similar mitochondrial content among sexes.** Mitochondrial enzymes activities were measured in homogenates of adult females (black bars) and males (white bars) previously maintained in HBSS containing 5.5mM glucose plus 5.5 mM glutamine (HBSS+Glc+Gln). (A) Citrate synthase (CS) activity was assessed following the increase in light absorption at 412 nm, while complex I-III and complex IV (B and C) activities were determined through reduction (for complex I-III) or oxidation (for complex IV) of cytochrome c at 550 nm. Comparative analyses of complex I-III (D) or complex IV (E) activities in females (black bars) and males (white bars) homogenates, normalized by their respective CS activities. Data are expressed as mean ± SEM of at least five different experiments. Comparisons between groups were done by Mann-Whitney’s test. Fig (B): \*  $p < 0.01$ . Fig (D): \*  $p < 0.05$ .

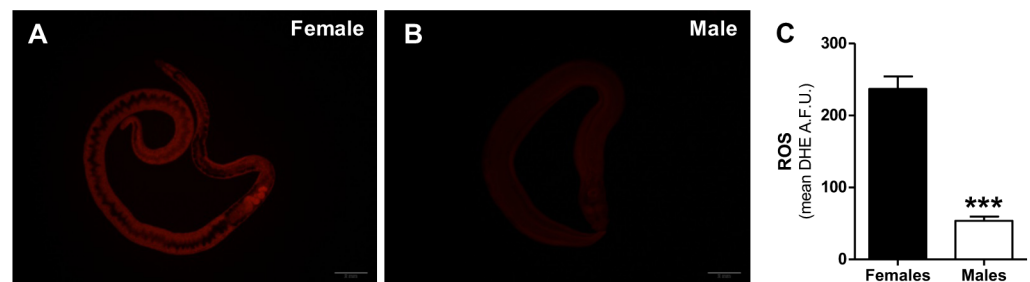
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media composition (Fig 3D and S9 Fig). This result contrasts with the recent transcriptome analyses of adult *Schistosoma* [78] which show that seven distinct complex I subunits were significantly more expressed in male worms compared to females. Since in our experiments, NADH-induced cytochrome *c* reduction depends on ubiquinone levels and complex III, it might be that these components compensate the reduced expression of complex I subunits to allow high complex I-III activity in female worms. Regarding complex IV activity, no differences were observed among parasite sexes and media composition (Fig 3E and S9D–S9F Fig). Of note, all enzyme activities shown in Fig 3B–3E were expressed normalized by CS, indicating that increased complex I-III activity observed in female worms is not a consequence of higher mitochondrial content. Therefore, higher complex I-III relative to complex IV activity in female worms would provide a more efficient mechanism for electron entry to ETS compared to males. Overall, the data shown in Fig 3 and S7–S9 Figs indicate that improved capacity to mediate nutrient oxidation and respiration observed in male worms (Fig 2A and 2E and S3 Fig) is not a consequence of higher mitochondrial content or to improved ETS complex activities, but rather to other mechanisms, such as efficient substrate transport and/or metabolism through specific TCA cycle enzymes [54,105–107]. Conceivably, limited mitochondrial pyruvate carrier activity in females may explain lower respiratory rates compared to males (Fig 2 and S3 Fig), even though exhibiting higher complex I-III activity (Fig 3D and S9A–S9C Fig). In addition, despite the similar capacity to oxidize fatty acids agrees with comparable CS and complex IV activities among sexes, the differences observed in female and male O<sub>2</sub> utilization (Fig 2 and S3 Fig) cannot be explained by these mechanisms. On the other hand, higher complex I-III activity observed in female worms, contrast with their lower respiratory rates relative to male worms, suggesting that increased electron flux in female mitochondria might have a distinct fate than fully reduce O<sub>2</sub> to water through complex IV. Therefore, it is possible that higher electron delivery to ETS through complex I-III activity in females would favor mitochondrial electron leak and ROS production, which is consistent with reduced respiratory rates and higher O<sub>2</sub> utilization through non-respiratory pathways (Fig 2 and S3 Fig).

### e) Female worms exhibit higher levels of intracellular oxidants relative to males

Despite the knowledge on parasite antioxidant mechanisms [11–14,65–72], and their relevance as a valuable chemotherapeutic target [25,65,68–70,74,75], direct evidence of endogenous ROS production by adult *Schistosoma* worms, and its biological significance, remains so far elusive. Of note is the sexual dimorphism that adult parasites exhibit in many distinct aspects, including the energy [47,52,57,79–84] and redox metabolism [67,78,85–87], with potential implications for rational and sex-specific drug design for schistosomiasis. In this sense, redox homeostasis was assessed in adult worms by evaluating the intracellular levels of oxidants through the use of the fluorescent probe dihydroethidium (DHE) [117]. Both female and male *S. mansoni* were kept in RPMI 1640 media without phenol red and images were acquired in a fluorescence stereoscope. Fig 4 shows representative DHE fluorescence images of both female (Fig 4A) and male (Fig 4B) worms, which stained quite homogeneously throughout the worm body in both sexes. In female worms, reduced fluorescence in the gut lumen could be due to interference of erythrocytes remnants and hemozoin crystals on light excitation or emission. Indeed, fluorescence intensity was clearly higher in female worms (Fig 4A), indicating higher levels of oxidants relative to male *S. mansoni* (Fig 4B). The differences observed in DHE staining are not due to limited probe uptake in males, as incubation of intact worms with an unrelated dye (DAPI) revealed the exact opposite trend (higher fluorescence in males compared to females) (S1 Methods and S10 Fig). These data are in agreement with the higher non-

## RPMI



**Fig 4. Intracellular oxidant levels are higher in females.** The levels of intracellular oxidants were assessed in whole female (A) and male (B) worms incubated in RPMI 1640 without phenol red and 50  $\mu$ M DHE. Fluorescence stereomicroscope images were collected for whole individual worms, the mean fluorescence intensity was quantified and expressed as arbitrary units (C). Data are expressed as mean  $\pm$  SEM of at least seventeen different experiments. Comparisons between groups were done by using Student's t-test. Fig (C): \*\*\* $p < 0.001$ .

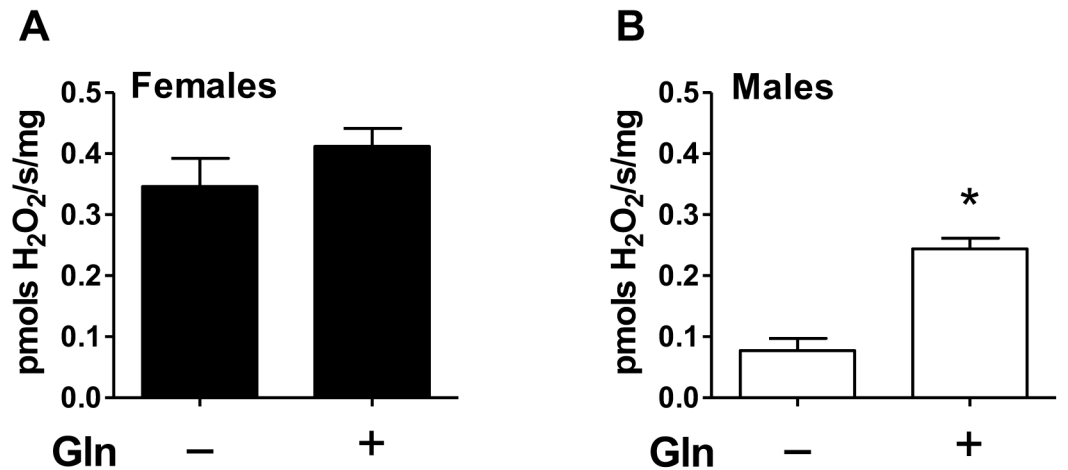
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respiratory  $O_2$  utilization observed in female worms relative to males, irrespective of the nutritional sources available (S3 Fig). Fig 4C shows the quantification of DHE fluorescence intensities in both parasite sexes, and confirms that oxidant levels in females were strikingly higher than in male worms. However, it is important to consider that the assessment of DHE fluorescence through imaging, as a proxy to detect intracellular ROS levels, has several limitations [117], and should be interpreted with caution and supported by alternative methods. Thus, in order to directly assess total ROS production, we next quantified  $H_2O_2$  production rates by whole parasites using the amplex red method.

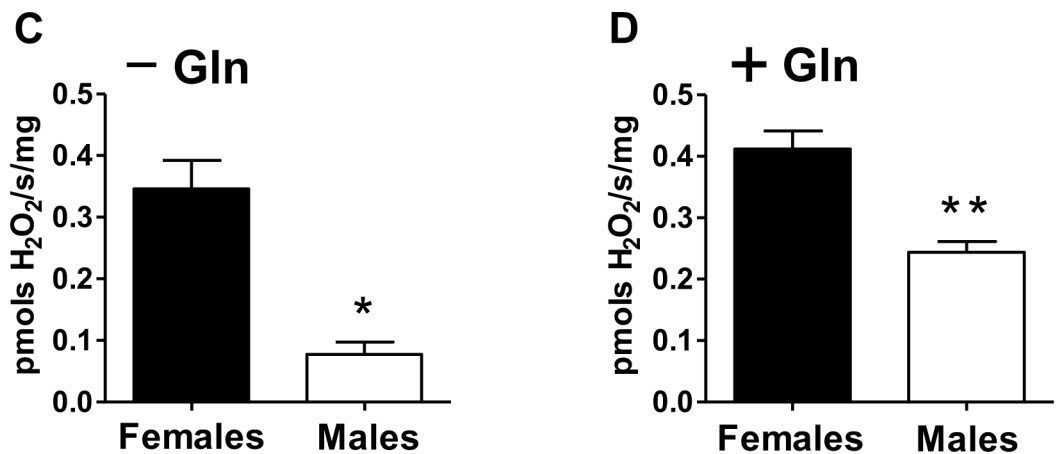
#### f) Sexual preferences in substrate utilization defines endogenous reactive oxygen species production in adult *S. mansoni*

