# Endothelial dysfunction and body mass index: is there a role for plasma peroxynitrite?

Theresa Chikopela[1]* , Douglas C. Heimburger[2,3], Longa Kaluba[4], Pharaoh Hamambulu[1], Newton Simfukwe[5], Wilbroad Mutale[6], John R. Koethe[7] and Fastone Goma[5]

## Abstract

**Background:** Endothelial function is dependent on the balance between vasoconstrictive and vasodilatory substances. The endothelium ability to produce nitric oxide is one of the most crucial mechanisms in regulating vascular tone. An increase in inducible nitric oxide synthase contributes to endothelial dysfunction in overweight persons, while oxidative stress contributes to the conversion of nitric oxide to peroxynitrite (measured as nitrotyrosine in vivo) in underweight persons. The objective of this study was to elucidate the interaction of body composition and oxidative stress on vascular function and peroxynitrite. This was done through an experimental design with three weight groups (underweight, normal weight and overweight), with four treatment arms in each. Plasma nitrotyrosine levels were measured 15–20 h post lipopolysaccharide (LPS) treatment, as were aortic ring tension changes. Acetylcholine (ACh) and sodium nitroprusside (SNP) challenges were used to observe endothelial-dependent and endothelial-independent vascular relaxation after pre-constriction of aortic rings with phenylephrine.

**Results:** Nitrotyrosine levels in saline-treated rats were similar among the weight groups. There was a significant increase in nitrotyrosine levels between saline-treated rats and those treated with the highest lipopolysaccharide doses in each of the weight groups. In response to ACh challenge, $R_{max}$ (percentage reduction in aortic tension) was lowest in overweight rats (112%). In response to SNP, there was an insignificantly lower $R_{max}$ in the underweight rats (106%) compared to the normal weight rats (112%). Overweight rats had a significant decrease in $R_{max}$ (83%) in response to SNP, signifying involvement of a more chronic process in tension reduction changes. A lower $R_{max}$ accompanied an increase in peroxynitrite after acetylcholine challenge in all weight groups.

**Conclusions:** Endothelial dysfunction, observed as an impairment in the ability to reduce tension, is associated with increased plasma peroxynitrite levels across the spectrum of body mass. In higher-BMI rats, an additional role is played by vascular smooth muscle in the causation of endothelial dysfunction.

**Keywords:** Endothelial dysfunction, Body mass index, Peroxynitrite, Nitrotyrosine, Endothelial-dependent vascular relaxation

* Correspondence: theresachikopela@yahoo.com;
theresa.sikazwe@lamu.edu.zm
[1]Department of Human Physiology, Faculty of Medicine, Lusaka Apex
Medical University, Lusaka, Zambia
Full list of author information is available at the end of the article

## 1 Background

The endothelium plays a crucial role in regulating haemodynamics by the production and release of multiple biochemical factors [1], among which are endothelium-derived vasoactive substances that can mediate either vasoconstriction (e.g., endothelin-1) or vasodilation (e.g., nitric oxide) [2]. The normal function of the endothelium depends on the balance of these factors, and a mechanism leading to endothelial dysfunction is reduced production of nitric oxide. Nitric oxide produced in the endothelial cells diffuses to vascular smooth muscle cells. It then activates nitric oxide-sensitive guanylyl cyclase, which induces vascular smooth muscle relaxation by modifying intracellular calcium concentrations. Reduced production of nitric oxide is a central feature of endothelial dysfunction and a loss of arterial vascular compliance [3].

Expression of inducible nitric oxide synthase (iNOS), a calcium-independent enzyme, is increased in response to increased signalling molecules, e.g., proinflammatory cytokines [4]. Klabunde et al. [5] reported that bacterial endotoxins (e.g., lipopolysaccharide) also stimulate the activity of iNOS in the setting of inflammation, and this effect may persist for several days [6]. In certain disease states, such as HIV infection, atherosclerosis and other inflammatory conditions [7], there is an increase in oxidative stress. This results from a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defences. The oxygen splits into single atoms with unpaired electrons called free radicals which seek electrons from other molecules to gain stability, destabilising the affected molecule and turning it into a free radical (e.g., superoxide ($O_2^-$)). In high amounts, $O_2^-$ non-enzymatically reacts with nitric oxide to produce peroxynitrite, a highly aggressive oxidant [1, 8].

Administration of bacterially derived lipopolysaccharide (LPS) in rats increases the production of iNOS and oxygen free-radical production, mimicking what occurs in humans during oxidative stress [9]. This involves the activation of toll-like receptors (TLR4) on endothelial cells [10]. The LPS binds to TLR4, activating nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and P38 mitogen-activated protein kinase (P38 MAP kinase). This leads to increased production of nitric oxide in many cells, such as macrophages and vascular smooth muscle cells. LPS also activates nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase, triggering excessive $O_2^-$ generation [11]. The $O_2^-$ and nitric oxide reaction produces peroxynitrite (Fig. 1) [12].

