Intracerebroventricular tempol administration in old rats reduces oxidative stress in the hypothalamus but does not change STAT3 signalling and SIRT1/AMPK pathway

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<td>Toklu, Hale; University of Florida College of Medicine, Pharmacology and Therapeutics; North Florida South Georgia Veterans Health System, Geriatric Research Education Clinical Center</td>
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Intracerebroventricular tempol administration in old rats reduces oxidative stress in the hypothalamus but does not change STAT3 signalling and SIRT1/ AMPK pathway

Hale Z. Toklu$^{1,2,\ast}$, Philip J. Scarpace$^{2,\ast}$, Yasemin Sakarya$^{2}$, Nataliya Kirichenko$^{1,2}$, Michael Matheny$^{2}$, Erin B. Bruce$^{2}$, Christy S. Carter$^{3}$, Drake Morgan$^{4}$, Nihal Tümer$^{1,2,\ast}$

1) Geriatric Research Education & Clinical Center, Malcolm Randall Veterans Affairs Medical Center, Gainesville, FL, USA

University of Florida Departments of

2) Pharmacology & Therapeutics

3) Aging and Geriatric Research

4) Psychiatry

Correspondence: Nihal Tümer, PhD.

Geriatric Research Education & Clinical Center, Malcolm Randall Veterans Affairs Medical Center, Gainesville, 32608 FL, USA

ntumer@ufl.edu

Tel: 352-376-1611 Ext: 6575

Fax: 352-374-6142

Hale Zerrin Toklu, PhD

haletoklu@yahoo.com
Abstract

Hypothalamic inflammation and increased oxidative stress are believed to be contributory mechanisms underlying obesity. Tempol, a free radical scavenger, has been shown to reduce inflammation and oxidative stress. We hypothesized that brain infusion of tempol would reduce oxidative stress, and thus reduce food intake and body weight, and improve body composition in aged-related obese rats with known elevated oxidative stress. Further, we predicted an associated increase in markers of leptin signalling, including the SIRT1/AMPK and STAT3 pathways. For this purpose, mini osmotic pumps were placed in the intracerebroventricular region for the continuous infusion of tempol or vehicle for two weeks in young (3 months) and aged (23 months) male F344xBN rats. Tempol significantly decreased (p<0.01) NADPH oxidase activity in the hypothalamus but failed to reduce food intake or weight gain and did not alter body composition. SIRT-1 activity and Acetyl p53 were decreased and pAMPK increased with age, but were unchanged with tempol. Basal pSTAT3 was unchanged with age or tempol. These results indicate that tempol decreases oxidative stress, but fails to alter feeding behaviour, body weight, or body composition. Moreover, tempol does not modulate the SIRT1/AMPK/p53 pathway and does not change leptin signalling. Thus, a reduction in hypothalamic oxidative stress is not sufficient to reverse age-related obesity.

Keywords: tempol; icv; brain; hypothalamus; aging; obesity; SIRT1; AMPK; leptin; p53; FOXO; STAT3; oxidative stress
Introduction