Cellular ROS production is mediated by distinct mechanisms [15–17], with quite specific substrate dependencies [15]. Beyond their central energetic role, glucose and glutamine metabolism also regulate redox homeostasis, through the pentose phosphate pathway [118], and glutaminase-mediated lactate production [119] respectively. In both cases, these substrates contribute to maintain redox balance by generating reducing potential as NADPH to allow buffering of antioxidant defenses. Although glutamine metabolism directly participates on glutathione synthesis, reducing ROS production and limiting redox imbalance [120], in specific conditions this amino acid contributes to cellular ROS production [121–123]. Thus, in order to assess how nutrient mediate endogenous ROS production in adult parasites, we next determined the rates of  $H_2O_2$  production in whole intact *S. mansoni* worms kept under two distinct nutritional conditions: 5.5 mM glucose and 5.5 mM glucose + 5.5 mM glutamine. Fig 5A shows that  $H_2O_2$  production rates by female worms were high and similar in both media composition (19% higher in glucose + glutamine relative to glucose), suggesting that glucose metabolism plays a dominant role in female ROS production. The rates of  $H_2O_2$  production were clearly lower in male worms (Fig 5B) compared to females, especially when glucose was the only substrate available. Interestingly, glutamine supplementation caused a significant increase ( $p < 0.02$ ) in  $H_2O_2$  generation rates relative to glucose in male worms (215% higher in glucose + glutamine relative to glucose), suggesting that this amino acid plays a major role in male *S. mansoni* ROS production (Fig 5B). These data agree with a 117% increase in non-respiratory  $O_2$  utilization relative to glucose provided by glutamine in male worms (Fig 2B). Therefore, adult parasites exhibit clear sexual substrate preferences for  $H_2O_2$  generation (glucose in

## HBSS+Glc



## HBSS+Glc

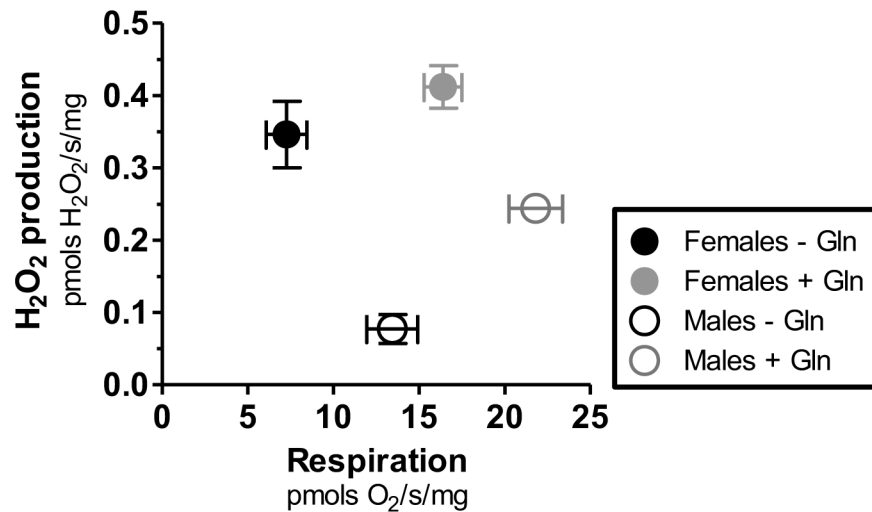


**Fig 5. Higher H<sub>2</sub>O<sub>2</sub> production rates in females is independent of nutritional source.** H<sub>2</sub>O<sub>2</sub> production rates were quantified in adult intact females (black bars) and males (white bars) previously maintained in HBSS containing 5.5 mM glucose (HBSS+Glc), or HBSS containing 5.5 mM glucose plus 5.5 mM glutamine (HBSS+Glc+Gln), by following the fluorescence of Amplex red oxidation. (A) Females and (B) males H<sub>2</sub>O<sub>2</sub> production rates promoted by different media composition. (C) Comparative analyses of H<sub>2</sub>O<sub>2</sub> production rates among parasite sexes in HBSS+Glc (C) or HBSS+Glc+Gln (D). Data are expressed as mean ± SEM of at least four different experiments. Comparisons between groups were done by Mann-Whitney test. Fig (B): \* *p*<0.05. Fig (C): \* *p*<0.05. Fig (D): \* *p*<0.01.

doi:10.1371/journal.pone.0158429.g005

females and glutamine in males). Direct comparisons among sexes revealed that H<sub>2</sub>O<sub>2</sub> production rates were significantly higher in female worms, regardless the available substrates. Indeed, the rates of H<sub>2</sub>O<sub>2</sub> formation in females were 4.4 and 1.7 times higher than in males in glucose and glucose+glutamine, respectively (Fig 5C and 5D). This result fully agrees with the higher oxidant levels found in female worms revealed by DHE staining (Fig 4). Also, the sexual differences in H<sub>2</sub>O<sub>2</sub> production are in line with the higher non-respiratory O<sub>2</sub> utilization observed in female worms relative to males, irrespective of the nutritional sources available (Fig 2E).

HBSS+Glc



**Fig 6. Low respiratory rates in females are linked to increased H<sub>2</sub>O<sub>2</sub> production.** Respiratory OCR values and H<sub>2</sub>O<sub>2</sub> production rates were determined in females (closed circles) and males (open circles) in HBSS+5.5 mM Glc (black circles) and HBSS+5.5 mM Glc+5.5 mM Gln (gray circles). Data were derived from Figs 1 and 5 are expressed as mean ± SEM of at least four different experiments.

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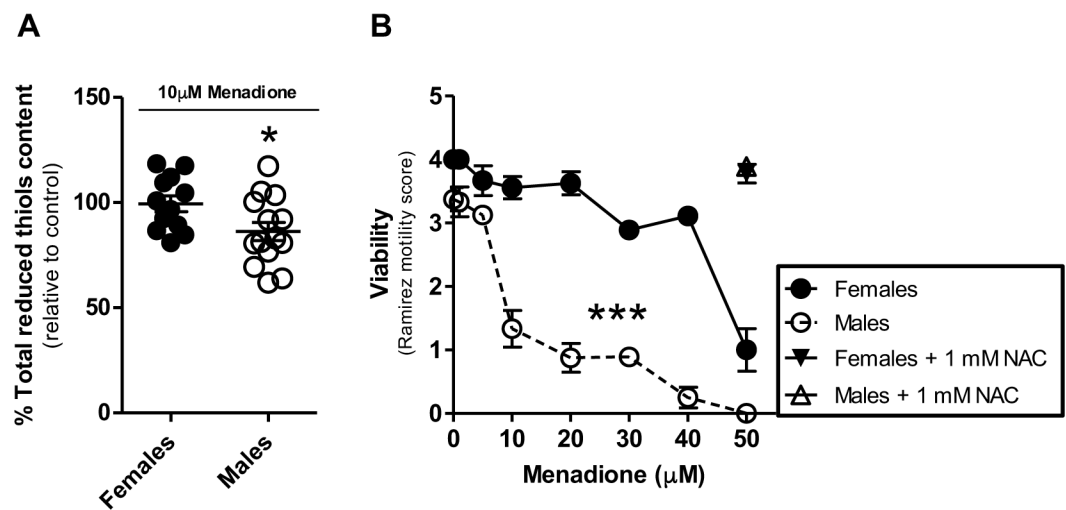
Fig 6 shows comparative analyses of H<sub>2</sub>O<sub>2</sub> generation rates in female and male worms kept under glucose and glucose + glutamine (Fig 5A and 5B), by their respective respiratory rates (Fig 1B and 1D). We observed that lower respiratory rates in females were associated to higher H<sub>2</sub>O<sub>2</sub> production in both substrate conditions, compared to males. Interestingly, the increase in respiratory rates provided by glutamine in females hardly affected H<sub>2</sub>O<sub>2</sub> production, suggesting that glutamine utilization is not a limiting factor for ROS production. This is consistent with our observation that glutamine supplementation in female worms increased OCR mostly through respiration rather than non-respiratory pathways (Fig 1B). Conceivably, despite glutamine oxidation promoted higher respiratory rates through the glutaminase-TCA cycle-OXPHOS path in females, the resulted increase in mitochondrial electron flow would not be followed by higher ROS production at ETS, as the antioxidant capacity would increase H<sub>2</sub>O<sub>2</sub> detoxification by increasing glutathione and NADPH production, which would avoid redox imbalance [119–120]. On the other hand, glutamine caused a proportional increase in both parameters in male worms, suggesting that increased glutamine oxidation and mitochondrial electron flow result in higher ROS production, as the capacity to synthesize glutathione and NADPH from glutamine would be limited in male worms. Alternatively, αKGDH complex structure might be different in male worms, facilitating electron leak and ROS production at this site [99–101], without compromising its activity and respiratory rates. In conclusion, these observations suggest that H<sub>2</sub>O<sub>2</sub> production in adult *S. mansoni* worms is sustained by sexual preferences in substrate utilization towards glucose in females, and glutamine in male worms.

g) Female worms are more resistant to a pro-oxidant challenge

A number of observations support the concept that sustained mild oxidative stress conditions trigger protective antioxidant responses in different models [124–134]. These adaptive responses not only confer protection to redox insults [125,126], but also provide other beneficial outputs, such as increased motor capacity [133], insulin sensitivity [134], improved glucose

homeostasis [134], and longevity [124,127,128,130,133]. Similar protective antioxidant responses were also reported for *S. mansoni* [135–139]. For example, the expression of parasite redox defense mechanisms was induced by either redox imbalance conditions or by chemotherapeutic agents [135–137], while hyperbaric O<sub>2</sub> therapy in *S. mansoni*-infected hamsters increased the number of recovered worms and parasite eggs trapped within tissues [138]. Curiously, the antioxidant n-acetylcysteine (NAC) potentiated the effect of artemether on parasite killing in a murine schistosomiasis model [139]. These evidence indicate that adult *S. mansoni* worms are able to regulate ROS production and/or their antioxidant defenses in order to cope with oxidant insult, thus avoiding parasite death. Although total oxidant levels and H<sub>2</sub>O<sub>2</sub> generation were consistently higher in female worms (Figs 4–6), it remains unknown whether endogenous ROS production is associated to protective adaptive responses in adult *S. mansoni*. In order to address this issue, we acutely exposed adult worms to menadione, a known pro-oxidant that stimulates endogenous ROS production and intracellular thiol oxidation [140–144]. Interestingly, Fig 7A shows that the content of total reduced thiols in female worms were not affected after 1h challenge with 10 μM menadione, while significant reductions (~13%) were observed male worms. When parasites were acutely exposed with different concentrations of menadione, we observed a dose-dependent reduction in viability of both parasite sexes, assessed by their motility [91] (Fig 7B). Strikingly, we observed that female worms were significantly more resistant to menadione challenge than male worms (Fig 7B). Indeed, motility of female worms hardly changed up to 20 μM menadione treatment, whereas strong reductions in this parameter were observed in male worms within this concentration range. Also noteworthy, the loss of parasite viability was mediated by redox imbalance, as this effect was completely blunted in both parasite sexes by co-treatment with 50 μM menadione plus 1 mM NAC.

