Cardioprotective Effects of Metformin in Ovariectomized Albino Wistar Rats

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Abstract

The current study used a human-equivalent therapeutic dose of metformin to address cardioprotective properties of the drug in ovariectomized rats. Sixty adult female rats were divided equally into four groups. Animals in Group 1 were sham-operated (n = 15), and rats in Groups 2–4 (n = 45) were ovariectomized. After one month, animals Groups 3 and 4 were treated with E₂ (100µg/kg, i.m., every other day, n = 15) and MF, (100 mg/kg/day orally, n = 15), respectively. These treatments continued for one month. Ovariectomized rats (Group 2) showed significant elevation in serum heart enzyme markers. MF-treated animals (Group4) showed a superior response to treatment compared to E₂ treated rats (Group 3) in restoring enzyme levels of CK-MB and LDH toward control levels. Compared to Group 2 animals, MF and E₂-treated rats showed a significant decrease in serum angiotensin II, cardiac MDA, and NOx levels and a significant increase in cardiac CAT activity and Total antioxidant capacity. Group 2 animals showed dyslipidemia. MF treatment renormalized lipid profile markers, but E₂ treatment did not induce a significant effect. Group 2 animals also showed significant elevation in proinflammatory markers. Both MF and E₂ treatment significantly decrease levels of TNF-α and IL-6. A focus on cardiac dysfunction markers, dyslipidemia and cardiac oxidative insult allowed the demonstration that MF is superior to E₂ for attenuating cardiotoxicity. Further, MF and estradiol are similar in their ability to mitigate inflammation.

Introduction

Cardiovascular disease (CVD) results from multiple metabolic disturbances and pathophysiological dysfunctions. Dyslipidemia, a disorder in lipid metabolism, leads to elevated low-density lipoprotein – cholesterol (LDL-C) that subsequently induces lipid peroxidation and overproduction of reactive oxygen species (ROS)(Levitan, Volkov and Subbaiah, 2010; Parthasarathy *et al.*, 2010; Ito, Sono and Ito, 2019; Yamada *et al.*, 2020). Several studies document that cardiovascular disease is associated with low concentrations of antioxidant enzymes(Chrysohoou *et al.*, 2007; Murr *et al.*, 2009). An imbalance in free radical production and antioxidant enzymes that metabolize ROS leads to oxidative stress. This stress is implicated in many pathological dysfunctions, such as activation of immune cells, enhancement of inflammatory cytokine production, induction of endothelial dysfunction leading to myocardial infiltration, and disturbance of cell membrane permeability, leading to cardiac enzyme leakage. Further, elevated ROS may affect the renin-angiotensin axis via elevated levels of angiotensin II. Excess angiotensin leads to hypertension(Manabe *et al.*, 2005; Yang *et al.*, 2007; Benigni, Cassis and Remuzzi, 2010; Mangge, 2014; Mittal *et al.*, 2014).

CVD is associated with menopause worldwide, especially in developing countries. The disease is a leading cause of morbidity and mortality(Roth *et al.*, 2018; Zhu *et al.*, 2019). Estradiol (E₂) is commonly used after menopause as hormonal replacement therapy. Estradiol displays antioxidant and anti-atherosclerotic activity and may attenuate inflammation caused by ischemia/perfusion injury. It may also

act as a short-term vasodilating agent (Mendelsohn and Karas, 1999; Xu *et al.*, 2006). However, (E_2) treatment may induce undesirable adverse side-effects, such as cancer (Brusselsaers *et al.*, 2018).

Metformin (MF), an insulin sensitizer, is an oral antihyperglycemic diguanide drug. It is commonly used for management of type 2 diabetes mellitus (T2DM), mostly in obese patients (Nasri and Rafieian-Kopaei, 2014). Several studies report that high doses of MF show favorable cardiovascular effects, such as improvement in vascular dysfunction and reduction in vascular oxidative stress (Trott *et al.*, 2011; Lobato *et al.*, 2012; Oliveira *et al.*, 2014). The maximum human therapeutic dose of MF is not to exceed 2550 mg/day. The low effective dose is 500 mg twice daily (UK Prospective Diabetes Study (UKPDS) Group, 1998). An allometric dose scaled from humans to rats is 100 mg/kg/day This dose was used in the current study to represent a daily low human therapeutic dose (Nair and Jacob, 2016).