The hypothalamus is a critical brain region that regulates food intake, body weight and glucose homeostasis. The hypothalamus directly senses and controls the metabolic signals from the periphery and projects neuronal inputs to the corresponding tissues and organs, accordingly. In recent years, abundant research has demonstrated that normal reactive oxygen species (ROS) levels produced in mitochondria are vital physiological sensors for hypothalamic glucose and fatty acids (Benani et al. 2007). ROS are chemically reactive molecules containing oxygen. They are formed as a natural by-product of the normal metabolism of O\textsubscript{2} and have important roles in cell signalling and homeostasis. However, the excessive production of O\textsubscript{2} radicals, superoxide, H\textsubscript{2}O\textsubscript{2}, nitric oxide, and peroxynitrite cause excitotoxicity and impair the energy metabolism of cells. Indeed, elevated ROS production causes cellular dysfunction and death (Gyengesi et al. 2012). The endogenous antioxidant system i.e. glutathione peroxidase, superoxide dismutase, catalase, and uric acid serve to convert/ neutralize these ROS to less toxic derivatives, thus preventing the reaction of ROS with DNA, RNA, lipids or proteins. The production of excessive amounts of ROS depletes the endogenous antioxidants resulting in the increased peroxidation of membrane lipids or oxidation of proteins leading to DNA fragmentation and inhibition of the mitochondrial electron transport system (Devasagayam et al. 2004). Due to the delicate balance required for efficient cellular function, increased ROS production is thought to be a causative factor in multiple disease states including obesity, and is also regarded as a possible cause of aging (Bonomini et al. 2015).
Within the hypothalamus there are several important nuclei regulating appetite and body weight, including the lateral hypothalamic area (LH), the paraventricular (PVN), the dorsomedial (DM), the ventromedial (VMH), and the arcuate hypothalamic nuclei (Arc). Some neurotransmitters have been shown to stimulate food intake and increase body weight while some others have an anorectic effect (Ahima and Antwi 2008). Several studies indicate that AMPK in the hypothalamus regulates energy metabolism by integrating inputs from hormones, peptides, neurotransmitters, and nutrients. Leptin, synthesized in adipose tissue, is one of the most important peptides involved in energy homeostasis and this hormone communicates to the hypothalamus and other important reward centers the nutritional/satiety state. The anorectic effect of leptin occurs via the increased phosphorylation of STAT3 (pSTAT3) as well as its interaction with AMPK/SIRT1 pathway (Figure 1). In the hypothalamus, leptin inhibits AMPK; thus leading to decreased food intake and increased energy expenditure (Lim et al. 2010). SIRT-1 and AMPK activity have been shown to control intracellular energy balance, and SIRT-1, in particular, affects longevity (Lim et al. 2010). SIRT-1 expression decreases FOXO1 acetylation suggesting that SIRT-1 regulates the central melanocortin system in a FOXO1 dependent manner. In addition, hypothalamic Sirt1 regulates S6K (mTOR pathway) signalling such that, inhibition of the fasting induced Sirt1 activity results in up-regulation of the S6K pathway (Cakir et al. 2009). Impaired hypothalamic leptin signalling (leptin resistance) is associated with increased adiposity and obesity. Furthermore, leptin resistance increases with age, as does the incidence of obesity.

Hypothalamic inflammation and increased oxidative stress is thought to be one of the underlying mechanisms of obesity (Cai and Liu 2012; Williams 2012). Moreover, aged rats demonstrate an
even greater increase in oxidative stress (Erdoes et al. 2011). Therefore, we hypothesized that central infusion of tempol, a free radical scavenger, would reduce oxidative stress in the hypothalamus of aged-obese rats, and potentially decrease food intake/ body weight via restoration of the AMPK/ SIRT1 dependent pathway or the leptin coupled pSTAT3 pathway.

Methods

Animals

Male Fischer 344 x Brown Norway (F344xBN) rats 3 and 23 month old (N= 5/group) were obtained from the National Institute on Aging Colony at Harlan Laboratories (Indianapolis, IN, USA). These rats constitute a good model for aging studies (Wolden-Hanson 2006, Tümer et al. 2014). Animals were cared for in accordance with the principles of the Guide to the Care and Use of Experimental Animals; and protocol (#201203230) was approved by the University of Florida Institutional Animal Care and Use Committee. Rats were maintained on a 12:12 hour light-dark cycle and provided a standard rodent chow (17% kcal from fat, 25% kcal from protein, no sucrose, 3.1 kcal/g, diet 7912, Harlan Teklad; (Madison, WI) and water *ad libitum* throughout the experimental protocol.