## RPMI



**Fig 7. Female worms are more resistant to a pro-oxidant challenge.** (A) Total reduced thiol contents were determined by light absorption at 412 nm in females (closed circles) or males (open circles) homogenates after 1h pre-incubation of intact worms with 10 μM menadione in RPMI 1640. (B) Viability of females (closed circles) or males (open circles) was determined by light microscopy after 2h pre-incubation of intact worms with different concentrations of menadione in RPMI 1640 using a previously reported motility scale [91]. Females (closed triangle) or males (open triangle) viability incubated for 2h with 50 μM menadione and 1 mM n-acetylcysteine (NAC) were also determined. Data were expressed as mean ± SEM of four (A) or three (B) different experiments. Comparisons between groups were done by Student's t-test (A) and two-way ANOVA and a *posteriori* Bonferroni (B). Fig (A): \*  $p < 0.05$ . Fig (B): \*\*\*  $p < 0.001$ .

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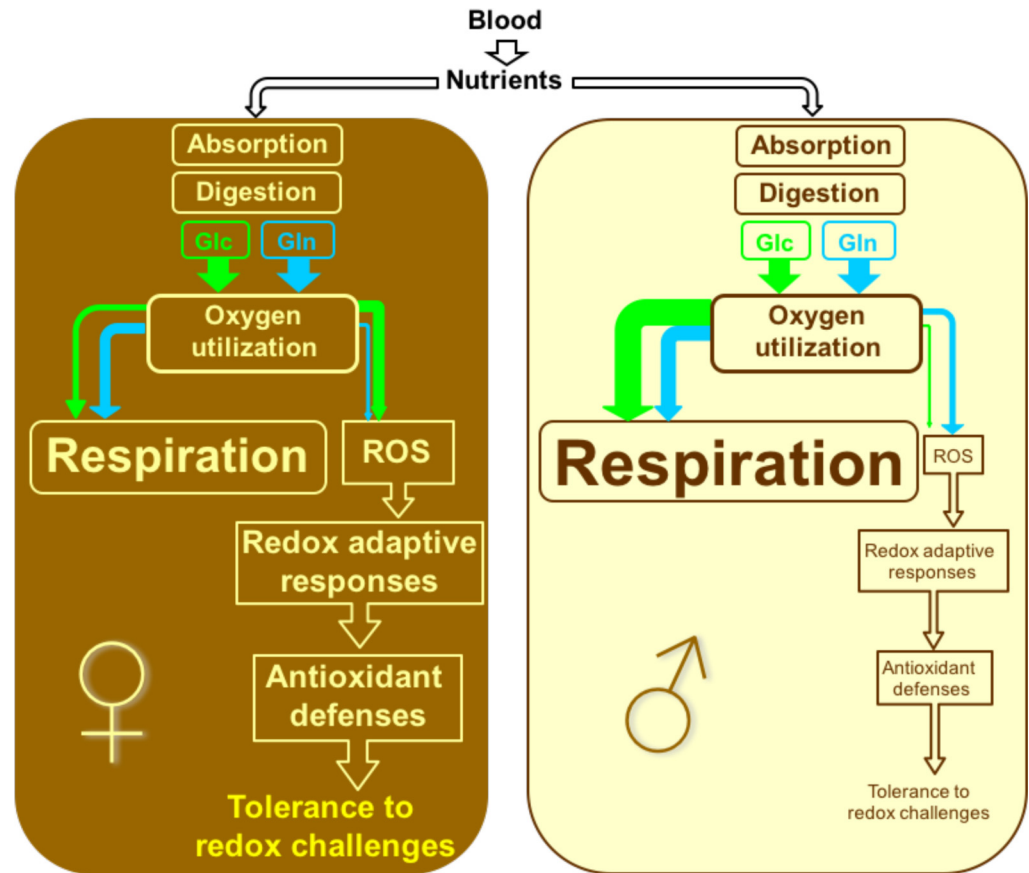


Together, these results indicate that female worms exhibit higher resistance to oxidative stress conditions generated by endogenous ROS production, which agrees with their higher content of antioxidant defenses compared to male parasites [67,78,85–87].

## Conclusions and Final Remarks

In the present work, we demonstrate that in adult *S. mansoni* worms sexual preferences in nutrient utilization regulate endogenous parasite O<sub>2</sub> consumption and ROS generation, which might differently contribute to redox biology among parasite sexes. A summary of the results presented here is schematically depicted in Fig 8. Blood-derived nutrients are absorbed through worm tegument or gut, and metabolized to their fundamental units. Glucose and glutamine are differentially oxidized through respiratory and non-respiratory pathways in adult worms, demanding distinct rates of O<sub>2</sub> utilization among nutrients and parasite sexes. Glutamine metabolism plays a dominant role to sustain respiratory pathways in female worms, while glucose is the preferential substrate for this purpose in males. Interestingly, the opposite trend is observed in both non-respiratory O<sub>2</sub> utilization and ROS formation among sexes, since glucose is the preferential substrate to generate ROS in females, while in male worms, glutamine prevails. Overall, respiratory rates are higher in males compared to females, while the rates of ROS production are higher in females. To cope with higher ROS levels generated endogenously by their own metabolism, redox adaptive mechanisms are more intensely activated in females than in males, mediating the build-up of antioxidant defenses, which ultimately confer higher tolerance to oxidative stress conditions. Therefore, we suggest that the higher levels of antioxidant defenses [67,78,85–87], and tolerance to redox challenges observed in female worms, seem to be a consequence of their higher endogenous ROS production generated by the parasite metabolism. Given that schistosomiasis clinically manifests by the inflammatory reaction triggered by antigens released by parasite eggs deposited in host tissues, targeting redox adaptive response mechanisms in adult worms might reveal an interesting venue for the development of new classes of schistosomicidal agents.

A number of evidence support the notion that ROS are key intracellular messengers, mediating adaptive stress response mechanisms, and promoting a number of positive physiological and cytoprotective effects [124–134,145–149]. For instance, improved stress resistance and longevity of *Caenorhabditis elegans* worms are induced by a variety of stress signals that have in common their ability to stimulate mitochondrial ROS formation [124,127,128,130,133,146,148,149]. Also, strains of the causative agent of Chagas' disease (*Trypanosoma cruzi*), with increased antioxidant defenses are more resistant to redox insults and are more virulent, suggesting a relationship between redox balance and infectivity [150]. In this regard, our group demonstrated that *T. cruzi* bloodstream trypomastigote forms were strikingly resistant to oxidative challenges, but were able to generate higher levels of H<sub>2</sub>O<sub>2</sub> than epimastigote forms [151]. Mechanistically, increased complex II-III and reduced complex IV activity observed in bloodstream forms, create an electron bottleneck facilitating electron leakage to produce O<sub>2</sub><sup>•−</sup> and H<sub>2</sub>O<sub>2</sub>, which seems to provide an oxidative pre-conditioning conferring protection against redox imbalance conditions. Indeed, despite the acute exposure to high levels of ROS is unquestionably cytotoxic, chronic mild oxidative stress conditions clearly promote beneficial outputs for many different models [127–133,146]. Conceivably, adult *S. mansoni* worms are able to respond differently to oxidative insults through hormetic responses, in a way that female redox homeostasis would be mostly controlled by the detoxification rates rather than their generation, whereas in males the opposite would be true. In this regard, *S. mansoni* worms are capable of inducing an adaptive response against redox challenges, by increasing the expression of antioxidant [136,152,153], and repair enzymes [152].



**Fig 8. Schematic representation on how sexual preferences in nutrient utilization regulate  $O_2$  consumption and ROS generation in adult *S. mansoni* and the potential implications to parasite redox biology.** Brown and light yellow boxes represent female and male worms, respectively. Nutrients derived from the bloodstream are absorbed by the parasites through either transtegumental transport or through the gut upon digestion by luminal enzymes. The present work focused on the contribution of glucose (green) and glutamine (blue) on  $O_2$  utilization pathways and the consequences to energy and redox metabolism. Nutrient oxidation is carried out by different enzymes of the parasite energy metabolism, releasing electrons, which are ultimately utilized by  $O_2$  demanding processes as a final acceptor to allow: *i*) respiration at cytochrome *c* oxidase activity (Respiration), which is directly connected to energy metabolism; *ii*) reactive oxygen species (ROS) generation that take place at multiple cellular sites. ROS produced by the parasite triggers redox adaptive mechanisms, essentially involving antioxidant defenses, which finally mediate tolerance to endogenous and/or physiological redox challenges. Sexual differences in pathways described in the present work are highlighted by the sizes of arrows, fonts and boxes. Female *S. mansoni* exhibit higher contribution of glutamine to respiration, compared to glucose, whereas in male worms, glucose metabolism has a major role to support respiration, compared to glutamine. As a whole, male *S. mansoni* exhibit higher  $O_2$  consumption rates by means of respiration than females, while  $O_2$  utilization through non-respiratory pathways was higher in females, which was mostly linked to glucose metabolism. In males, non-respiratory  $O_2$  utilization was essentially provided by glutamine metabolism. Non-respiratory  $O_2$  consumption rates are directly connected to  $H_2O_2$  and ROS production, exhibiting the same substrates dependencies among sexes.  $H_2O_2$  production was mostly sustained by glucose oxidation in females, while in males, glutamine was the preferred substrate to generate ROS. Conceivably, increased ROS formation in females activate redox adaptive responses, mediating the build-up of antioxidant defenses, and allowing improved tolerance to oxidative stress.

doi:10.1371/journal.pone.0158429.g008

We are aware of the potential limitations inherent of the experimental approach employed in the present work. Firstly, all processes investigated here were carried out with worm *sex vivo*, in  $O_2$  tensions much higher than those found in mesenteric veins of the vertebrate host. Potentially, the exposure to high  $O_2$  tensions might overwhelm the worm's antioxidant capacity, exacerbating their  $O_2$  utilization for both energy transduction and ROS generation.