Peroxynitrite can cross cell membranes, exposing the cell to its effects [13–15]. This allows it to have specific actions resulting in cellular dysfunction [13]. It affects both the structure and function of the endothelium [1,
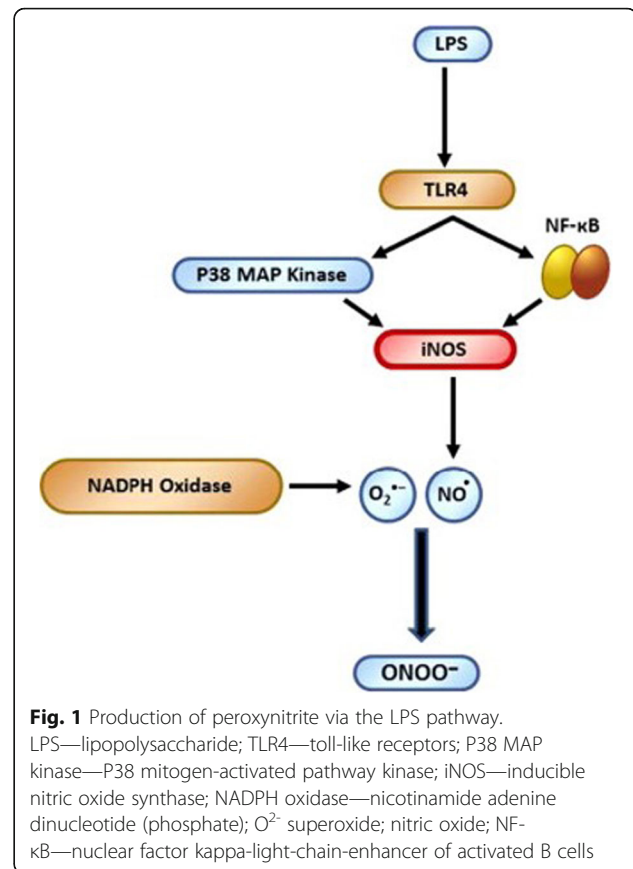


**Fig. 1** Production of peroxynitrite via the LPS pathway. LPS—lipopolysaccharide; TLR4—toll-like receptors; P38 MAP kinase—P38 mitogen-activated pathway kinase; iNOS—inducible nitric oxide synthase; NADPH oxidase—nicotinamide adenine dinucleotide (phosphate); $O^{2-}$ superoxide; nitric oxide; NF-κB—nuclear factor kappa-light-chain-enhancer of activated B cells

12, 13, 16] and can trigger apoptosis and poly (ADP-ribose) polymerase (PARP)-dependent cell death [17]. Mihm et al. [1] reported an increase in DNA fragmentation associated with a reduction in vascular relaxation, suggesting a pathway of apoptosis as an essential mechanism of endothelial dysfunction in vivo. The ability of peroxynitrite to limit the function of endothelial nitric oxide synthase (eNOS) by inactivating it results in a reduction in the bioavailability of nitric oxide.

Adipose tissue is a major source of iNOS [18], which increases oxidative stress [19]. Maslov et al. [19] reported that patients with increased adipose tissue had higher levels of serum nitrotyrosine, which could contribute to the endothelial dysfunction observed in patients with higher body mass index (BMI). However, endothelial dysfunction is also reported in individuals with low BMI [20]. This may be associated with increased oxidative stress and peroxynitrite causing DNA damage [20–22]. This mechanism may also be related to peroxynitrite increment and its effect on the endothelium. While several studies have investigated the effects of peroxynitrite on vascular function, there are few data on whether body composition alters this relationship. We therefore investigated the effects of peroxynitrite on endothelial function in rats with varying body mass. We

hypothesised that exposure of the endothelial cells to high levels of peroxynitrite in vivo would impair the ability to reduce tension through the endothelial properties. This would further the understanding of the mechanism of peroxynitrite and body mass on endothelium-dependent vascular relaxation.

## 2 Methods

### 2.1 Animal model
Male Wistar rats ranging from 100 to 450 g were selected and housed in the animal unit of the Department of Physiological Sciences, at the University. The animals were maintained according to standard nutritional and environmental conditions, and they had free access to a standard diet (Bendel Feeds and Flour Mill) and water ad libitum, in their own single animal cages. Animal studies were conducted according to standard guidelines for the use of laboratory animals [23] and ethical approval was sought for from the University Ethics Committee (Ref 004-01-18).

### 2.2 Acclimatisation and LPS treatment
The body mass index (BMI) was calculated according to Ahmed et al. [24] as the weight (g) divided by the square of the length (tip of the nose to the start of the tail) in centimeters. Forty-eight rats were subdivided, equally, into weight groups categorised as (a) underweight (BMI below 0.45 g/cm$^2$), (b) normal weight (BMI between 0.45 and 0.68 g/cm$^2$) and (c) overweight (BMI above 0.68 g/cm$^2$) [25]. Each of these groups was further divided into four treatment groups (with four rats each). The LPS was administered to the rats in non-fatal doses of 10 mg/kg, 3.16 mg/kg and 1 mg/kg intraperitoneally. The fourth subgroup was a sham group treated with saline intraperitoneally [9]. To avoid bias, animal selection was based only on weight requirement, and this was done the animal unit superintendent. When the sample size in each of the 4 groups is approximately 4, a one-way analysis of variance will have over 80% power to detect at the 0.05 level a difference in means characterised by a variance of means, V, of 29268.75, assuming that the typical standard deviation is 28.55 based on results from Kamisaki et al. [9].

### 2.3 Blood sample collection
The rats were sacrificed 15 to 20 h after treatment with LPS [9] by cervical dislocation, and 2 ml of blood was obtained through cardiac puncture and placed into tubes with EDTA and kept at 2–8 °C for a maximum of 2 h. This was done in a laboratory, specifically designed for animal experiments. The blood was then centrifuged at 2700× for 15 min. The resulting plasma supernatant was immediately transferred into two aliquots, labelled with the ID (weight group and number), date of sacrifice and treatment type and stored at − 80 °C.

### 2.4 Nitrotyrosine measurement
Plasma nitrotyrosine levels were measured in the blood samples using ELISA kits (ab210603, Abcam, Cambridge, USA). The absorbance of the samples was read at 450-nm wavelength by ELISA 96-well microplate reader (BioTek Model EL800, BioTek Instruments, USA) and recorded in nanomoles.