The present study aimed to assess effects of a low dose of MF on biochemical dysfunction markers of CVD in ovariectomized rats in comparison with estradiol typically used for hormonal replacement therapy.

Materials And Methods

Chemicals

17 β -estradiol (E_2) was purchased from Sedico Pharmaceutical Company; Giza, Egypt. E_2 was dissolved in olive oil to prepare a dose of 100 μ g/kg. Metformin (MF) powder was purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). MF was freshly dissolved distilled water to prepare a dose of 100 mg/kg. All other chemicals and reagents were analytical grade.

Animals

Forty adult Wister female albino rats weighing 250–280 g, were obtained from the National Organization for Drug Control and Research. Animals were acclimated for two weeks at 25°C $\pm$ 2°C and 55% $\pm$ 5% humidity under a 12 h light/dark cycle and allowed free access to a standard diet and water. before starting the experiment. All experimental protocols were approved by the Institutional Animal Ethics Committee at the National Organization for Drug Control and Research (Approval no. 8/231/2019). Procedures followed the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996).

Experimental design

Animals were randomly allocated into four groups of 15: Group 1 included sham (SH) operated rats. Animals in the remaining Groups (2–4) ovariectomized under aseptic conditions. Rats in Groups 1 and 2, received intramuscular injection of olive oil every other day. One month after surgery, rats in Groups 3 and 4 treated as follows, Group 3I (OVX + E_2) received intramuscular injections of E_2 dissolved in olive oil at a dose of 100 μ g/kg, every other day (Nomikos *et al.*, 1987). Group 4 rats (OVX + MF) received oral doses of MF 100 mg/kg every second day. (Wang *et al.*, 2011). All treatments were conducted for one month.

Rats were anesthetized with 60 mg/kg thiopental. Ovariectomy was carried out by removing the bilateral ovaries under aseptic conditions (Lasota and Danowska-Klonowska, 2004). All treatments were performed after a one-month postoperative recovery period.

Preparation of cardiac homogenate for biochemical assays

After treatments, all rats were decapitated under mild anesthesia. Blood samples were collected from the retro-orbital vein from all animals into serum preparation tubes. Sera samples were divided into aliquots and stored at -20°C for further biochemical evaluation. Hearts were immediately removed and washed in ice-cold saline. Ten hearts per group were weighed and homogenized in ice-cold potassium chloride (KCl, 1.15%) using a glass homogenizer to yield 20% homogenates for analyses of antioxidant parameters.

Determination of serum estradiol level

Serum estradiol was evaluated using an ELISA kit purchased from BioSource co., Ltd., CA, USA.

Determination of serum biochemical risk factors for CVD:

Serum activity of lactate dehydrogenase (LDH) and creatine kinase (CK-MB) were measured using commercial kits purchased from Reactivos GPL Barcelona, Spain. Serum total cholesterol (TC), HDL-C, and TG were assessed using kits purchased from Biodiagnostic, Egypt. Low-density lipoprotein (LDL-C) was calculated as: $\text{LDL-C} = \text{TC} - \text{HDL-C} - \text{TG}/5$ (Friedewald, Levy and Fredrickson, 1972). Atherogenic index 1 and atherogenic index 2 were calculated for as TC/HDL-C and LDL-C/HDL-C ratios, respectively (Cai *et al.*, 2017). Tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and angiotensin II (ANG-II) were quantified using ELISA kits purchased from Cusabio, Germany.