Secondly, given that pairing regulates physiological aspects of adult *S. mansoni* [82–84], it might be possible that the conjugal biology might also be determinant to parasite redox homeostasis. An example in this regard is the higher expression of antioxidant enzymes in paired *S. japonicum* females compared to unpaired ones [154,155]. However, it is important to emphasize that the experimental approach employed here to interrogate the sexual trends in energy and redox metabolism of adult worms would be nearly impossible to achieve using paired worms, as the particular metabolic features for one sex would be masked by the other. Despite the growing need to understand basic aspects of schistosomiasis, the specific sites and pathways (i.e. mitochondria, NADPH oxidase, peroxisomes) involved on endogenous ROS production remain elusive. The data presented here indicate that unpaired adult *S. mansoni* worms exhibit sexual preferences in nutrient utilization that regulate O<sub>2</sub> consumption and ROS generation pathways which might provide differential tolerance to oxidative stress among parasite sexes. Future research will provide insights over key aspects described here, especially those related to the mechanisms that mediate redox adaptive responses in *S. mansoni* females, and the potential implications for parasite survival.

## Supporting Information

**S1 Fig. Nutrients regulate total O<sub>2</sub> utilization in adult worms.** Comparative analyses of total O<sub>2</sub> consumption of adult female (A, black bars) and male (B, white bars) worms determined in four different nutrient compositions as following: HBSS + 5.5 mM glucose, HBSS + 5.5 mM glucose + 5.5 mM glutamine, RPMI 1640, or RPMI 1640 + serum. Data are expressed as mean ± SEM of at least four different experiments. Comparisons between groups were done by Mann-Whitney's (letters) or Student's *t* tests (symbols). Fig (A): <sup>a</sup>  $p = 0.0025$  relative to +Gln, Fig (B): <sup>a</sup>  $p < 0.004$  relative to +Serum, Fig (C): <sup>a</sup>  $p = 0.014$  relative to +Gln, Fig (D): <sup>a</sup>  $p < 0.004$  relative to +Serum, Fig (E): \*  $p < 0.015$  relative to males.

(TIF)

**S2 Fig. Relative contributions of respiratory and non-respiratory O<sub>2</sub> utilization in adult worms in different nutritional conditions.** The relative contribution of respiration (red bars) and non-respiration (green bars) to total OCR of adult female (A) and male (B) worms were determined in four different nutrient compositions as following: HBSS + 5.5 mM glucose, HBSS + 5.5 mM glucose + 5.5 mM glutamine, RPMI 1640, or RPMI 1640 + serum. Data shown are mean ± SEM of at least four different experiments.

(TIF)

**S3 Fig. Sexual differences in relative contribution of respiratory and non-respiratory O<sub>2</sub> utilization among diets in adult worms.** Comparative analyses of respiratory (red bars) and non-respiratory (green bars) OCR in adult female and male worms was determined by high resolution respirometry in media containing HBSS + 5.5 mM glucose (A), HBSS + 5.5 mM glucose + 5.5 mM glutamine (B), RPMI 1640 (C), or RPMI 1640 + serum (D). Data are expressed as mean ± SEM of at least four different experiments. Comparisons between groups were done by Student's *t*-test or Mann-Whitney's test. Red letters and green symbols over bars represent statistical differences between sexes in respiration and non-respiration, respectively. Statistical symbols are relative to males. In all comparisons, Mann-Whitney's test was applied. Fig (A): <sup>a</sup>  $p < 0.0001$ , \*  $p < 0.0001$ . Fig (B): <sup>b</sup>  $p < 0.05$ , \*\*  $p < 0.05$ . Fig (C): <sup>b</sup>  $p < 0.05$ , \*\*  $p < 0.05$ . Fig (D): <sup>c</sup>  $p < 0.002$ , #  $p < 0.002$ .

(TIF)

**S4 Fig. Isolated mitochondria from male worms exhibit higher respiratory capacity.** Typical traces of O<sub>2</sub> consumption in isolated mitochondria from female (A) and male (B) worms,

using 10 mM glutamate + 1 mM malate (Glu-Mal) and 10mM succinate (Suc) as substrates (See [S1 Methods](#)). To assess state 2 respiration, the OCR induced by Glu+Mal+Suc was subtracted from that resistant to AA, while the state 3 was determined after addition of 1 mM ADP. (C) Comparison of respiratory states 2 and 3 demonstrate higher OCR in male mitochondria. Data are expressed as mean  $\pm$  SEM of  $n = 1$  for females and  $n = 2$  for males. (TIF)

**S5 Fig. Respiration linked to oxidative phosphorylation is quite similar between sexes.**  $O_2$  consumption rates (OCR) of intact adult *S. mansoni* worms were determined by high resolution respirometry in media containing four different nutrient compositions as following: HBSS containing 5.5 mM glucose. (A) Representative OCR trace obtained from 30 intact *S. mansoni* males. The OCR coupled to oxidative phosphorylation was assessed by inhibiting ATP synthase with multiple injections of oligomycin. The OCR sensitive to 25  $\mu$ g/mL oligomycin was used to determine OXPHOS (B), while the residual OCR insensitive to oligomycin, but inhibited by AA, was considered as “proton leak” (C). Data are expressed as mean  $\pm$  SEM of four different experiments. (TIF)

**S6 Fig. Limited contribution of mitochondrial  $\beta$ -oxidation to respiration in adult *S. mansoni* worms is not distinct among sexes.** The contribution of lipid metabolism through beta-oxidation ( $\beta$ -ox, blue bars) and by other substrates (OS, pink bars) were determined in adult female and male worms by high resolution respirometry in media containing RPMI 1640 + serum. To determine the contribution of mitochondrial  $\beta$ -oxidation to respiration, basal OCR was measured followed by addition of 200  $\mu$ M etomoxir to each respirometer chamber. The etomoxir-sensitive component of the respiration was considered as  $\beta$ -ox, while the remaining etomoxir-insensitive one (OS), as derived from oxidation of substrates other than lipids. (TIF)

**S7 Fig. Similar mitochondrial content among parasite sexes regardless the nutrient sources.** Citrate synthase activity was measured in adult female (black bars) and male (white bars) worms kept in three different nutrient compositions, as following: HBSS + 5.5 mM glucose, HBSS + 5.5 mM glucose + 5.5 mM glutamine, or RPMI 1640 + serum. Data shown are mean  $\pm$  SEM of at least five different experiments. (TIF)

**S8 Fig. Complex I-III is specifically higher in female worms regardless the nutrient sources.** Complex I-III (C I-III) and complex IV (C IV) activities were measured in adult female (black bars) and male (white bars) worms kept in three different nutrient compositions, as following: HBSS + 5.5 mM glucose, HBSS + 5.5 mM glucose + 5.5 mM glutamine, or RPMI 1640 + serum. Data shown are mean  $\pm$  SEM of at least five different experiments. Comparisons between groups were done by Student's t-test (A) or Mann-Whitney's test (B and C). Fig (A): \*  $p < 0.01$  relative to C I-III. Fig (B): \*  $p < 0.02$  relative to C I-III. Fig (C): \*  $p < 0.01$  relative to C I-III. (TIF)

**S9 Fig. Female complex I-III activity is higher than in males regardless the nutrient sources.** Complex I-III (C I-III) and complex IV (C IV) activities were measured in adult female (black bars) and male (white bars) worms kept in three different nutrient compositions, as following: HBSS + 5.5 mM glucose, HBSS + 5.5 mM glucose + 5.5 mM glutamine, or RPMI 1640 + serum. Data shown are mean  $\pm$  SEM of at least five different experiments. Comparisons

between groups were done by Student's t-test (A) or Mann-Whitney's test (B and C). Fig (A): \*  $p < 0.001$  relative to females. Fig (C): \*  $p < 0.02$  relative to females. (TIF)

**S10 Fig. DAPI uptake is higher in males.** Worms were cultured for 24 h in RPMI + serum, and subsequently incubated in RPMI 1640 without phenol red supplemented with 1  $\mu\text{g}/\text{mL}$  DAPI for 20 minutes at 37°C and 5%  $\text{CO}_2$  (See [S1 Methods](#)). Fluorescence microscopy images were collected for whole individual worms, the average fluorescence intensity was quantified and expressed as arbitrary units. Data are expressed as mean  $\pm$  SEM of at least three different experiments. Comparisons between groups were done by using Mann-Whitney's test. \*\*\*  $p < 0.001$ . (TIF)

**S1 Methods.**  
(DOCX)

## Acknowledgments

We would like to express our gratitude to Mrs. Terezinha de Paula Braz at IBqM-UFRJ animal facility, to Mr. Paulo César dos Santos from Laboratório de Malacologia at Fundação Instituto Oswaldo Cruz (RJ) and to Mr. Charlion Cosme at IBqM-UFRJ for the excellent technical assistance. We also thank Prof. Patricia H. Alvarenga, Dr. Luciana E.S.F. Machado and BSc. Nathanael A. Miller for the valuable critical reading of this manuscript. This work is dedicated with loving memory to Erly de Souza Gomes (1946–2015).

## Author Contributions

Conceived and designed the experiments: MPO JBRCS MFO. Performed the experiments: MPO JBRCS. Analyzed the data: MPO JBRCS MFO. Contributed reagents/materials/analysis tools: JBRCS MFO. Wrote the paper: MPO MFO.

## References

1. Steinmann P, Keiser J, Bos R, Tanner M, Utzinger J. Schistosomiasis and water resources development: systematic review, meta-analysis, and estimates of people at risk. *Lancet Infect. Dis.* 2006; 6: 411–425. PMID: [16790382](#)
2. Gazzinelli G, Lambertucci JR, Katz N, Rocha RS, Lima MS, Colley DG. Immune responses during human Schistosomiasis mansoni. XI. Immunologic status of patients with acute infections and after treatment. *J. Immunol.* 1985; 135: 2121–2127. PMID: [4020142](#)
3. Boros DL. Immunopathology of Schistosoma mansoni infection. *Clin. Microbiol. Rev.* 1989; 2: 250–269. PMID: [2504481](#)
4. Warren KS. The pathology, pathobiology and pathogenesis of schistosomiasis. *Nature.* 1978; 273: 609–612. PMID: [351411](#)
5. Grzych JM, Pearce E, Cheever A, Caulada ZA, Caspar P, Heiny S, et al. Egg deposition is the major stimulus for the production of Th2 cytokines in murine schistosomiasis mansoni. *J. Immunol.* 1991; 146: 1322–1327. PMID: [1825109](#)
6. Pearce EJ, Caspar P, Grzych JM, Lewis FA, Sher A. Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth, Schistosoma mansoni. *J. Exp. Med.* 1991; 173: 159–166. PMID: [1824635](#)
7. Bout DT, Joseph M, David JR, Capron AR. In vitro killing of S. mansoni schistosomula by lymphokine-activated mouse macrophages. *J. Immunol.* 1981; 127: 1–5. PMID: [7240738](#)
8. Braschi S, Wilson RA. Proteins exposed at the adult schistosome surface revealed by biotinylation. *Mol. Cell. Proteomics.* 2006; 5: 347–356. PMID: [16269422](#)
9. Skelly PJ, Wilson RA. Making sense of the schistosome surface. *Adv. Parasitol.* 2006; 63: 185–284. PMID: [17134654](#)