### 2.5 Isolation of rat aortae and organ bath technique
Following sacrifice, the thoracic aorta was rapidly isolated for tension evaluation. The aortic ring segments (3–4 mm broad) were mounted in a 25-ml organ bath with two L-shaped stainless-steel hooks running parallel to one another through the aortic lumen. The isometric force transducer was connected to one of the hooks (Myograph ADInstruments, AU). The rings were incubated in a 25-ml organ bath containing physiological saline solution (PSS) and aerated 37 °C. After 20 min of equilibration (resting tension 50 mN), a wash with fresh 25 ml PSS was performed, and tension reading was zeroed. A second wash with PSS was done, followed by another 20-min rest before resetting to zero again.

The rings were stimulated by replacing the PSS with warm 25 mL 60 mM high-potassium PSS (KPSS) for 15 min. Tension changes were recorded using the ML856 PowerLab 26 T (Model: ML 856) and displayed by the LabTutor. After this, the aortic rings were washed with PSS three times at 10-min intervals. The rings were stimulated a second time with KPSS for 15 min and then washed with PSS three times over at 10-min intervals [26, 27].

To the PSS in the organ bath, 1 μM (5 μl of $10^{-3}$ stock) of phenylephrine was added. The contraction was observed for 20 min. The maximum tension attained was considered as 100% contraction. Without washing, 1.09 × $10^{-9}$ to 1.04 × $10^{-4}$ $M$ acetylcholine (ACh) was added in ascending order. The maximal effect on the tension of each dose was recorded with each addition of ACh. The vessels were then washed three times over 10 min with 5 mL PSS before being stimulated again with KPSS and treated with phenylephrine to observe the effect of ACh. Without washing, another addition to the organ bath of the increasing concentrations of ACh was made.

The endothelial dysfunction induced by the reactive peroxynitrite was indicated by the reduction in the percentage maximum relaxation value ($R_{max}$). This is the ability of the aorta to have vascular smooth muscle tension reduction through the endothelial contribution of nitric oxide. A disturbance in the endothelium's ability to produce nitric oxide would cause the inability to

reduce tension. The log concentration it would take to get to 50% inhibition or 50% reduction in tension is $logIC_{50}$. An increase in $logIC_{50}$ would imply that more of the ACh was required to cause tension reduction. To determine the sensitivity of vascular smooth muscle only to nitric oxide, sodium nitroprusside (SNP) was used with the protocol described above [28]. The concentrations used were $1.09 \times 10^{-9}$ to $1.04 \times 10^{-4} M$.

## 3 Statistical analyses

Data were recorded using the PowerLab data acquisition system (PowerLab 26 T, ADInstruments), and tension data were analysed with data acquisition and analysis software (LabChart 7, ADInstruments). Contractile and relaxant response data were fitted using GraphPad Prism Software (GraphPad Software, Inc., San Diego, CA. vs 8.1) [29]. Data are presented as mean ± SEM. Statistical evaluations for dose-response curves were performed using GraphPad Prism Software. The endothelial dysfunction induced by the reactive peroxynitrite was indicated by the reduction in the percentage maximum relaxation value ($R_{max}$), and an increase in $logIC_{50}$.

Differences in levels of nitrotyrosine after treatment with LPS were analysed using one-way ANOVA comparing the weight groups within the same LPS treatment. Another analysis included nitrotyrosine level comparison among doses of LPS within the same weight group. A comparison of the means was made using Bonferroni correction; mean comparisons were made using STATA software and presented as mean ± SD. In all cases, $P < 0.05$ was deemed statistically significant.

## 4 Results

### 4.1 Characteristics of the rats in the study

The BMIs of the three weight groups are shown in Table 1. Within the LPS treatment groups, A to D, BMI values were similar in each of the weight groups.

**Table 1** BMI of rats by weight group and LPS treatment group (mean ± SD)

| Treatment groups | Weight groups | | |
|---|---|---|---|
| | Underweight | Normal weight | Overweight |
| A | 0.37 ± 0.05[a] | 0.54 ± 0.04[b] | 0.71 ± 0.04[c] |
| B | 0.38 ± 0.04[a] | 0.48 ± 0.04[b] | 0.75 ± 0.05[c] |
| C | 0.40 ± 0.02[a] | 0.56 ± 0.04[b] | 0.74 ± 0.13[c] |
| D | 0.41 ± 0.01[a] | 0.53 ± 0.05[b] | 0.69 ± 0.05[c] |

Treatment groups comprising groups A—saline, B—1 mg/kg, C—3.16 mg/kg and D—10 mg/kg. A letter has been assigned to each treatment. Different letters in the table columns represent significant differences among the treatment groups (A, B, C and D) at $P < 0.05$. Across each row, weight groups (underweight, normal weight, and overweight) have been marked by the same letter if there were not significantly different ($P > 0.05$).

### 4.2 Plasma nitrotyrosine concentrations post-LPS treatment

LPS treatment induced a significant increase in the levels of nitrotyrosine in the aorta of rats treated with 10 mg/kg dose of LPS (group D) compared to that observed in the saline-treated rats in all the weight groups. In the underweight rats, saline-treated rats (group A) had nitrotyrosine levels of 492 ± 259 nM as compared to 827 ± 137 nM ($P = 0.02$) in the group D (high-dose LPS) rats. In the normal weight rats, group A rats had nitrotyrosine of 595 ± 153 nM vs. 1065 ± 284 nM in the group D rats ($P = 0.01$). In the overweight rats, group A rats had nitrotyrosine levels of 619 ± 118 nM vs. 962 ± 148 nM in group D rats ($P < 0.01$). In all the weight groups, the intermediate doses (1 mg/kg and 3.16 mg/kg) did not show a significant rise in nitrotyrosine levels from saline-treated rats; only LPS at 10 mg/kg increased nitrotyrosine levels. These relationships are depicted in Fig. 2.

### 4.3 Relationship of body mass index with endothelial-dependent relaxation

There were no significant differences in nitrotyrosine levels ($P = 0.42$) among the three weight groups in the saline-treated rats (underweight—492 ± 259 nM; normal weight rats—595 ± 153 nM; overweight—619 ± 118 nM; $P = 0.42$). The dose-dependent responses to ACh and SNP are shown in Fig. 3.