Determination of antioxidant/oxidant parameters in cardiac tissues

Antioxidant and oxidant parameters were evaluated in cardiac tissue homogenate. Catalase activity was estimated following the method in Claiborne (Claiborne, 1985). Total antioxidant capacity (TAC) was evaluated using a commercial kit supplied by Biodiagnostic, Cairo, Egypt. Malondialdehyde (MDA) was assayed following the method by Mihara and Uchiyama, 1978. Total nitrates (NO_x) were determined using the method by Green *et al.*, 1982.

Statistical analysis

Statistical analyses were used data from ten samples for each parameter ($n = 10$). Data are reported as mean $\pm$ SD. Statistical significance was assessed using one-way analysis of variance followed by Tukey's multiple comparison test. $p < 0.05$ was considered significant. Data analyses used GraphPad Prism7 (GraphPad Software, San Diego CA, USA).

Results

Effect of treatment on serum estradiol (E₂) levels and serum CK-MB and LDH enzyme activities

OVX-treated animals (Group 2) displayed significantly low levels of serum E₂, along with a significant increase in CK-MB and LDH activities, compared to SH rats (Group 1) (Table 1). OVX- MF-treated rats (Group 3) showed more normal levels of serum estradiol and LDH enzyme activity. Moreover, MF reduced the increase in CK-MB enzyme activity compared to Group 2 animals. Group 4 rats also showed a significant increase in serum E₂ level rats compared with animals in Groups 1 and 2. Finally, a significant inhibition in CK-MB and LDH enzyme activity was observed compared to ovariectomized animals. Collectively, MF treatment was superior to E₂ in protecting cardiac function as measured by activities of heart enzymes and estradiol levels in serum.

Table 1
Effect of ovariectomy (OVX), oestradiol (OVX + E₂) and metformin (OVX + MF) treatments on serum 17 β estradiol, CK-MB, and LDH

Treatment	17 β estradiol (pg/ml)	CK-MB (U/L)	LDH (U/L)
SH	88.14 $\pm$ 4.80	10.91 $\pm$ 1.79	200.56 $\pm$ 80.58
OVX	57.66 $\pm$ 5.47 ^{a***}	185.30 $\pm$ 12.85 ^{a***}	3070.69 $\pm$ 692.14 ^{a***}
OVX + E ₂	131.60 $\pm$ 5.19 ^{a*** b***}	78.17 $\pm$ 4.52 ^{a*** b***}	683.99 $\pm$ 141.57 ^{a*** b***}
OVX + MF	81.22 $\pm$ 6.54 ^{b***c***}	30.61 $\pm$ 5.40 ^{a***b***c***}	463.68 $\pm$ 102.47 ^{b***}

The data are presented as the mean $\pm$ SD, n = 10. ^a: significant difference versus the sham (SH) group; ^b: significant difference versus the ovariectomized (OVX) group and ^c: significant difference versus estradiol treatment. * = p < 0.05, ** = p < 0.01, and*** = p < 0.001

Effects of treatment on cardiac antioxidant/oxidative parameters and serum ANG-II levels

Ovariectomized rats showed significant elevation in cardiac MDA and serum ANG-II levels, along with significant depletion in cardiac CAT activity and TAC compared to control animals (Table 2). MF and E₂ treated rats showed renormalized CAT enzyme activity. E₂ treatment induced a significant decline in cardiac MDA levels, and MF-treatment restored MDA to normal levels. TAC was significantly elevated in MF compared to E₂ treated animals.

Table 2

Effect of ovariectomy (OVX), oestradiol (OVX + E₂) and metformin (OVX + MF) on serum ANG-II level and cardiac MDA, CAT and TAC

Treatment	ANG-II (pg./ml)	MDA (μmol/g tissue)	CAT (U/mg protein)	TAC (mmol/L)
SH	8.75 ± 0.78	70.83 ± 5.40	0.23 ± 0.04	0.87 ± 0.18
OVX	64.91 ± 6.45 a***	97.92 ± 15.94 a***	0.04 ± 0.01 a***	0.48 ± 0.14 a***
OVX + E ₂	21.88 ± 2.19 a***b***	40.79 ± 12.25 b***	0.20 ± 0.03 a*b***	0.90 ± 0.11 b***
OVX + MF	25.95 ± 4.15 a***b***	72.33 ± 8.69 b***c***	0.23 ± 0.04 b***	1.34 ± 0.13 a***b*** c***