Surgical procedure and treatment

Rats were anesthetized with isofluurane (2-3%) and their heads were prepared for surgery. Animals were placed into a stereotaxic frame and a small incision (1.5 cm) was made over the midline of the skull to expose the landmarks of the cranium (bregma and lambda). The following coordinates were used for injection into the third ventricle: 1.1 mm posterior to bregma and 1.6 mm ventral from the skull surface on the midline (medial fissure), with the nose bar set at 4 mm
below the ear bars (below zero). A small hole was drilled through the skull and a 23-gauge stainless steel guide cannula was placed into the third ventricle. 2400 mg of tempol was dissolved in 4 ml saline. Either ACSF (artificial cerebrospinal fluid) or tempol was infused (5µg/min) by an Alzet osmotic mini pump (Alza, Palo Alto, CA) into the lateral ventricle via an implanted cannula as described previously (Scarpace et al. 2007). This maintained a tempol dose of 300 µg/h. The tempol dose was based on our previous study (Erdos et al. 2006) and other studies (Kang et al. 2009; Xue et al. 2011). The cannula is attached to the mini pumps by a polyethylene (PE)50 tubing long enough to implant on the back, between or slightly posterior to the scapulae. A small incision is made at the base of the neck and a subcutaneous pocket to receive the pump is created by blunt dissection. All the pumps are all filled with ACSF. After a recovery and equilibrium period of a week, the dummy pumps are replaced with saline or tempol containing pumps and brain infusion is continued for 2 more weeks (Figure 2).

Food intake, energy intake and delta body weight

Rats were housed individually and food consumption and body weight were recorded in grams daily throughout the experiment.

Body composition of fat and lean mass

Body composition was assessed using time-domain nuclear magnetic resonance (TD-NMR) in restrained but fully conscious rats (TD-NMR Minispec, Bruker Optics, The Woodlands, TX, USA) 2 days before and 2 weeks after the pump implantation.

Serum leptin
Enzyme immunoassays were used to determine the levels of leptin (rat leptin ELISA kit, EZRL-83K; Milipore, Massachusetts, USA). Leptin was assayed in fed state blood that was collected at death.

**Tissue harvesting and preparation**

Rats were euthanized with isofluorane. The circulatory system was perfused with 20 ml of ice-cold saline, and perirenal and retroperitoneal white adipose tissues (PWAT and RTWAT, respectively), brown adipose tissue (BAT), and hypothalamus were excised. The hypothalamus was removed by making an incision medial to piriform lobes, caudal to the optic chiasm and anterior to the cerebral crus, to a depth of 2 to 3 mm. The hypothalamus was sonicated in 50 mMTris-HCl, pH 6.8, plus protease inhibitors. Protein concentrations were determined using the DC protein assay kit (Bio-Rad, Hercules, CA).

**NADPH oxidase activity**

NADPH oxidase activity was measured with a lucigenin-enhanced chemiluminescence assay using hypothalamus homogenates. The micro-plate containing approximately 15µg of hypothalamus homogenates was maintained at 37°C while luminescence was recorded using a micro-plate reader. Relative light units were obtained for 30 min in the presence of NADH (474 µm) and lucigenin (218 µM), and background-corrected values were normalized to protein content.

**Western Blot Analysis**

Briefly, an equal amount of protein for each sample was separated by 10-12.5% SDS-PAGE for 1 hour at 100 mA. After electrophoresis, the proteins were transferred to nitrocellulose...
membranes and blocked with 5% skimmed milk in Tris-buffered saline containing 0.1% Tween 20. All membranes were incubated overnight at 4°C with primary antibody. Immunoreactivity was visualized by ECL Plus detection system (GE Healthcare, Piscataway, NJ) and quantified by ImageQuant TL (GE Healthcare).

**NADPH subunits**

NADPH oxidase subunits (phox p47, p67 and gp91) were assessed by Western blot assays. 10 µg (for phox p47), 5 µg (for p67) and 15µg (for gp91) protein were loaded and antibodies (all from Millipore, Billerica, MA) were used in 1/2000 concentration.

**Total antioxidant capacity**

Total antioxidant capacity in whole brain homogenates was measured using the total antioxidant capacity kit (Abcam, Cambridge, UK, catalog number: ab65329) according to the manufacturer's instructions. Briefly, plasma was allowed to reduce Cu²⁺ for 1.5 h at room temperature. Reduced Cu⁺ was chelated with a colorimetric probe and absorbance was measured at 570 nm. Results were expressed as trolox equivalent according to a trolox standard curve.