After treatment with ACh, the underweight rat aortic rings had an $R_{max}$ of 137%, and in the normal weight group, an $R_{max}$ of 121% was observed ($P = 0.03$). The overweight rats had a lower but statistically insignificant $R_{max}$ of 112% ($P = 0.8$) compared to the normal weight rats. The underweight rat aortic rings had a $logIC_{50}$ of − 7.91. This increased to − 7.87 in normal weight and overweight rats (− 7.87). The $logIC_{50}$ values, as shown in Fig. 4 below, were not significantly different among the weight groups.

Response to SNP treatment was used to observe endothelial-independent tension reduction. The $R_{max}$ value in the underweight was 106%, that in the normal weight was 112% ($P = 0.66$), and that in the overweight rats was 83%, significantly lower than that in the normal weight rats ($P = 0.01$). Figure 4 shows the underweight with a $logIC_{50}$ of − 7.60, − 7.40 in normal weight rats, and − 7.70 in overweight rats ($P = 0.43$).

### 4.4 Effect of peroxynitrite on endothelial-dependent relaxation

There was no significant difference in the nitrotyrosine levels from saline-treated rat aorta through the first two dosages of LPS (1 mg/kg and 3.16 mg/kg) in all the weight groups, as shown in Fig. 2. The comparison of the effects of changes in peroxynitrite levels on tension
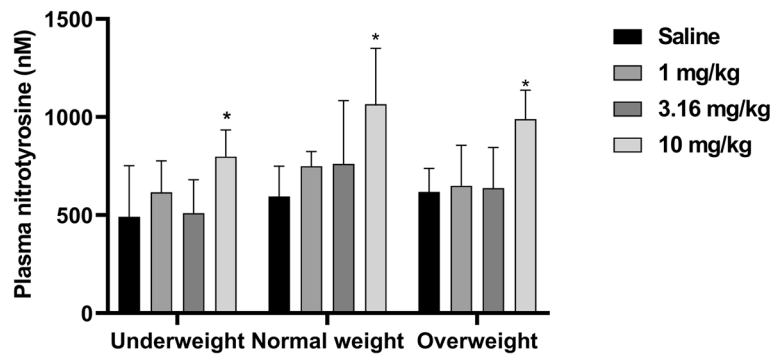
**Fig. 2** Dose-dependent effects of LPS on plasma concentrations of nitrotyrosine (nM) 15 to 20 h after the injection of LPS (treatment groups A through D). Data are expressed as the mean ± SD; $n = 4$. *$P < 0.05$ compared with that of the aorta from rats treated with saline (treatment groups A and D)

reduction, therefore, focused on groups A and D. Figure 5 summarises the dose-dependent decrease in tension observed from treatment with ACh or SNP in all the weight types.

As illustrated in Fig. 6, in the treatment of the aortae with ACh, underweight rats had an $R_{max}$ of 137% in group A vs. 92% in group D ($P < 0.001$). Group A had $logIC_{50}$ of $-7.91$, and this increased in group D ($-7.8$) but not significantly ($P = 0.67$).

In the normal weight rats, ACh treatment produced an $R_{max}$ of 121% in group A vs. 94% in group D rats ($P < 0.001$). The $logIC_{50}$ in group A was $-7.87$; it was insignificantly lower in group D ($-8.03$, $P = 0.41$). In the overweight rats, $R_{max}$ was 112% in group A vs. 89% in group D ($P < 0.001$). The $logIC_{50}$ in group A was lower than that in group D, which was $-7.80$ but was statistically insignificant ($P = 0.69$). As depicted in Fig. 6, when treated with SNP, the aorta from underweight rats had an $R_{max}$ of 106% in group A vs. 93% in group D ($P = 0.08$). The $logIC_{50}$ was $-7.09$ and $-7.92$ in groups A and D, respectively ($P = 0.07$). In the normal weight rats, $R_{max}$ in group A rat aortae was 112% vs. 108% in group D ($P = 0.54$). The $logIC_{50}$ in group A was $-7.11$ vs. $-$

7.35 in group D ($P = 0.44$). Overweight rats, when treated with SNP, showed an $R_{max}$ of 83% in the group A rat aortae vs. 103% in group D ($P = 0.04$). $LogIC_{50}$ in group A was $-7.43$ vs. $-7.30$ in group D ($P = 0.52$).
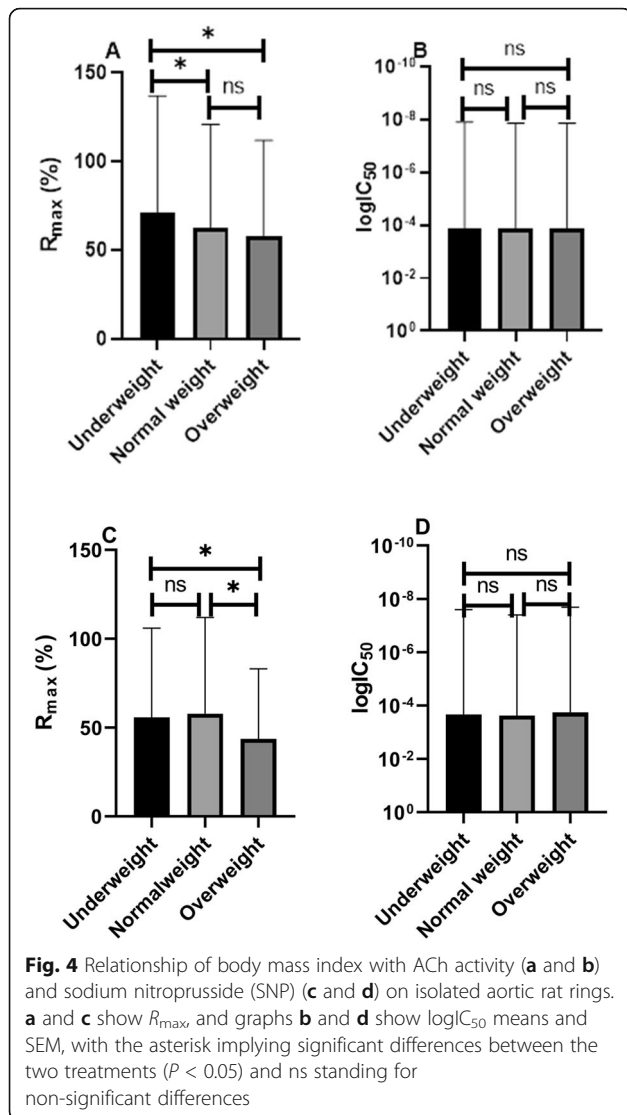
## 5 Discussion