The data are presented as the mean ± SD, n = 10. ^a: significant difference versus the sham (SH) group; ^b: significant difference versus the ovariectomized (OVX) group and ^c: significant difference versus estradiol treatment. * = p < 0.05, ** = p < 0.01, and *** = p < 0.001

Effect of treatment on serum lipid profile and atherogenic indices (AI-I & AI-II)

Compared to SH rats (Group 1), ovariectomy induced a state of dyslipidemia evidenced by a significant increase in serum TC, TG, LDL-C levels and a significant depletion in HDL-C level. Further, OVC rats displayed significant elevation in atherogenic indices I & II (Table 3). MF treatment was more effective than E₂ in countering the impact of ovariectomy, as indicated by restoration of normal lipid profile parameters and atherogenic indices (Table 3). E₂ induced a significant elevation in TC and LDL-C compared to SH - and OVX -treated rats (Groups 1 and 2). Compared to controls, E₂ treatment induced a significant elevation in TG and atherogenic indices with a significant reduction in HDL-C concentrations. MF treatment was more effective in renormalizing dyslipidemia than E₂.

Table (3) Effect of ovariectomy (OVX), oestradiol (OVX + E₂) and metformin (OVX + MF) on total cholesterol (TC), triglycerides (TG), HDL-C, LDL-C and atherogenic indices (AI-I & AI-II)

Treatment	TC (mg/dl)	TG (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	AHI	AHII
SH	111.60 ± 7.39	156.13 ± 4.20	41.62 ± 10.45	47.94 ± 8.04	1.89 ± 0.49	1.63 ± 0.41
OVX	162.52 ± 24.20 a***	228.66 ± 11.09 a***	85.18 ± 7.33 a***	19.81 ± 4.80 a***	7.25 ± 1.43 a***	7.97 ± 1.43 a***
OVX + E ₂	189.04 ± 21.88 a***b*	186.88 ± 7.71 a*** b***	119.46 ± 15.47 a***b***	30.90 ± 7.27 a***b*	4.46 ± 1.40 a***b***	5.07 ± 1.27 a***b***
OVX + MF	122.15 ± 12.60 b***c***	87.87 ± 10.93 a*** b***c***	48.00 ± 13.28 b***c***	62.55 ± 13.11 a**b***c***	1.65 ± 0.34 b***c***	1.31 ± 0.32 b***c***

The data are presented as the mean ± SD, n = 10. ^a - significant difference versus the sham (SH) control animals; ^b - significant difference versus ovariectomized (OVX) animals and ^c - significant difference versus estradiol treated animals. * = $p < 0.05$, ** $p < 0.01$, and *** = $p < 0.001$

Effect of ovariectomy and different treatments on serum TNF- α , IL-6 and cardiac total NOx

Figure 1 reveals that OVX-group showed a significant elevation in serum proinflammatory markers, TNF- α (89.56; $P < 0.0001$) and IL-6 (81.23; $P < 0.0001$) and cardiac total NOx (195.3; $P < 0.0001$), compared with SH-group (21.39, 18.59 and 69.14, respectively). OVX-MF and OVX-E₂ groups showed a significant low level of inflammatory cytokines; serum TNF- α (43.35 and 38.22; $P < 0.0001$), IL-6 (42.78 and 37.12; $P < 0.0001$), compared to OVX-group, along with normal level of cardiac total NOx (69.38 and 69.87; $P > 0.999$), compared to control animals.

Discussion

Menopause is associated with many cardiovascular diseases (CVD) (Roth *et al.*, 2018; Zhu *et al.*, 2019). The present study demonstrated significant increases in cardiac enzyme activities, LDH and CK-MB following ovariectomized rats (Table 1).