10. Goldring OL, Clegg JA, Smithers SR, Terry RJ. Acquisition of human blood group antigens by *Schistosoma mansoni*. Clin. Exp. Immunol. 1976; 26: 181–187. PMID: [1033797](#)
11. Mkoji GM, Smith JM, Prichard RK. Antioxidant systems in *Schistosoma mansoni*: correlation between susceptibility to oxidant killing and the levels of scavengers of hydrogen peroxide and oxygen free radicals. Int. J. Parasitol. 1988; 18: 661–666. PMID: [2844688](#)
12. Mkoji GM, Smith JM, Prichard RK. Antioxidant systems in *Schistosoma mansoni*: evidence for their role in protection of the adult worms against oxidant killing. Int. J. Parasitol. 1988; 18: 667–673. PMID: [2844689](#)
13. Nare B, Smith JM, Prichard RK. *Schistosoma mansoni*: levels of antioxidants and resistance to oxidants increase during development. Exp. Parasitol. 1990; 70: 389–397. PMID: [2323392](#)
14. Mei H, LoVerde PT. *Schistosoma mansoni*: the developmental regulation and immunolocalization of antioxidant enzymes. Exp. Parasitol. 1997; 86: 69–78. PMID: [9149242](#)
15. Boveris A, Oshino N, Chance B. The cellular production of hydrogen peroxide. Biochem. J. 1972; 128: 617–630. PMID: [4404507](#)
16. Boveris A, Chance B. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. Biochem. J. 1973; 134: 707–716. PMID: [4749271](#)
17. Babior BM, Kipnes RS, Curnutte JT. Biological defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent. J. Clin. Invest. 1973; 52:741–744. PMID: [4346473](#)
18. Figueira TR, Barros MH, Camargo AA, Castilho RF, Ferreira JC, Kowaltowski AJ, et al. Mitochondria as a source of reactive oxygen and nitrogen species: from molecular mechanisms to human health. Antioxid. Redox Signal. 2013; 18: 2029–2074. doi: [10.1089/ars.2012.4729](#) PMID: [23244576](#)
19. Martins D, Titorenko VI, English AM. Cells with impaired mitochondrial H<sub>2</sub>O<sub>2</sub> sensing generate less •OH radicals and live longer. Antioxid. Redox Signal. 2014; 21: 1490–1503. doi: [10.1089/ars.2013.5575](#) PMID: [24382195](#)
20. Pelletier M, Lepow TS, Billingham LK, Murphy MP, Siegel RM. New tricks from an old dog: mitochondrial redox signaling in cellular inflammation. Semin. Immunol. 2012; 24: 384–392. doi: [10.1016/j.smim.2013.01.002](#) PMID: [23391428](#)
21. Esparza I, Ruppel A, Mestan J, Krammer PH. Preactivation of macrophages in mice acutely infected with *Schistosoma mansoni*. Immunobiology. 1988; 177:105–119. PMID: [3136079](#)
22. Smith JM, Mkoji GM, Prichard RK. Depression of hydrogen peroxide dependent killing of schistosomula in vitro by peritoneal exudate cells from *Schistosoma mansoni* infected mice. Am. J. Trop. Med. Hyg. 1989; 40:186–194. PMID: [2537585](#)
23. Oliveira MF, d'Avila JC, Torres CR, Oliveira PL, Tempone AJ, Rumjanek FD, et al. Haemozoin in *Schistosoma mansoni*. Mol. Biochem. Parasitol. 2000; 111: 217–221. PMID: [11087932](#)
24. Oliveira MF, d'Avila JC, Tempone AJ, Soares JB, Rumjanek FD, Ferreira-Pereira A, et al. Inhibition of heme aggregation by chloroquine reduces *Schistosoma mansoni* infection. J. Infect. Dis. 2004; 190: 843–852. PMID: [15272414](#)
25. Corrêa Soares JB, Menezes D, Vannier-Santos MA, Ferreira-Pereira A, Almeida GT, Venancio TM, et al. Interference with hemozoin formation represents an important mechanism of schistosomicidal action of antimalarial quinoline methanols. PLoS Negl. Trop. Dis. 2009; 3: e477. doi: [10.1371/journal.pntd.0000477](#) PMID: [19597543](#)
26. Oliveira MF, Timm BL, Machado EA, Miranda K, Attias M, Silva JR, et al. On the pro-oxidant effects of haemozoin. FEBS Lett. 2002; 512: 139–144. PMID: [11852068](#)
27. Yazdanbakhsh M, Tai PC, Spry CJ, Gleich GJ, Roos D. Synergism between eosinophil cationic protein and oxygen metabolites in killing of schistosomula of *Schistosoma mansoni*. J. Immunol. 1987; 138: 3443–3447. PMID: [3571976](#)
28. Perevoshchikova IV, Quinlan CL, Orr AL, Gerencser AA, Brand MD. Sites of superoxide and hydrogen peroxide production during fatty acid oxidation in rat skeletal muscle mitochondria. Free Rad. Biol. Med. 2013; 61: 298–309. doi: [10.1016/j.freeradbiomed.2013.04.006](#) PMID: [23583329](#)
29. Treberg JR, Quinlan CL, Brand MD. Evidence for two sites of superoxide production by mitochondrial NADH-ubiquinone oxidoreductase (complex I). J. Biol. Chem. 2011; 286: 27103–27110. doi: [10.1074/jbc.M111.252502](#) PMID: [21659507](#)
30. Orr AL, Quinlan CL, Perevoshchikova IV, Brand MD. A refined analysis of superoxide production by mitochondrial sn-glycerol 3-phosphate dehydrogenase. J. Biol. Chem. 2012; 287: 42921–42935. doi: [10.1074/jbc.M112.397828](#) PMID: [23124204](#)
31. Hey-Mogensen M, Goncalves RL, Orr AL, Brand MD. Production of superoxide/H<sub>2</sub>O<sub>2</sub> by dihydroorotate dehydrogenase in rat skeletal muscle mitochondria. Free. Rad. Biol. Med. 2014; 72: 149–155. doi: [10.1016/j.freeradbiomed.2014.04.007](#) PMID: [24746616](#)

32. Quinlan CL, Goncalves RL, Hey-Mogensen M, Yadava N, Bunik VI, Brand MD. The 2-oxoacid dehydrogenase complexes in mitochondria can produce superoxide/hydrogen peroxide at much higher rates than complex I. *J. Biol. Chem.* 2014; 289:8312–8325. doi: [10.1074/jbc.M113.545301](https://doi.org/10.1074/jbc.M113.545301) PMID: [24515115](https://pubmed.ncbi.nlm.nih.gov/24515115/)
33. Quinlan CL, Perevoshchikova IV, Hey-Mogensen M, Orr AL, Brand MD. Sites of reactive oxygen species generation by mitochondria oxidizing different substrates. *Redox Biol.* 2013; 1: 304–312. doi: [10.1016/j.redox.2013.04.005](https://doi.org/10.1016/j.redox.2013.04.005) PMID: [24024165](https://pubmed.ncbi.nlm.nih.gov/24024165/)
34. Gonçalves RL, Barreto RF, Polycarpo CR, Gadelha FR, Castro SL, Oliveira MF. A comparative assessment of mitochondrial function in epimastigotes and bloodstream trypomastigotes of *Trypanosoma cruzi*. *J. Bioenerg. Biomembr.* 2011; 43:651–661. doi: [10.1007/s10863-011-9398-8](https://doi.org/10.1007/s10863-011-9398-8) PMID: [22081211](https://pubmed.ncbi.nlm.nih.gov/22081211/)
35. Soares JB, Gaviraghi A, Oliveira MF. Mitochondrial physiology in the major arbovirus vector *Aedes aegypti*: substrate preferences and sexual differences define respiratory capacity and superoxide production. *PLoS ONE.* 2015; 10: e0120600. doi: [10.1371/journal.pone.0120600](https://doi.org/10.1371/journal.pone.0120600) PMID: [25803027](https://pubmed.ncbi.nlm.nih.gov/25803027/)
36. Prichard RK, Schofield PJ. *Fasciola hepatica*: cytochrome c oxidoreductases and effects of oxygen tension and inhibitors. *Exp. Parasitol.* 1971; 29:215–222. PMID: [4326588](https://pubmed.ncbi.nlm.nih.gov/4326588/)
37. Tielens AG, van der Meer P, van den Bergh SG. The aerobic energy metabolism of the juvenile *Fasciola hepatica*. *Mol. Biochem. Parasitol.* 1981; 3:205–214. PMID: [6792538](https://pubmed.ncbi.nlm.nih.gov/6792538/)
38. Tielens AG, van den Heuvel JM, van den Bergh SG. The energy metabolism of *Fasciola hepatica* during its development in the final host. *Mol. Biochem. Parasitol.* 1984; 13:301–307. PMID: [6527693](https://pubmed.ncbi.nlm.nih.gov/6527693/)
39. Takamiya S, Wang H, Hiraishi A, Yu Y, Hamajima F, Aoki T. Respiratory chain of the lung fluke *Paragonimus westermani*: facultative anaerobic mitochondria. *Arch. Biochem. Biophys.* 1994; 312:142–150. PMID: [8031121](https://pubmed.ncbi.nlm.nih.gov/8031121/)
40. Girardini JE, Khayath N, Amirante A, Dissous C, Serra E. *Schistosoma mansoni*: ferredoxin-NADP(H) oxidoreductase and the metabolism of reactive oxygen species. *Exp. Parasitol.* 2005; 110:157–161. PMID: [15888298](https://pubmed.ncbi.nlm.nih.gov/15888298/)
41. Takamiya S, Fukuda K, Nakamura T, Aoki T, Sugiyama H. *Paragonimus westermani* possesses aerobic and anaerobic mitochondria in different tissues, adapting to fluctuating oxygen tension in micro-aerobic habitats. *Int. J. Parasitol.* 2010; 40:1651–1658. doi: [10.1016/j.ijpara.2010.07.009](https://doi.org/10.1016/j.ijpara.2010.07.009) PMID: [20716443](https://pubmed.ncbi.nlm.nih.gov/20716443/)
42. Huang SC, Freitas TC, Amiel E, Everts B, Pearce EL, Lok JB, et al. Fatty acid oxidation is essential for egg production by the parasitic flatworm *Schistosoma mansoni*. *PLoS Pathog.* 2012; 8:e1002996. doi: [10.1371/journal.ppat.1002996](https://doi.org/10.1371/journal.ppat.1002996) PMID: [23133378](https://pubmed.ncbi.nlm.nih.gov/23133378/)
43. Schiller EL, Bueding E, Turner VM, Fisher J. Aerobic and anaerobic carbohydrate metabolism and egg production of *Schistosoma mansoni* in vitro. *J. Parasitol.* 1975; 61:385–389. PMID: [1169294](https://pubmed.ncbi.nlm.nih.gov/1169294/)
44. Seed JL, Boff M, Bennett JL. Phenol oxidase activity: Induction in female schistosomes by in vitro incubation. *J. Parasitol.* 1978; 64: 283–289. PMID: [417162](https://pubmed.ncbi.nlm.nih.gov/417162/)
45. Coles GC. Oxidative phosphorylation in adult *Schistosoma mansoni*. *Nature.* 1972; 240:488–489. PMID: [4565950](https://pubmed.ncbi.nlm.nih.gov/4565950/)
46. Van Oordt BEP, Van Deen Heuvel JM, Tielens AGM, Van Den Bergh SG. The energy production of adult *Schistosoma mansoni* is for a part large aerobic. *Mol. Biochem. Parasitol.* 1985; 16: 117–126. PMID: [3929086](https://pubmed.ncbi.nlm.nih.gov/3929086/)
47. Bueding E. Carbohydrate metabolism of *Schistosoma mansoni*. *J. Gen. Physiol.* 1950; 33: 475–95. PMID: [15422103](https://pubmed.ncbi.nlm.nih.gov/15422103/)
48. Bueding E, Charms B. Cytochrome c, cytochrome oxidase, and succinoxidase activities of helminths. *J. Biol. Chem.* 1952; 196: 615–627. PMID: [12981005](https://pubmed.ncbi.nlm.nih.gov/12981005/)
49. Saz HJ, Bueding E. Relationships between anthelmintic effects and biochemical and physiological mechanisms. *Pharmacol. Rev.* 1966; 18: 871–894. PMID: [5325211](https://pubmed.ncbi.nlm.nih.gov/5325211/)
50. Bueding E, Peters L, Koletsky S, Moore DV. Effect of respiratory inhibition on *Schistosoma mansoni*. *Brit. J. Pharmacol.* 1953; 8:15–18. PMID: [13066685](https://pubmed.ncbi.nlm.nih.gov/13066685/)
51. Thompson DP, Morrison DD, Pax RA, Bennett JL. Changes in glucose metabolism and cyanide sensitivity in *Schistosoma mansoni* during development. *Mol. Biochem. Parasitol.* 1984; 13: 39–51. PMID: [6513987](https://pubmed.ncbi.nlm.nih.gov/6513987/)
52. Bruce JI, Ruff MD, Davidson DE, Crum JW. *Schistosoma mansoni* and *Schistosoma japonicum*: comparison of selected aspects of carbohydrate metabolism. *Comp. Biochem. Physiol. B.* 1974; 49:157–164. PMID: [4415689](https://pubmed.ncbi.nlm.nih.gov/4415689/)
53. Skelly PJ, Stein LD, Shoemaker CB. Expression of *Schistosoma mansoni* genes involved in anaerobic and oxidative glucose metabolism during the cercaria to adult transformation. *Mol. Biochem. Parasitol.* 1993; 60:93–104. PMID: [8396206](https://pubmed.ncbi.nlm.nih.gov/8396206/)