**Superoxide dismutase (SOD)**

Superoxide Dismutase (SOD) activity in whole brain homogenates was determined using a commercial kit (Abcam, ab65354). SOD activity was determined by using xanthine oxidase method based on O2⁻ generation. The rate of the reduction with a superoxide anion is linearly related to the xanthine oxidase (XO) activity and is inhibited with SOD. The inhibition activity of SOD was determined by a colorimetric method.
**Catalase**

Catalase was assessed to determine antioxidant capacity in hypothalamus. 6 µg protein was loaded and antibody (Anti-Catalase; EMD Chemicals) was used in 1/2000 concentration.

**Reduced total glutathione (GSH) assay**

Glutathione measurements were performed using a modification of the Ellman procedure (Beutler et al. 1963). Briefly, after centrifugation at 2000 g for 10 min, 0.5 ml of supernatant was added to 2 ml of 0.3 mol/l Na₂HPO₄·2H₂O solution. A 0.2 ml solution of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) (0.4 mg/ml 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. The results are expressed as µmol GSH/g tissue.

**AMPK pathway/ SIRT-1 activity**

Total AMPK, pAMPK, AcP53, FOXO1 and SIRT-1 were assayed by Western blot analysis. 20 µg (for AMPK, pAMPK and FOXO1), 10 µg (for AcP53), 40µg (for SIRT-1) protein were loaded and antibodies (AMPK, pAMPK, FOXO1, SIRT-1 & Cell Signaling, Danvers, MA; AcP53 & Abcam, Cambridge, MA) were used in 1/1000 (AMPK, pAMPK and SIRT-1), 1/2000 (AcP53, FOXO1) concentrations.

**Leptin signalling**

Leptin signalling was assessed by total STAT and pSTAT protein which were assayed by Western blot analysis. 60 µg (for pSTAT) and 30 µg (for STAT) protein was loaded and antibodies (Cell Signaling, Danvers, MA) were used in 1/5000 (STAT), 1/800 (pSTAT) concentrations.
Statistical analysis

Statistical analysis was carried out using GraphPad Prism 5.0 (GraphPad Software, San Diego; CA; USA). All data were expressed as means ± SEM. Groups were compared with Kruskal Wallis test followed by Dunn’s test for multiple comparison test or Student’s t-test. Values of p<0.05 were considered as significant.

Results

Prior to tempol treatment, body weight was different across age (p<.001), but not within age groups (Figure 3). Treatment with tempol did not affect food intake or body weight when compared with their respective control. Also, the cumulative food consumption was not significantly different among groups.

Adipose tissue weight at death, as expected, was greater in the aged compared with young animals, but there was no influence with tempol treatment (Table 1). Similarly, brown adipose tissue (BAT) was more abundant in aged versus young rats with no change with tempol. When percent whole body lean mass and fat mass were examined by TDNMR, both measures were significantly higher in old compared with young rats, but again, there was no difference with tempol treatment (Table 1).

NADPH oxidase catalyzes the one electron reduction of oxygen into superoxide using either NADPH or NADH as the electron donor. Aged controls had significantly higher level of NADPH oxidase enzyme and this was partially reversed by icv tempol treatment. However, subunits of NADPH oxidase, p-47, p67 and gp-91 were not significantly different among groups (Figure 4).
Total antioxidant capacity and superoxide dismutase activity were determined in whole brain, and antioxidant enzyme levels, including catalyse enzyme and total reduced glutathione, were determined in the hypothalamus. With age, there was a decline in the total antioxidant capacity and superoxide dismutase activity in the brain. Antioxidant capacity was significantly elevated by tempol treatment in old rats, while increase in SOD activity was not significant (Figure 5 a and b). Hypothalamic catalase levels were significantly lower in old rats (Figure 5c), however tempol failed to reverse this decline. Total GSH levels in hypothalamus were not different among groups (Fig. 5d).