We observed no differences in levels of plasma peroxynitrite in the saline-treated rats of all three weight groups, as reflected by insignificant differences of nitrotyrosine levels. However, with increases in BMI, a significant decrease in the ability to reduce tension through the endothelial-dependent vascular relaxation pathway was noted. The overweight rats had a significantly lower ability to reduce tension, even with a direct contribution of nitric oxide by SNP. Treatment with 10 mg/kg LPS induced a significant increase in the levels of nitrotyrosine in all weight groups as compared with saline-treated rats. We demonstrated that an increase in peroxynitrite leads to a decrease in the reduction of tension in the aortae in the underweight, normal weight and overweight rats.

Endothelial function is subject to alterations in biochemical factors, some of which are related to changes
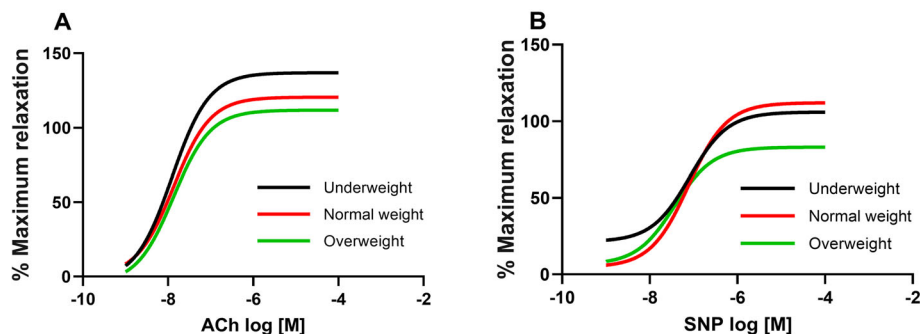


**Fig. 3** Vascular relaxation responses in isolated aortic rat rings showing endothelial-dependent relaxation (**a**) and endothelial-independent relaxation (**b**) by body mass index categories

**Fig. 4** Relationship of body mass index with ACh activity (**a** and **b**) and sodium nitroprusside (SNP) (**c** and **d**) on isolated aortic rat rings. **a** and **c** show $R_{max}$, and graphs **b** and **d** show $logIC_{50}$ means and SEM, with the asterisk implying significant differences between the two treatments ($P < 0.05$) and ns standing for non-significant differences

in body mass. Both endothelial function and plasma peroxynitrite are related to body mass [18, 19], and its effects on the levels of peroxynitrite were observed in the saline-treated rats. BMI significantly impacted peroxynitrite levels as measured by nitrotyrosine. The levels of nitrotyrosine were proportional to BMI: the higher the BMI, the higher the plasma nitrotyrosine. The elevated levels of peroxynitrite, as shown by higher nitrotyrosine levels, produced in the overweight rats, could be because of an increase in oxidative stress from the larger amount of adipose tissue [18]. In other literature, lower body mass has also been associated with higher levels of peroxynitrite. This has been attributed to the damage that peroxynitrite causes to endothelium DNA [21, 22]. This was not so in our study, where the underweight rats had the lowest levels of nitrotyrosine.

We observed that in saline-treated rats, higher BMI was significantly associated with endothelial dysfunction:

normal weight rats had significantly lower $R_{max}$ (121%) compared to underweight rats ($R_{max}$ = 137%) ($P = 0.03$). Further decrease in endothelial-dependent vascular relaxation in overweight rats was observed. Adipose tissue, a major source of iNOS [18], is observed in overweight. This may lead to an elevation in oxidative stress, as observed by the increase in peroxynitrite [19] which causes reduced endothelial-dependent vascular relaxation. Several literature have outlined that the higher levels of plasma peroxynitrite in overweight and underweight rats may be responsible for endothelial dysfunction in these weight groups [20–22]. In this study, acute changes in tension reduction among the different weight groups and varying peroxynitrite levels have been demonstrated in rat aortae.

To study only the effect of the endothelium, aortic ring response to endothelial-independent vascular relaxation was assessed using SNP. SNP is an exogenous agent that generates nitric oxide through nonenzymatic and enzymatic pathways in smooth muscle cells. It does not require a functional endothelium or NOS enzymes for its activity. Once infused, SNP interacts with oxyhaemoglobin, dissociating immediately and forming methaemoglobin while releasing cyanide and nitric oxide. Nitric oxide activates the enzyme guanylate cyclase found within the vascular smooth muscle. This results in increased intracellular concentrations of cyclic guanosine monophosphate, which inhibits calcium entry into vascular smooth muscle cells. The mechanism results in the increase of calcium uptake by the smooth endoplasmic reticulum to produce vasodilation [30]. In the current study, the effectiveness of this pathway was noted by a significant change in $R_{max}$ after treatment with SNP.