54. Huang TY. The energy metabolism of *Schistosoma japonicum*. *Int. J. Biochem.* 1980; 12: 457–464. PMID: [7418939](#)
55. Tielens AGM, van Oordt BEP, van Den Bergh SG. Carbohydrate metabolism in adult schistosomes of different strains and species. *Int. J. Parasitol.* 1989; 19, 447–449. PMID: [2777465](#)
56. Lane CA, Pax RA, Bennett JL. L-glutamine: an amino acid required for maintenance of the tegumental membrane potential of *Schistosoma mansoni*. *Parasitology.* 1987; 94:233–242. PMID: [3588012](#)
57. Bruce JI, Ruff MD, Belusko RJ, Werner JK. *Schistosoma mansoni* and *Schistosoma japonicum*: utilization of amino acids. *Int. J. Parasitol.* 1972; 2:425–430. PMID: [4661477](#)
58. Clegg JA. In vitro cultivation of *Schistosoma mansoni*. *Exp. Parasitol.* 1965; 16:133–147. PMID: [14281210](#)
59. Foster A, Chen G, Vandewaa A, Pax RA, Bennett JL. Glutamine- vs Glucose-Supported Motor Activity in *Schistosoma mansoni*: Physiological Relevance of Aerobic Metabolism. *Exp. Parasitol.* 1989; 69: 44–53. PMID: [2499487](#)
60. Meyer F, Meyer H, Bueding E. Lipid metabolism in the parasitic and free-living flatworms *Schistosoma mansoni* and *Dugesia dorotocephala*. *Biochim.Biophys.Acta.* 1970; 21: 257–266.
61. Rumjanek FD, Simpson AJ. The incorporation and utilization of radiolabelled lipids by adult *Schistosoma mansoni* in vitro. *Mol. Biochem.Parasitol.* 1980; 1: 31–44. PMID: [7442709](#)
62. Pearce EJ, Huang SC. The metabolic control of schistosome egg production. *Cell. Microbiol.* 2015; 17:796–801. doi: [10.1111/cmi.12444](#) PMID: [25850569](#)
63. Cioli D, Pica-Mattocchia L, Basso A, Guidi A. Schistosomiasis control: praziquantel forever? *Mol. Biochem. Parasitol.* 2014; 195:23–29. doi: [10.1016/j.molbiopara.2014.06.002](#) PMID: [24955523](#)
64. Ismail M, Botros S, Metwally A, William S, Farghally A, Tao LF, et al. Resistance to praziquantel: direct evidence from *Schistosoma mansoni* isolated from Egyptian villagers. *Am. J. Trop. Med. Hyg.* 1999; 60: 932–935. PMID: [10403323](#)
65. Mkoji GM, Smith JM, Prichard RK. Glutathione redox state, lipid peroxide levels, and activities of glutathione enzymes in oltipraz-treated adult *Schistosoma mansoni*. *Biochem. Pharmacol.* 1989; 38:4307–4313. PMID: [2512935](#)
66. Alger HM, Williams DL. The disulfide redox system of *Schistosoma mansoni* and the importance of a multifunctional enzyme, thioredoxin glutathione reductase. *Mol. Biochem.Parasitol.* 2002; 121:129–139. PMID: [11985869](#)
67. Sayed AA, Cook SK, Williams DL. Redox balance mechanisms in *Schistosoma mansoni* rely on peroxiredoxins and albumin and implicate peroxiredoxins as novel drug targets. *J. Biol. Chem.* 2006; 281:17001–17010. PMID: [16606626](#)
68. Kuntz AN, Davioud-Charvet E, Sayed AA, Califf LL, Dessolin J, Arnér ES, et al. Thioredoxin glutathione reductase from *Schistosoma mansoni*: an essential parasite enzyme and a key drug target. *PLoS Med.* 2007; 4:e206. PMID: [17579510](#)
69. Simeonov A, Jadhav A, Sayed AA, Wang Y, Nelson ME, Thomas CJ, et al. Quantitative high-throughput screen identifies inhibitors of the *Schistosoma mansoni* redox cascade. *PLoS Negl. Trop. Dis.* 2008; 2:e127. doi: [10.1371/journal.pntd.0000127](#) PMID: [18235848](#)
70. Angelucci F, Sayed AA, Williams DL, Boumis G, Brunori M, Dimastrogiovanni D, et al. Inhibition of *Schistosoma mansoni* thioredoxin-glutathione reductase by auranofin: structural and kinetic aspects. *J. Biol. Chem.* 2009; 284:28977–28985. doi: [10.1074/jbc.M109.020701](#) PMID: [19710012](#)
71. Sayed AA, Williams DL. Biochemical characterization of 2-Cys peroxiredoxins from *Schistosoma mansoni*. *J. Biol.Chem.* 2004; 279:26159–26166. PMID: [15075328](#)
72. Mei H, Thakur A, Schwartz J, Lo Verde PT. Expression and characterization of glutathione peroxidase activity in the human blood fluke *Schistosoma mansoni*. *Infect.Immun.* 1996; 64:4299–4306. PMID: [8926102](#)
73. Kwatia MA, Botkin DJ, Williams DL. Molecular and enzymatic characterization of *Schistosoma mansoni* thioredoxin peroxidase. *J. Parasitol.* 2000; 86:908–915. PMID: [11128509](#)
74. Frappier F, Azoulay M, Leroy JP. Effect of oltipraz on the metabolism of glutathione in *Schistosoma mansoni*. *Biochem.Pharmacol.* 1988; 37: 2864–2866. PMID: [3395363](#)
75. Sayed AA, Simeonov A, Thomas CJ, Inglese J, Austin CP, Williams DL. Identification of oxadiazoles as new drug leads for the control of schistosomiasis. *Nat. Med.* 2008; 14:407–412. doi: [10.1038/nm1737](#) PMID: [18345010](#)
76. Hoffmann KF. An historical and genomic view of schistosome conjugal biology with emphasis on sex-specific gene expression. *Parasitology.* 2004; 128:S11–22. PMID: [16454894](#)
77. Cornford EM, Oldendorf WH. Transintegumental uptake of metabolic substrates in male and female *Schistosoma mansoni*. *J. Parasitol.* 1979; 65: 357–363. PMID: [480063](#)