At death, several signalling molecules that are normally affected by feeding were examined. The rats were euthanized in the early morning, during a time when feeding is normally minimal, thus these baseline values reflect this non-food consumption state.

SIRT1 was significantly lower (p<0.001) in the hypothalamus of old rats. On the other hand, tempol treatment failed to reverse this decline (Figure 6a).

No significant change was detected in FOXO1 protein, whereas acP53 was significantly (p<0.05) lower in old controls. Tempol treatment also did not have any effect on either of these signalling molecules (Fig 6b, c).

The baseline pAMPK levels were increased (p<0.05) with age in the hypothalamus, whereas total AMPK levels remained unchanged. Thus, pAMPK/ AMPK ratio was also significantly higher in old control. Tempol treatment prevented the increased pAMPK and pAMPK/AMPK ratio with age (Fig 6d, e, f).

*Intracerebroventricular tempol administration in old rats reduces oxidative stress in hypothalamus but does not change STAT3 signalling and SIRT1/ AMPK pathway*
Basal levels of pSTAT and total STAT protein were not significantly different among age groups and were not affected by tempol treatment (Fig. 7).

**Discussion**

Hypothalamic inflammation and increased oxidative stress are thought to be two mechanisms underlying obesity and aging (Cai and Liu 2012; Williams 2012; Erdos et al. 2011). Tempol, (4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl), a free radical scavenger, has been shown to decrease sympathetic activity in brain (Lu et al. 2004); and possess neuroprotective effects in various models of brain injuries including stroke (Wilcox 2010; Hall et al, 2010; Dohare et al. 2014). It has been proposed that tempol's neuroprotective efficacy is largely due to its ability to catalytically scavenge peroxynitrite radicals (Xiong et al. 2009). In the present study we hypothesized that central infusion of tempol, a free radical scavenger, would reduce oxidative stress in the hypothalamus and decrease food intake/ body weight by affecting leptin signalling through the AMPK/ SIRT1 dependent pathway or pSTAT3 pathway in rats with aged-related obesity. For this purpose, we administered tempol at a dose of 5 µg/ min as a continuous infusion for 2 weeks. In other studies (Kang et al. 2009; Xue et al. 2011), demonstrated that the effective dose ranged between 1.3 -8.6 µg/ min. Therefore, our dose may be considered as a medium dose.

However, our results indicate that centrally administered tempol neither affected food intake nor body composition (lean and fat mass), despite, the significantly decreased oxidative stress in hypothalamus in old rats by tempol treatment. There are three categories of antioxidant species: enzyme systems (GSH reductase, SOD, catalase, peroxidase, etc.), small molecules (ascorbate, uric acid, GSH, vitamin E, etc.) and proteins (albumin, transferrin, etc.). Total antioxidant
capacity measurement can detect combination of both small molecule antioxidants and proteins or small molecules. Measurement of the combined non-enzymatic antioxidant capacity provides an indication of the overall capability of the tissue to counteract reactive oxygen species, resist oxidative damage and combat oxidative stress-related diseases.

In the present study, catalase activity, which is responsible for the conversion of hydrogen peroxide to water and oxygen, was slightly increased in young with tempol; but there was no effect in old rats. On the other hand, in aged rats, tempol reduced NADPH oxidase, a marker for oxidative stress, in the hypothalamus. Both total SOD enzyme activity and the antioxidant capacity of the whole brain also decreased significantly with age. Tempol treatment significantly increased brain antioxidant capacity in old rats, whereas a slight, non-significant increase was observed in SOD. Other researchers have shown that tempol treatment was effective in increasing SOD activity and GSH activity in young brains (Ali et al. 2016). However, our findings did not confirm any increase in antioxidant enzymes in either brain or hypothalamus. On the other hand, we detected an increase total antioxidant capacity, which is mainly conferred by non-enzymatic antioxidants. Our findings can be interpreted as tempol’s failure to increase the enzymatic antioxidants in aged brain. The possible reason for this failure may be the irreversible decrease in antioxidant enzyme activity that cannot be restored. Hence, early and / or long-term treatment can be the key. Overall, tempol treatment reduced oxidative stress, by enhancing antioxidant system, but failed to alter feeding behaviour, body weight, or body composition, suggesting oxidative stress has a minimal role in aged-related obesity in rats.