Post SNP challenge, the underweight and normal weight rats showed insignificant differences in $R_{max}$ and $logIC_{50}$ in the curves of saline-treated rats compared to those treated with LPS. This may be a demonstration of impairment of endothelial production and delivery of nitric oxide, rather than vascular smooth muscle sensitivity to nitric oxide. This is similar to other findings which have reported a decrease in endothelial dependant vascular relaxation, though not stratified by BMI [1, 15]. We observed that higher BMI was significantly associated with lower tension reduction propensity even when the pathway of direct nitric oxide donation (SNP mechanism) to the smooth muscle was used. This implies that there may be a more chronic reason for the increase in endothelial dysfunction in overweight rats. This gives an in-depth characterisation of the role of BMI in endothelial dysfunction via the increase in peroxynitrite levels. These findings are similar to those of O'Brien et al. [31] who also observed an
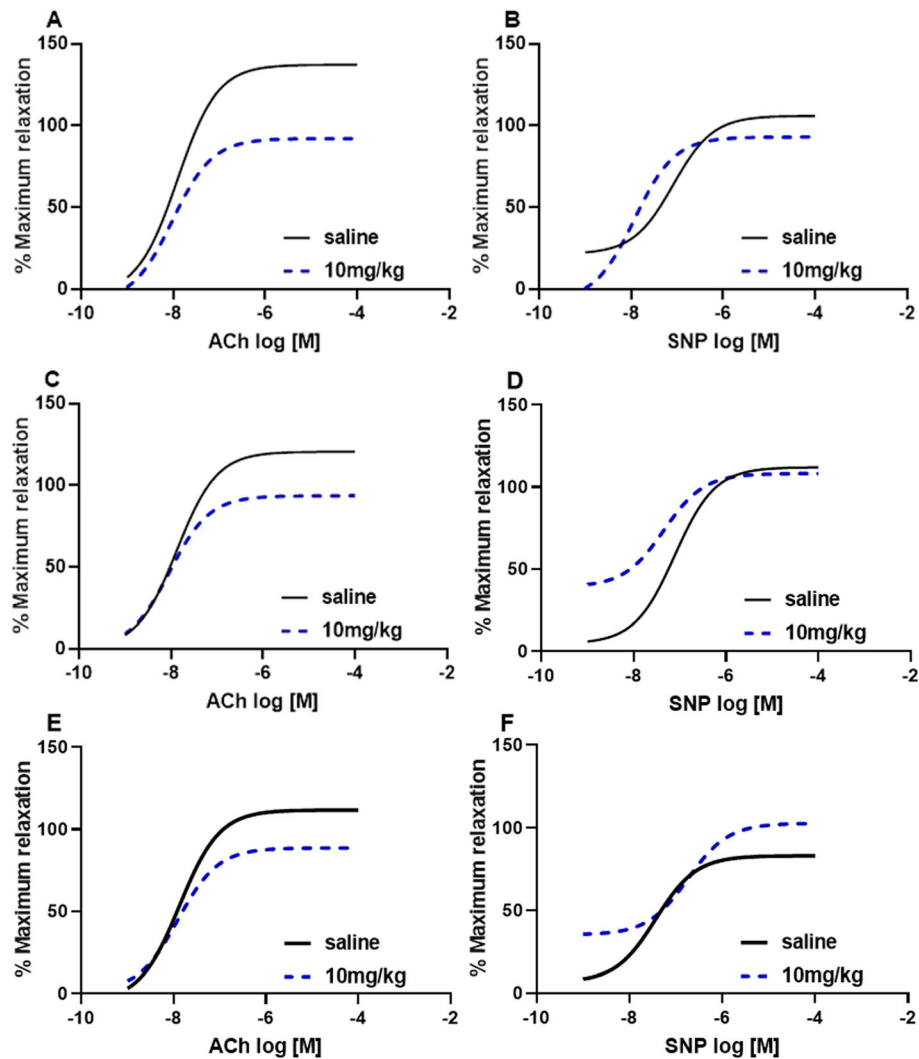
**Fig. 5** Vascular relaxation responses in isolated aortic rat rings exposed to 0 and 10 mg/kg of LPS (treatments A and D). Left side: change in vascular endothelium-dependent relaxation response to acetylcholine (ACh) rats. Right side panel: endothelium-independent relaxation response to nitric oxide donor sodium nitroprusside (SNP). **a** and **b**: underweight rats, **c** and **d**: normal weight rats, **e** and **f**: overweight rats

impairment in endothelial-dependent and endothelial-independent vascular relaxation among obese rats.

We also observed the effect of increasing peroxynitrite levels (i.e., increased nitrotyrosine levels via LPS administration at 10 mg/kg) on endothelial function in all weight groups. When the characteristics of the dose-dependent curves of untreated rats and those treated with 10 mg/kg LPS were compared, a statistically significant reduction in endothelial-dependent ability to reduce tension was observed between saline-treated rats and those treated with the highest LPS dosage. In treatment with ACh, this reduction in endothelium-dependent vascular relaxation was noted by a significant decrease in $R_{max}$. In a contracted aorta, the addition of ACh should typically result in vascular smooth muscle relaxation through the activation of protein kinase G [1, 32, 33],

leading to dilation of the blood vessel. Increase in peroxynitrite causes significant endothelial dysfunction across the spectrum of body mass, as shown by reduced $R_{max}$ in all the weight groups. The diminished response to ACh supports the idea that increased levels of peroxynitrite cause a reduction in endothelium-dependent vascular relaxation. Others have reported that exposure of rat aortic rings to high concentrations of peroxynitrite caused a marked impairment of the endothelium-dependent vascular relaxation [15, 32]. Mihm et al. [1] found that brief incubations with relevant concentrations of nitrotyrosine elicited significant impairment of ACh-induced relaxation in a concentration-dependent manner.