78. Anderson L, Amaral MS, Beckedorff F, Silva LF, Dazzani B, Oliveira KC, et al. Schistosoma mansoni Egg, Adult Male and Female Comparative Gene Expression Analysis and Identification of Novel Genes by RNA-Seq. PLoS Negl. Trop. Dis. 2015; 9:e0004334. doi: [10.1371/journal.pntd.0004334](https://doi.org/10.1371/journal.pntd.0004334) PMID: [26719891](https://pubmed.ncbi.nlm.nih.gov/26719891/)
79. Cornford EM, Crane PD. In vitro estimation of the rate of hexose phosphorylation, by sequential pulsing with [3H]- and [14C]2-deoxy-D-glucose. Comp. Biochem. Physiol. B. 1985; 82:25–28. PMID: [4053573](https://pubmed.ncbi.nlm.nih.gov/4053573/)
80. Uglem GL, Read CP. Sugar transport and metabolism in Schistosoma mansoni. J. Parasitol. 1975; 61:390–397. PMID: [1138037](https://pubmed.ncbi.nlm.nih.gov/1138037/)
81. Cornford EM, Fitzpatrick AM, Quirk TL, Diep CP, Landaw EM. Tegumental glucose permeability in male and female Schistosoma mansoni. J. Parasitol. 1988; 74:116–128. PMID: [3357096](https://pubmed.ncbi.nlm.nih.gov/3357096/)
82. Cornford EM, Fitzpatrick AM. Comparative glucose utilization rates in separated and mated schistosomes. Exp. Parasitol. 1987; 64:448–457. PMID: [3678450](https://pubmed.ncbi.nlm.nih.gov/3678450/)
83. Cornford EM, Huot ME. Glucose transfer from male to female schistosomes. Science. 1981; 213:1269–1271. PMID: [7268436](https://pubmed.ncbi.nlm.nih.gov/7268436/)
84. Cornford EM, Fitzpatrick AM. The mechanism and rate of glucose transfer from male to female schistosomes. Mol. Biochem. Parasitol. 1985; 17:131–141. PMID: [4069156](https://pubmed.ncbi.nlm.nih.gov/4069156/)
85. Girardini JE, Dissous C, Serra E. Schistosoma mansoni ferredoxin NADP(H) oxidoreductase and its role in detoxification. Mol. Biochem. Parasitol. 2002; 124:37–45. PMID: [12387848](https://pubmed.ncbi.nlm.nih.gov/12387848/)
86. Alger HM, Sayed AA, Stadecker MJ, Williams DL. Molecular and enzymatic characterisation of Schistosoma mansoni thioredoxin. Int. J. Parasitol. 2002; 32:1285–1292. PMID: [12204228](https://pubmed.ncbi.nlm.nih.gov/12204228/)
87. Fitzpatrick JM, Johansen MV, Johnston DA, Dunne DW, Hoffmann KF. Gender-associated gene expression in two related strains of Schistosoma japonicum. Mol. Biochem. Parasitol. 2004; 136:191–209. PMID: [15478798](https://pubmed.ncbi.nlm.nih.gov/15478798/)
88. Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Guide for the Care and Use of Laboratory Animals. 8th ed. Popul. (French Ed. Washington, D.C.: The National Academies Press; 2011.
89. Smithers SR, Terry RJ. The infection of laboratory hosts with cercariae of Schistosoma mansoni and the recovery of adult worms. Parasitology. 1965; 55: 695–700. PMID: [4957633](https://pubmed.ncbi.nlm.nih.gov/4957633/)
90. Srere PA, Gonen L, Brazil H. Citrate condensing enzyme of pigeon breast muscle and moth flight muscle. Acta. Chem. Scand. 1963; 17: 129–134.
91. Pon LA, Schon EA. Mitochondria. In: Methods in Cell Biology, 2<sup>nd</sup> edition. 2007; San Diego: Academic Press. 944 p.
92. Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem. 1968; 25:192–205. PMID: [4973948](https://pubmed.ncbi.nlm.nih.gov/4973948/)
93. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: an open-source platform for biological-image analysis. Nat. Methods. 2012; 9: 676–682. doi: [10.1038/nmeth.2019](https://doi.org/10.1038/nmeth.2019) PMID: [22743772](https://pubmed.ncbi.nlm.nih.gov/22743772/)
94. Ramirez B, Bickle Q, Yousif F, Fakorede F, Mouries MA, Nwaka S. Schistosomes: challenges in compound screening. Expert Opin. Drug Discov. 2007; 2:S53–S61.
95. Tenhunen R, Marver HS, Schmid R. Microsomal heme oxygenase. Characterization of the enzyme. J. Biol. Chem. 1969; 244:6388–6394. PMID: [4390967](https://pubmed.ncbi.nlm.nih.gov/4390967/)
96. Fujimoto D, Prockop DJ. Protocollagen proline hydroxylase from Ascaris lumbricoides. J. Biol. Chem. 1969; 244:205–210. PMID: [5773284](https://pubmed.ncbi.nlm.nih.gov/5773284/)
97. Katz IR. Oxygen affinity of tyrosine and tryptophan hydroxylases in synaptosomes. J. Neurochem. 1980; 35:760–763. PMID: [6108999](https://pubmed.ncbi.nlm.nih.gov/6108999/)
98. Gibian MJ, Galaway RA. Steady-state kinetics of lipoxygenase oxygenation of unsaturated fatty acids. Biochemistry. 1976; 15:4209–4214. PMID: [822867](https://pubmed.ncbi.nlm.nih.gov/822867/)
99. Tretter L, Adam-Vizi V. Generation of reactive oxygen species in the reaction catalyzed by alpha-ketoglutarate dehydrogenase. J. Neurosci. 2004; 24:7771–7778. PMID: [15356188](https://pubmed.ncbi.nlm.nih.gov/15356188/)
100. Starkov AA, Fiskum G, Chinopoulos C, Lorenzo BJ, Browne SE, Patel MS, Beal MF. Mitochondrial alpha-ketoglutarate dehydrogenase complex generates reactive oxygen species. J. Neurosci. 2004; 24:7779–7788. PMID: [15356189](https://pubmed.ncbi.nlm.nih.gov/15356189/)
101. Quinlan CL, Goncalves RL, Hey-Mogensen M, Yadava N, Bunik VI, Brand MD. The 2-oxoacid dehydrogenase complexes in mitochondria can produce superoxide/hydrogen peroxide at much higher rates than complex I. J. Biol. Chem. 2014; 289:8312–8325. doi: [10.1074/jbc.M113.545301](https://doi.org/10.1074/jbc.M113.545301) PMID: [24515115](https://pubmed.ncbi.nlm.nih.gov/24515115/)

102. Fitzpatrick JM, Hirai Y, Hirai H, Hoffmann KF. Schistosome egg production is dependent upon the activities of two developmentally regulated tyrosinases. *FASEB J.* 2007; 21:823–835. PMID: [17167065](#)
103. Cornford EM. Schistosomatium douthitti: Effects of thyroxine. *Exp. Parasitol.* 1974; 36: 210–221. PMID: [4370352](#)
104. Cornford EM. Effects of insulin on Schistosomatium douthitti. *Gen. Comp. Endocr.* 1974; 23: 286–293. PMID: [4851755](#)
105. Coles GC. Enzyme levels in cercariae and adult Schistosoma mansoni. *Int. J. Parasitol.* 1973; 3: 505–510. PMID: [4199254](#)
106. Bueding E, Saz HJ. Pyruvate kinase and phosphoenolpyruvate carboxykinase activities of Ascaris muscle, Hymenolepis diminuta and Schistosoma mansoni. *Comp. Biochem. Physiol.* 1968; 24: 511–518. PMID: [5651288](#)
107. Conde-del Pino E, Pérez-Vilar M, Cintrón-Rivera AA, Señeriz R. Studies in Schistosoma mansoni. I. Malic and lactic dehydrogenase of adult worms and cercariae. *Exp. Parasitol.* 1966; 18: 320–326.
108. Hamdan FF, Ribeiro P. Characterization of a stable form of tryptophan hydroxylase from the human parasite Schistosoma mansoni. *J. Biol. Chem.* 1999; 274:21746–21754. PMID: [10419488](#)
109. Saeed HM, Mostafa MH, O'Connor PJ, Rafferty JA, Doenhoff MJ. Evidence for the presence of active cytochrome P450 systems in Schistosoma mansoni and Schistosoma haematobium adult worms. *FEBS Lett.* 2002; 519: 205–209. PMID: [12023046](#)
110. Mair GR, Niciu MJ, Stewart MT, Brennan G, Omar H, Halton DW, et al. A functionally atypical amidating enzyme from the human parasite Schistosoma mansoni. *FASEB J.* 2004; 18: 114–121. PMID: [14718392](#)
111. Newport GR, Weller TH. Deposition and maturation of eggs of Schistosoma mansoni in vitro: importance of fatty acids in serum-free media. *Am. J. Trop. Med. Hyg.* 1982; 31: 349–357. PMID: [7200332](#)
112. Lopaschuk GD, Wall SR, Olley PM, Davies NJ. Etomoxir, a carnitine palmitoyltransferase I inhibitor, protects hearts from fatty acid-induced ischemic injury independent of changes in long chain acylcarnitine. *Circ. Res.* 1988; 63: 1036–1043. PMID: [3197271](#)
113. Okumura-Noji K, Miura Y, Lu R, Asai K, Ohta N, Brindley PJ, et al. CD36-related protein in Schistosoma japonicum: candidate mediator of selective cholesteryl ester uptake from high-density lipoprotein for egg maturation. *FASEB J.* 2013; 27: 1236–1244. doi: [10.1096/fj.12-219816](#) PMID: [23195036](#)
114. Ponsot E, Zoll J, N'guessan B, Ribera F, Lampert E, Richard R, et al. Mitochondrial tissue specificity of substrates utilization in rat cardiac and skeletal muscles. *J. Cell. Physiol.* 2005; 203: 479–486. PMID: [15521069](#)
115. Rasmussen UF, Vielwerth SE, Rasmussen HN. Skeletal muscle bioenergetics: a comparative study of mitochondria isolated from pigeon pectoralis, rat soleus, rat biceps brachii, pig biceps femoris and human quadriceps. *Comp. Biochem. Physiol.* 2004; 137: 435–446.
116. Halangk W, Bohnensack R, Frank K, Kunz W. Effect of various substrates on mitochondrial and cellular energy state of intact spermatozoa. *Biomed. Biochim. Acta.* 1985; 44: 411–420. PMID: [4004841](#)
117. Zielonka J, Kalyanaraman B. Hydroethidine- and MitoSOX-derived red fluorescence is not a reliable indicator of intracellular superoxide formation: another inconvenient truth. *Free Rad. Biol Med.* 2010; 48:983–1001. doi: [10.1016/j.freeradbiomed.2010.01.028](#) PMID: [20116425](#)
118. Aw TY, Rhoads CA. Glucose regulation of hydroperoxide metabolism in rat intestinal cells. Stimulation of reduced nicotinamide adenine dinucleotide phosphate supply. *J. Clin. Invest.* 1994; 94: 2426–2434. PMID: [7989600](#)
119. DeBerardinis RJ, Mancuso A, Daikhin E, Nissim I, Yudkoff M, Wehrli S, Thompson CB. Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis. *Proc. Natl. Acad. Sci. U.S.A.* 2007; 104:19345–19350. PMID: [18032601](#)
120. Suzuki S, Tanaka T, Poyurovsky MV, Nagano H, Mayama T, Ohkubo S, et al. Phosphate-activated glutaminase (GLS2), a p53-inducible regulator of glutamine metabolism and reactive oxygen species. *Proc. Natl. Acad. Sci. U.S.A.* 2010; 107: 7461–7466. doi: [10.1073/pnas.1002459107](#) PMID: [20351271](#)
121. Furukawa S, Saito H, Matsuda T, Inoue T, Fukatsu K, Han I, et al. Relative effects of glucose and glutamine on reactive oxygen intermediate production by neutrophils. *Shock.* 2000; 13: 274–278. PMID: [10774615](#)
122. Furukawa S, Saito H, Inoue T, Matsuda T, Fukatsu K, Han I, et al. Supplemental glutamine augments phagocytosis and reactive oxygen intermediate production by neutrophils and monocytes from post-operative patients in vitro. *Nutrition.* 2000; 16: 323–329. PMID: [10793298](#)