Our hypothesis was based on the idea that decreasing oxidative stress would improve aging related obesity and impaired hypothalamic signalling in brain. Aging, however, is a
multifactorial process. Besides the increased oxidative stress, a decrease in SIRT-1 and AMPK activity have been shown to control intracellular energy balance and affect longevity (Lim et al. 2010). Cell cycle regulation by AMPK is mediated by inhibition of the mTOR (mammalian target of rapamycin) pathway as well as the upregulation of the p53, FOXO1/ FOXO3a axis. The mTOR pathway is a major controller of protein biosynthetic processes (Wang et al. 2011). It was previously shown that pAMPK levels decrease in peripheral tissues with age (Reznick et al. 2007); however, it seems this is not the case in the brain. Previously, Liu et al. (2012) reported that baseline levels of phosphorylated AMPK were higher in aged mice brains compared to young (Liu et al. 2012). In keeping with Liu et al, 2012, our study demonstrates significantly higher pAMPK in old control vs young control rats, though all groups had similar total AMPK levels.

Recently, studies focused on the relation between SIRT-1, AMPK and p53 in terms of caloric restriction and longevity. P53 is a tumor suppressor protein and regulates autophagy. P53 interacts with SIRT1 and mTOR pathways (Paraiso et al. 2013; Lee and Gu 2013; Salmien et al. 2013; Duan et al. 2013; Carling 2004). A stress signal like glucose starvation or DNA damage rapidly activates p53 and AMPK. Increases in AMPK can also induce p53. Increased p53 levels results in mTOR inhibition, decreased levels of pS6K, and activation of autophagy (Tucci 2012). Another downstream marker in this pathway is FOXO1 and its expression in brain was previously shown to decrease with aging in frontal, parietal, occipital cortex and hippocampus (Zemva et al. 2012). In the present study, FOXO1 levels in the hypothalamus displayed a variation among groups, although without significance. Moreover, acetyl p53 levels were significantly higher in the old rats and tempol had a tendency to increase the levels in old rats.
We also evaluated the pSTAT3/STAT3 levels, a marker for leptin signalling; because we previously demonstrated that leptin receptor blockade disrupts body weight regulation (Matheny et al. 2014). Although there was a tendency for decline of leptin signalling in old versus young, neither baseline levels, nor levels after tempol treatment were significantly different. In conclusion, these results indicate that although tempol decreases oxidative stress, it fails to modulate leptin-signalling pathways. Moreover, tempol fails to alter feeding behaviour, body weight, or body composition. Thus, a reduction in hypothalamic oxidative stress is not sufficient to reverse age-related obesity.

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**Conflict of interest:** None

**References**


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Table 1: Serum leptin and distribution of fats, lean and fat mass % of young and old rats treated with vehicle or tempol. Results are presented as mean ±sd. P<0.001 old vs young