There was no significant change in $logIC_{50}$ in groups A and D of all the weight groups upon ACh treatment.
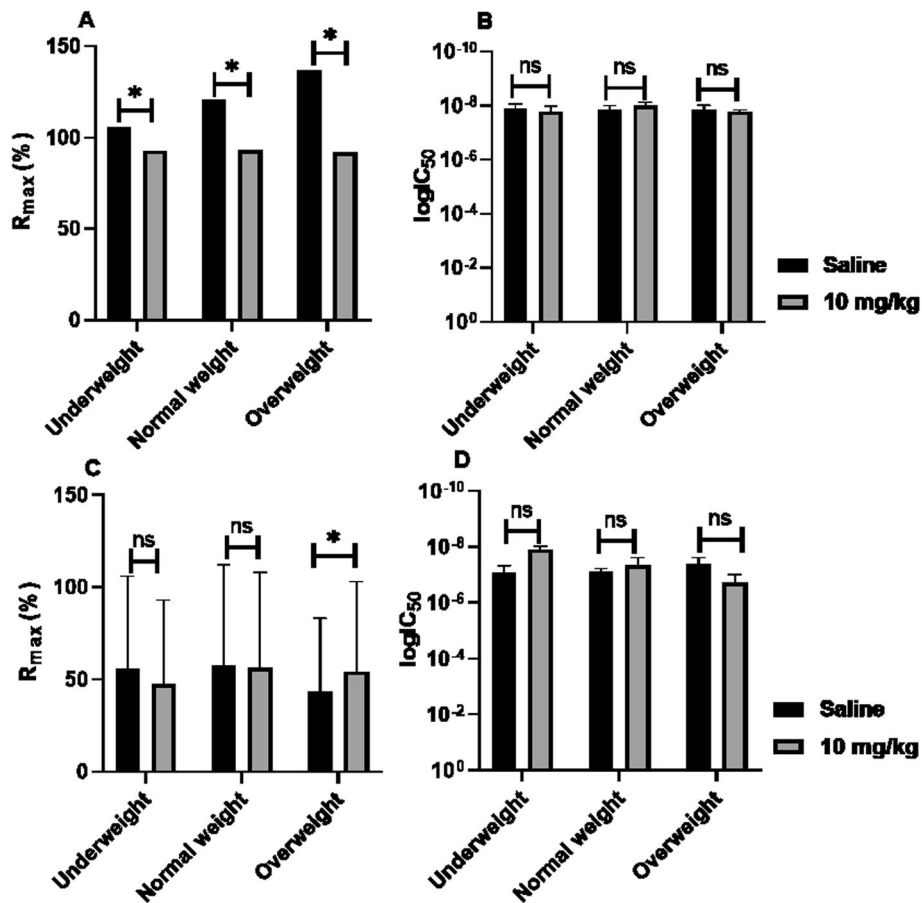
**Fig. 6** Effect of peroxynitrite on ACh (**a** and **b**) and SNP (**c** and **d**) activity on isolated aortic rat rings exposed to saline and 10 mg/kg of LPS (treatments A and D). Graphs **a** and **c** show $R_{max}$, and graphs **b** and **d** show $logIC_{50}$ means and SEM with the asterisk implying significant differences between the two treatments ($P < 0.05$) and ns standing for the non-significant difference

Other literature has shown that increased peroxynitrite impairs tension reduction, as demonstrated by an increase in $logIC_{50}$ [32]. This increase was not statistically different.

Although there were significant differences in nitrotyrosine levels between rats treated with saline vs. those treated with the highest LPS dosage, more precise levels might have been recorded if nitrotyrosine levels were measured at a different time post-LPS treatment. Furthermore, we could have recorded more accurate levels of nitrotyrosine if chromatography/mass spectrometry were used instead of ELISA.

## 6 Conclusion
Higher BMI in male Wistar rats is associated with higher plasma peroxynitrite levels. Across the spectrum of body mass, higher peroxynitrite levels are associated with impaired ability to reduce tension as measured by $R_{max}$ (endothelium-dependent vascular relaxation). As noted by the insignificant changes in tension after addition of SNP in the underweight and normal weight rats, the

pathway involved in these two groups seems to involve reduced nitric oxide bioavailability and not smooth muscle relaxation incapability. The overweight rats seem to have impairment of both endothelial-dependent vascular relaxation pathway and a more chronic vascular change that may lead to failure of smooth muscle relaxation. In our study, we explain the dose-dependent effect of plasma nitrotyrosine (peroxynitrite) levels on endothelial function and the role of BMI. This could be vital in explaining the different responses to oxidative stress that occur in human of different BMI. We recommend future studies with larger sample size should be done to confirm the reliability of our results.

## Availability of data and materials
Data will be made available upon request from the principal investigator (corresponding author)

## Ethics approval
Approval was from the University of Zambia Biomedical Research Ethics committee (UNZABREC) - REF. 004-01-18.

## Consent for publication
Not applicable.

## Competing interests
The authors declare that they have no competing interests.

## Author details
¹Department of Human Physiology, Faculty of Medicine, Lusaka Apex Medical University, Lusaka, Zambia. ²Vanderbilt Institute for Global Health and Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA. ³Department of Medicine, School of Medicine, University of Zambia, Lusaka, Zambia. ⁴School of Medicine, Cavendish University, Lusaka, Zambia. ⁵Department of Physiological Sciences, School of Medicine, University of Zambia, Lusaka, Zambia. ⁶Department of Health Policy and Management, School of Public Health, University of Zambia, Lusaka, Zambia. ⁷Division of Infectious Diseases, Vanderbilt University Medical Center, Nashville, TN, USA.