123. Goossens V, Grooten J, Fiers W. The oxidative metabolism of glutamine. A modulator of reactive oxygen intermediate-mediated cytotoxicity of tumor necrosis factor in L929 fibrosarcoma cells. *J. Biol. Chem.* 1996; 271: 192–196. PMID: [8550558](#)
124. Schulz TJ, Zarse K, Voigt A, Urban N, Birringer M, Ristow M. Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell. Metab.* 2007; 6:280–293. PMID: [17908557](#)
125. Vanden Hoek TL, Becker LB, Shao Z, Li C, Schumacker PT. Reactive oxygen species released from mitochondria during brief hypoxia induce preconditioning in cardiomyocytes. *J. Biol. Chem.* 1998; 273:18092–18098. PMID: [9660766](#)
126. Forbes RA, Steenbergen C, Murphy E. Diazoxide-induced cardioprotection requires signaling through a redox-sensitive mechanism. *Circ. Res.* 2001; 88:802–809. PMID: [11325872](#)
127. Schmeisser S, Schmeisser K, Weimer S, Groth M, Priebe S, Fazius E, et al. Mitochondrial hormesis links low-dose arsenite exposure to lifespan extension. *Aging Cell.* 2013; 12:508–517. doi: [10.1111/ace.12076](#) PMID: [23534459](#)
128. Yang W, Hekimi S. A mitochondrial superoxide signal triggers increased longevity in *Caenorhabditis elegans*. *PLoS Biol.* 2010; 8: e1000556. doi: [10.1371/journal.pbio.1000556](#) PMID: [21151885](#)
129. Fabrizio P, Battistella L, Vardavas R, Gattazzo C, Liou LL, Diaspro A, et al. Superoxide is a mediator of an altruistic aging program in *Saccharomyces cerevisiae*. *J. Cell Biol.* 2004; 166:1055–1067. PMID: [15452146](#)
130. Zarse K, Schmeisser S, Groth M, Priebe S, Beuster G, Kuhlow D, et al. Impaired insulin/IGF1 signaling extends life span by promoting mitochondrial L-proline catabolism to induce a transient ROS signal. *Cell Metab.* 2012; 15:451–465. doi: [10.1016/j.cmet.2012.02.013](#) PMID: [22482728](#)
131. Ristow M, Zarse K. How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis). *Exp. Gerontol.* 2010; 45: 410–418. doi: [10.1016/j.exger.2010.03.014](#) PMID: [20350594](#)
132. Ristow M, Schmeisser S. Extending life span by increasing oxidative stress. *Free Rad. Biol Med.* 2011; 51:327–336. doi: [10.1016/j.freeradbiomed.2011.05.010](#) PMID: [21619928](#)
133. Maglioni S, Schiavi A, Runci A, Shaik A, Ventura N. Mitochondrial stress extends lifespan in *C. elegans* through neuronal hormesis. *Exp. Gerontol.* 2014; 56:89–98. doi: [10.1016/j.exger.2014.03.026](#) PMID: [24709340](#)
134. Ristow M, Zarse K, Oberbach A, Klötting N, Birringer M, Kiehnopf M, et al. Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc. Natl. Acad. Sci. U.S.A.* 2009; 106:8665–8670. doi: [10.1073/pnas.0903485106](#) PMID: [19433800](#)
135. Zelck UE, Von Janowsky B. Antioxidant enzymes in intramolluscan *Schistosoma mansoni* and ROS-induced changes in expression. *Parasitology.* 2004; 128:493–501. PMID: [15180317](#)
136. Messerli SM, Morgan W, Birkeland SR, Bernier J, Cipriano MJ, McArthur AG, et al. Nitric oxide-dependent changes in *Schistosoma mansoni* gene expression. *Mol. Biochem. Parasitol.* 2006; 150:367–370. PMID: [16962671](#)
137. Aragon AD, Imani RA, Blackburn VR, Cunningham C. Microarray based analysis of temperature and oxidative stress induced messenger RNA in *Schistosoma mansoni*. *Mol. Biochem. Parasitol.* 2008; 162:134–141. doi: [10.1016/j.molbiopara.2008.08.004](#) PMID: [18775750](#)
138. Kuntz RE, Tulloch GS, Van Riper D, Huang TC, Moore JA, Moore R. *Schistosoma mansoni* in vivo exposure to hyperbaric oxygen. *J. Parasitol.* 1980; 66:364–365. PMID: [7391882](#)
139. Seif el-Din SH, Al-Hroob AM, Ebeid FA. *Schistosoma mansoni*: N-acetylcysteine downregulates oxidative stress and enhances the antischistosomal activity of artemether in mice. *Exp. Parasitol.* 2011; 128: 230–235. doi: [10.1016/j.exppara.2011.03.006](#) PMID: [21426905](#)
140. Thor H, Smith MT, Hartzell P, Bellomo G, Jewell S, Orrenius S. The metabolism of menadione (2-methyl-1,4-naphthoquinone) by isolated hepatocytes. A study of the implications of oxidative stress in intact cells. *J. Biol. Chem.* 1982; 257: 12419–12425. PMID: [6181068](#)
141. Di Monte D, Ross D, Bellomo G, Eklöv L, Orrenius S. Alterations in intracellular thiol homeostasis during the metabolism of menadione by isolated rat hepatocytes. *Arch. Biochem. Biophys.* 1984; 235:334–342. PMID: [6097182](#)
142. Di Monte D, Bellomo G, Thor H, Nicotera P, Orrenius S. Menadione-induced cytotoxicity is associated with protein thiol oxidation and alteration in intracellular Ca<sup>2+</sup> homeostasis. *Arch. Biochem. Biophys.* 1984; 235:343–350. PMID: [6097183](#)
143. Tzeng WF, Chiou TJ, Wang CP, Lee JL, Chen YH. Cellular thiols as a determinant of responsiveness to menadione in cardiomyocytes. *J. Mol. Cell. Cardiol.* 1994; 26: 889–897. PMID: [7966357](#)

144. Criddle DN, Gillies S, Baumgartner-Wilson HK, Jaffar M, Chinje EC, Passmore S, et al. Menadione-induced reactive oxygen species generation via redox cycling promotes apoptosis of murine pancreatic acinar cells. *J. Biol. Chem.* 2006; 281: 40485–40492. PMID: [17088248](#)
145. Hermes-Lima M, Zenteno-Savín T. Animal response to drastic changes in oxygen availability and physiological oxidative stress. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 2002; 133: 537–556. PMID: [12458182](#)
146. Cypser JR, Johnson TE. Multiple stressors in *Caenorhabditis elegans* induce stress hormesis and extended longevity. *J. Gerontol. A Biol. Sci. Med. Sci.* 2002; 57: B109–B114. PMID: [11867647](#)
147. Yanase S, Hartman PS, Ito A, Ishii N. Oxidative stress pretreatment increases the X-radiation resistance of the nematode *Caenorhabditis elegans*. *Mutat. Res.* 1999; 426:31–39. PMID: [10320748](#)
148. Hosono R, Mitsui Y, Sato Y, Aizawa S, Miwa J. Life span of the wild and mutant nematode *Caenorhabditis elegans*: effects of sex, sterilization, and temperature. *Exp. Gerontol.* 1982; 17:163–172. PMID: [6213421](#)
149. Lee SJ, Hwang AB, Kenyon C. Inhibition of respiration extends *C. elegans* life span via reactive oxygen species that increase HIF-1 activity. *Curr. Biol.* 2010; 20: 2131–2136. doi: [10.1016/j.cub.2010.10.057](#) PMID: [21093262](#)
150. Piacenza L, Peluffo G, Alvarez MN, Martínez A, Radi R. Trypanosoma cruzi antioxidant enzymes as virulence factors in Chagas disease. *Antioxid. Redox Signal.* 2013; 19: 723–734. doi: [10.1089/ars.2012.4618](#) PMID: [22458250](#)
151. Gonçalves RL, Barreto RF, Polycarpo CR, Gadelha FR, Castro SL, Oliveira MF. A comparative assessment of mitochondrial function in epimastigotes and bloodstream trypomastigotes of *Trypanosoma cruzi*. *J. Bioenerg. Biomembr.* 2011; 43: 651–661. doi: [10.1007/s10863-011-9398-8](#) PMID: [22081211](#)
152. Aragon AD, Imani RA, Blackburn VR, Cunningham C. Microarray based analysis of temperature and oxidative stress induced messenger RNA in *Schistosoma mansoni*. *Mol. Biochem. Parasitol.* 2008; 162:134–141. doi: [10.1016/j.molbiopara.2008.08.004](#) PMID: [18775750](#)
153. Zelck UE, Von Janowsky B. Antioxidant enzymes in intramolluscan *Schistosoma mansoni* and ROS-induced changes in expression. *Parasitology.* 2004; 128:493–501. PMID: [15180317](#)
154. Sun J, Li C, Wang S. The up-regulation of ribosomal proteins further regulates protein expression profile in female *Schistosoma japonicum* after pairing. *PLoS ONE.* 2015; 10:e0129626. doi: [10.1371/journal.pone.0129626](#) PMID: [26070205](#)
155. Sun J, Wang SW, Li C, Hu W, Ren YJ, Wang JQ. Transcriptome profilings of female *Schistosoma japonicum* reveal significant differential expression of genes after pairing. *Parasitol. Res.* 2014; 113:881–892. doi: [10.1007/s00436-013-3719-2](#) PMID: [24297695](#)