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<tr>
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<th>Young Tempol</th>
<th>Old Control</th>
<th>Old Tempol</th>
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<td>Serum leptin</td>
<td>9.06 ± 1.49</td>
<td>7.75 ± 0.76</td>
<td>17.03 ± 1.00*</td>
<td>19.53 ± 2.13*</td>
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<td>RTWAT (g)</td>
<td>2.85 ± 0.16</td>
<td>3.07 ± 0.70</td>
<td>8.53 ± 1.00*</td>
<td>9.21 ± 0.47*</td>
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<td>PWAT (g)</td>
<td>0.87 ± 0.07</td>
<td>0.83 ± 0.16</td>
<td>1.67 ± 0.12*</td>
<td>1.72 ± 0.13*</td>
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<td>EWAT (g)</td>
<td>3.78 ± 0.17</td>
<td>4.36 ± 0.73</td>
<td>9.75 ± 0.90*</td>
<td>10.09 ± 0.27*</td>
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<tr>
<td>Sum of WATs (g)</td>
<td>7.50 ± 0.32</td>
<td>8.26 ± 1.57</td>
<td>19.96 ± 1.88*</td>
<td>21.03 ± 0.47*</td>
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<td>BAT (g)</td>
<td>0.35 ± 0.01</td>
<td>0.35 ± 0.02</td>
<td>0.64 ± 0.06*</td>
<td>0.65 ± 0.04*</td>
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<td>Lean mass %</td>
<td>Pre 59.66 ± 1.1</td>
<td>59.23 ± 0.3</td>
<td>56.43 ± 0.9*</td>
<td>56.45 ± 0.7*</td>
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<td></td>
<td>Post 58.98 ± 1.3</td>
<td>58.61 ± 1.1</td>
<td>54.38 ± 0.8*</td>
<td>54.82 ± 1.0*</td>
</tr>
<tr>
<td>Fat mass %</td>
<td>Pre 24.61 ± 0.7</td>
<td>23.49 ± 1.4</td>
<td>27.59 ± 0.6*</td>
<td>28.22 ± 0.4*</td>
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<tr>
<td></td>
<td>Post 24.83 ± 0.6</td>
<td>25.41 ± 1.3</td>
<td>27.92 ± 0.7*</td>
<td>28.41 ± 0.7*</td>
</tr>
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Fig. 1: Aging is a complicated process, often characterized by increased reactive oxygen species (ROS) and inflammation. Whether this increase is a cause or effect is still unclear. Furthermore, there is evidence that aging and increased ROS production leads to impaired FOXO1, AMPK, and SIRT1 signaling, possibly leading to a decrease in catabolic pathways promoting obesity. Furthermore, leptin resistance is associated with aging, and may further dampen catabolic signaling, which may further inflammation and ROS production.

Fig. 2: The schematic representation of the experimental protocol

Fig. 3: Body weight (BW), delta BW, food intake and cumulative food intake after the pump change in vehicle or tempol treated young and old rats

Fig. 4: NADPH oxidase activity and subunits in the hypothalamus of vehicle or tempol treated young and old rats. *: P<0.05 vs young control; ++: p<0.01 old control vs old tempol

Fig. 5: a) Total antioxidant capacity; b) SOD activity in brain; c) catalase, and d) reduced glutathione (GSH) levels in hypothalamus of vehicle or tempol treated young and old rats. *: P<0.05 old control vs young control; +: p<005 old control vs old tempol

Fig. 6: Cellular signalling markers of SIRT 1- AMPK pathway in the hypothalamus of vehicle or tempol treated young and old rats. P<0.05 vs young control

Fig. 7: Leptin signalling markers in the hypothalamus of vehicle or tempol treated young and old rats.
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Fig. 2: The schematic representation of the experimental protocol

236x100mm (150 x 150 DPI)
Fig. 3: Body weight (BW), delta BW, food intake and cumulative food intake after the pump change in vehicle or tempol treated young and old rats
Fig. 4: NADPH oxidase activity and subunits in the hypothalamus of vehicle or tempol treated young and old rats. *: P<0.05 vs young control; ++: p<0.01 old control vs old tempol

178x144mm (300 x 300 DPI)
Fig. 5: a) Total antioxidant capacity; b) SOD activity in brain; c) catalase, and d) reduced glutathione (GSH) levels in hypothalamus of vehicle or tempol treated young and old rats. *: P<0.05 old control vs young control; +: p<005 old control vs old tempol.
Fig. 6: Cellular signalling markers of SIRT 1-AMPK pathway in the hypothalamus of vehicle or tempol treated young and old rats. P<0.05 vs young control

164x243mm (150 x 150 DPI)
Fig. 7: Leptin signalling markers in the hypothalamus of vehicle or tempol treated young and old rats.

96x245mm (150 x 150 DPI)