Estrogen hormone deficiency activates the renin-angiotensin system (RAS) and leads to CVD (Yung *et al.*, 2011). Angiotensin II (ANG-II) is a potent vasoconstrictor and a key component of RAS for maintaining systemic arterial pressure (Manabe *et al.*, 2005; Benigni, Cassis and Remuzzi, 2010). An increase in ANG-II leads to endothelial dysfunction through binding with angiotensin II receptor 1 (ANG-II R1). This

interaction promotes ROS production through the activation of NOX4. OVX animals in the present study showed a significant elevation in ANG-II level, consistent with these previous results. (Table 2).

Oxidative stress is pivotal in multiple pathophysiological processes and may increase the risk of CVD via atherosclerotic plaques and coronary artery disease (Stocker and Kearney, 2004; Rezzani *et al.*, 2008; Kattoor *et al.*, 2017). Oxidative insult in postmenopausal women may be attributed to a deficit in estradiol. Estradiol might act as an antioxidant via its phenolic group. This group may scavenge ROS and help maintain normal oxidant status (Persky *et al.*, 2000). Further, estradiol may upregulate the expression of antioxidant enzymes (Suzuki, Brown and Wise, 2006). The incidence of dyslipidemia and elevated plasma atherogenic indices are higher after menopause (Phan and Toth, 2014; Cai *et al.*, 2017; Wu *et al.*, 2018; Ko and Kim, 2020). Higher levels of cholesterol and lipoprotein, especially LDL-C, are vulnerable to extensive oxidation by ROS. This oxidation leads to lipid peroxidation and the formation of MDA as an oxidation product. Also, cholesterol may accumulate in coronary arteries to cause CVD (Levitan, Volkov and Subbaiah, 2010; Parthasarathy *et al.*, 2010; Ito, Sono and Ito, 2019; Yamada *et al.*, 2020). These processes may explain the current results of oxidative insult in cardiac tissue (Table 2) associated with dyslipidemia and higher atherogenic indices (Table 3) following ovariectomy.

The significant elevation of proinflammatory cytokines, TNF α and IL6, in OVX-treated rats in the present study are considered subcellular risk factors associated with CVD, including ischemia/perfusion, atherosclerosis, and heart failure. (Torre-Amione, 2005; Kaur *et al.*, 2006) authors attributed the elevation of proinflammatory cytokines is a result of elevated ROS generation that triggers the immune cells to migrate to the injured tissues (Yang *et al.*, 2007; Mittal *et al.*, 2014).

Focusing on the current results of NO x , OVX-treated group showed a significant increase in total NO x level, compared to SH- group, this result could be attributed to many factors including biosynthesis factors of nitric oxide (NO).

The current results indicated the oxidative insult in cardiac tissue which resulted in overproduction of ROS, tetrahydrobiopterin (BH4) is a reducing cofactor in nitric oxide synthesis, BH4 is vulnerable to be oxidized via ROS leading to uncoupling NOS, and superoxide formation which reacts with nitric oxide leads to formation of reactive nitrogen species formed (RNS) especially, peroxynitrite (OONO $^{\cdot-}$) (Gross *et al.*, 2000; Heitzer *et al.*, 2000; Katusic, 2001; Kuzkaya *et al.*, 2003). Several factors induced upregulation of NOS enzyme family such as a depletion in estrogen and an elevation of inflammatory markers, those factors are associated in OVX-group with concomitant of increase of ROS lead to uncoupled NOS and release of OONO $^{\cdot-}$ (Ceccatelli, 1997; Naseem, 2005; Ronchetti *et al.*, 2016; Münzel *et al.*, 2017).