## References
1. Mihm MJ, Jing L, Bauer JA (2000) Nitrotyrosine causes selective vascular endothelial dysfunction and DNA damage. J Cardiovasc Pharmacol 36:182–187
2. Griendling KK, Alexander RW (1996) Endothelial control of the cardiovascular system: recent advances. FASEB J 10:283–292
3. Avogaro A, De Kreutzenberg SV, Fadini G (2008) Endothelial dysfunction: causes and consequences in patients with diabetes mellitus. Diabetes Res Clin Pract 82(Suppl 2):S94–S101
4. Stefano G, Goumon Y, Bilfinger T (2000) Basal nitric oxide limits immune, nervous and cardiovascular excitation: human endothelial express a mu opiate receptor. Prog Neurobiol 60:513–530
5. Klabunde RE (2008) Cardiovascular physiology concepts. In: Cardiovascular pharmacology concepts
6. Pacher P, Beckman JS, Liaudet L (2007) Nitric oxide and peroxynitrite in health and disease. Physiol Rev 87:315–424
7. Lubos E, Handy DE, Loscalzo J (2008) Role of oxidative stress and nitric oxide in atherothrombosis. Front Biosci 13:5323–5344
8. Huie RE, Padmaja S (1993) The reaction rate of nitric oxide with superoxide. Free Rad Res Commun 18:195–199
9. Kamisaki Y, Wada K, Ataka M et al (1997) Lipopolysaccharide-induced increase in plasma nitrotyrosine concentrations in rats. Biochim Biophys Acta 1362:24–28
10. Irene Guijarro-Muñoz MC, Álvarez-Cienfuegos A, Álvarez-Vallina L, Sanz L (2013) Lipopolysaccharide activates toll-like receptor 4 (TLR4)-mediated NF-κB signaling pathway and proinflammatory response in human pericytes. J Biol Chem 289(4):2457–2468
11. Chen C, Chen YH, Lin WW (1999) Involvement of p38 mitogen-activated protein kinase in lipopolysaccharide-induced iNOS and COX-2 expression in J774 macrophages. Immunology 97:124–129
12. Ashutosh Kumar S-HC, Kadiiska MB, Hong J-S, Zielonka J, Kalyanaraman B, Mason RP (2014) Inducible nitric oxide synthase is key to peroxynitrite-mediated, LPS-induced protein radical formation in murine microglial BV2 cells. Free Radic Biol Med 73:51–59 https://doi.org/10.1016/j.freeradbiomed.2014.04.014
13. Denicola A, Souza JM, Radi R (1998) Diffusion of peroxynitrite across erythrocyte membranes. Proc Natl Acad Sci U S A 95:3566–3571
14. Radi R, Beckman JS, Bush KM et al (1991) Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide. J Biol Chem 266:4244–4250
15. Szabó C, Harry I, Rafael R (2007) Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. Nat Rev Drug Discov 6:662–680
16. Kline ER, Kleinhenz DJ, Liang B et al (2008) Vascular oxidative stress and nitric oxide depletion in HIV-1 transgenic rats are reversed by glutathione restoration. Am J Physiol Heart Circ Physiol 294:H2792–H2804
17. Dickhout JG, Hossain GS, Pozza LM et al (2005) Peroxynitrite causes endoplasmic reticulum stress and apoptosis in human vascular endothelium: implications in atherogenesis. Arterioscler Thromb Vasc Biol 25:2623–2629
18. Ribiere C, Jaubert AM, Gaudiot N et al (1996) White adipose tissue nitric oxide synthase: a potential source for NO production. Biochem Biophys Res Commun 222(2):706–712
19. Maslov L, Natalia VN, Alla AB et al (2019) Is oxidative stress of adipocytes a cause or a consequence of the metabolic syndrome? J Clin Transl Endocrinol 15:1–5
20. Ivanov AV, Valuev-Elliston VT, Ivanova ON et al (2016) Oxidative stress during HIV infection: mechanisms and consequences. Oxid Med Cell Longev 2016:8910396
21. Mizoue T, Kasai H, Kubo T et al (2006) Leanness, smoking, and enhanced oxidative DNA damage. Cancer Epidemiol Biomarkers Prev 15:582–585
22. Zengi A, Ercan G, Caglayan O et al (2011) Increased oxidative DNA damage in lean normoglycemic offspring of type 2 diabetic patients. Exp Clin Endocrinol Diabetes 119:467–471
23. Sciences-Cfioom (1985) International guiding principles for biomedical research involving animals. World Health Organization. National Academies Press, Washington, D.C., United States.
24. Ahmed MR, Wale H, Garba K et al (2007) Body mass index of male and femal Wistar rats following administration of leptin hormone after a dietary regime. Ann Bioanthropoplogy 5:22–26
25. Novelli EL, Diniz YS, Galhardi CM et al (2007) Anthropometrical parameters and markers of obesity in rats. Lab Anim 41:111–119
26. Vanderbilt University Medical Center DOCP, Department of Medicine (2018) Manual. National Vanderbilt University, Nashville
27. Dmt-Normalisation-Guide (2018) In: ADInstruments
28. Bauer JA, Booth BP, Fung HL (1995) Nitric oxide donors: biochemical pharmacology and therapeutics. Adv Pharmacol 34:361–381
29. Harvey M, Christopoulos A (2004) Fitting models to biological data using linear and nonlinear regression. A practical guide to curve fitting. Oxford University Press, New York
30. Friederich JA, Butterworth JFT (1995) Sodium nitroprusside: twenty years and counting. Anesth Analg 81:152–162
31. O'brien SF, Davidge ST, Zhang Y et al (2001) Protection of vascular wall function in insulin-resistant rats from copper oxidative stress. Br J Pharmacol 133:477–484
32. Korkmaz S, Radovits T, Barnucz E et al (2009) Dose-dependent effects of a selective phosphodiesterase-5-inhibitor on endothelial dysfunction induced by peroxynitrite in rat aorta. Eur J Pharmacol 615:155–162
33. Szabo C (2009) Role of nitrosative stress in the pathogenesis of diabetic vascular dysfunction. Br J Pharmacol 156:713–727

## Publisher's Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.