The pleiotropic effect of metformin as antihyperlipidemic (Lin *et al.*, 2018), antioxidant (Diaz-Morales *et al.*, 2017), cardioprotective (Grant, 1996) with antidiabetic properties directed the current study to investigate its role in mitigating the risk factors of cardiovascular dysfunction induced by ovariectomy. Several studies were investigated the cardioprotective effect of high doses of MF ranging from 200 to 500 mg/kg on rats (Trott *et al.*, 2011; Lobato *et al.*, 2012; Oliveira *et al.*, 2014). In the current study, low dose of MF

was chosen simulated the daily human dose of metformin 1000 mg/kg on the basis of its cardioprotective effect on adult male rats (Wang *et al.*, 2011; Nair and Jacob, 2016). As expected (Yan *et al.*, 2009; Kheniser, Kashyap and Kasumov, 2019), MF normalized a level of serum estradiol, lipid profile markers as well as atherogenic indices compared with SH-group.

In vitro study suggested the potential hypolipidemic effect of metformin through stimulating the activity of AMPK leading to enhancing the rate of lipogenesis in the liver tissue, furthermore, it downregulated a sterol regulatory element binding protein-2 (SREBP-2) as well as), HMG-COA reductase, and HMG-COA synthase, all key factors in cholesterol synthesis(Zhang *et al.*, 2018). Estradiol lacks antihyperlipidemic ability(Liu *et al.*, 2004; Babaei *et al.*, 2010), which could explain impacts on lipid profiles

MF treatment renormalized serum estradiol levels in ovariectomized animals in the present study. MF also lowered ANG-II levels, concurrent with the normalization of lipid profiles. This activity could be important for lowering the production of ROS, NOS, and inflammation. Such changes were observed as decreased cardiac MDA, NOx, and serum TNF α and IL-6 levels, and augmented cardiac CAT activity and TAC level. Also, MF treatment demonstrated cardioprotective effects, evidenced by decreased serum CK-MB and LDH enzyme activities.(Alzoubi *et al.*, 2014; Zilinyi *et al.*, 2018; Lee *et al.*, 2019)

Conclusion

Antioxidant and antihyperlipidemic properties of MF allow this agent to ameliorate biochemical risk factors of cardiovascular dysfunction and inflammation following estradiol withdrawal. MF might be a safer and effective alternative to replace hormonal replacement therapy with estradiol.

Declarations

Funding 'Not applicable'

Conflicts of interest/Competing interests

'Not applicable'

Availability of data and material

All raw data were saved in spread sheet

Code availability

'Not applicable'

Authors' contributions

The authors were contributed equally in experimental design, data collection, writing

Ethics approval

All experimental protocols were approved by the Institutional Animal Ethics Committee at the National Organization for Drug Control and Research (Approval no. 8/231/2019). Procedures followed the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996).

References

1. Alzoubi, K., Khabour, O., Al-azzam, S., Tashtoush, M. and Mhaidat, N. (2014) 'Metformin Eased Cognitive Impairment Induced by Chronic L-methionine Administration: Potential Role of Oxidative Stress', *Current Neuropharmacology*. Bentham Science Publishers Ltd., 12(2), pp. 186–192. doi: 10.2174/1570159x11666131120223201.
2. Babaei, P., Mehdizadeh, R., Ansar, M. M. and Damirchi, A. (2010) 'Effects of ovariectomy and estrogen replacement therapy on visceral adipose tissue and serum adiponectin levels in rats', *Menopause International*. Menopause Int, 16(3), pp. 100–104. doi: 10.1258/mi.2010.010028.
3. Benigni, A., Cassis, P. and Remuzzi, G. (2010) 'Angiotensin II revisited: New roles in inflammation, immunology and aging', *EMBO Molecular Medicine*. Wiley-Blackwell, pp. 247–257. doi: 10.1002/emmm.201000080.
4. Brusselaers, N., Tamimi, R. M., Konings, P., Rosner, B., Adami, H. O. and Lagergren, J. (2018) 'Different menopausal hormone regimens and risk of Breast cancer', *Annals of Oncology*. Oxford University Press, 29(8), pp. 1771–1776. doi: 10.1093/annonc/mdy212.
5. Cai, G., Shi, G., Xue, S. and Lu, W. (2017) 'The atherogenic index of plasma is a strong and independent predictor for coronary artery disease in the Chinese Han population.', *Medicine*. Wolters Kluwer Health, 96(37), p. e8058. doi: 10.1097/MD.00000000000008058.
6. Ceccatelli, S. (1997) 'Expression and plasticity of NO synthase in the neuroendocrine system', *Brain Research Bulletin*. Brain Res Bull, 44(4), pp. 533–538. doi: 10.1016/S0361-9230(97)00239-6.
7. Chrysoshoou, C., Panagiotakos, D. B., Pitsavos, C., Skoumas, J., Economou, M., Papadimitriou, L. and Stefanadis, C. (2007) 'The association between pre-hypertension status and oxidative stress markers related to atherosclerotic disease: The ATTICA study', *Atherosclerosis*. Atherosclerosis, 192(1), pp. 169–176. doi: 10.1016/j.atherosclerosis.2006.04.030.
8. Claiborne, A. (1985) 'Catalase activity In: Greenwald RA (ed) Handbook of methods for oxygen free radical research', in. CRC Press, Boca Raton, Florida, pp. 283–284.
9. Diaz-Morales, N., Rovira-Llopis, S., Bañuls, C., Lopez-Domenech, S., Escribano-Lopez, I., Veses, S., Jover, A., Rocha, M., Hernandez-Mijares, A. and Victor, V. M. (2017) 'Does Metformin Protect Diabetic Patients from Oxidative Stress and Leukocyte-Endothelium Interactions?', *Antioxidants and Redox Signaling*. Mary Ann Liebert Inc., 27(17), pp. 1439–1445. doi: 10.1089/ars.2017.7122.
10. Friedewald, W. T., Levy, R. I. and Fredrickson, D. S. (1972) 'Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge.', *Clinical*

chemistry, 18(6), pp. 499–502.

11. Grant, P. J. (1996) 'The effects of high- and medium-dose metformin therapy on cardiovascular risk factors in patients with type II diabetes', *Diabetes Care*. American Diabetes Association Inc., 19(1), pp. 64–66. doi: 10.2337/diacare.19.1.64.
12. Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S. and Tannenbaum, S. R. (1982) 'Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids', *Analytical Biochemistry*. Academic Press, 126(1), pp. 131–138. doi: 10.1016/0003-2697(82)90118-X.
13. Gross, S. S., Jones, C. L., Hattori, Y. and Raman, C. S. (2000) 'Tetrahydrobiopterin: An essential cofactor of Nitric Oxide Synthase with an elusive role', in Loouis, I. J. (ed.) *Nitric Oxide: Biology and Pathobiology*. 1st edn. San Diego, CA: Academic Press, pp. 167–185.
14. Heitzer, T., Brockhoff, C., Mayer, B., Warnholtz, A., Mollnau, H., Henne, S., Meinertz, T. and Münzel, T. (2000) 'Tetrahydrobiopterin improves endothelium-dependent vasodilation in chronic smokers : evidence for a dysfunctional nitric oxide synthase.', *Circulation research*, 86(2), pp. E36–E41. doi: 10.1161/01.RES.86.2.e36.
15. Ito, F., Sono, Y. and Ito, T. (2019) 'Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: Oxidative stress in diabetes, atherosclerosis, and chronic inflammation', *Antioxidants*. MDPI AG, 8(3). doi: 10.3390/antiox8030072.
16. Kattoor, A. J., Pothineni, N. V. K., Palagiri, D. and Mehta, J. L. (2017) 'Oxidative Stress in Atherosclerosis', *Current Atherosclerosis Reports*. Current Medicine Group LLC 1. doi: 10.1007/s11883-017-0678-6.
17. Katusic, Z. S. (2001) 'Vascular endothelial dysfunction: Does tetrahydrobiopterin play a role?', *American Journal of Physiology - Heart and Circulatory Physiology*. American Physiological Society. doi: 10.1152/ajpheart.2001.281.3.h981.
18. Kaur, K., Sharma, A. K., Dhingra, S. and Singal, P. K. (2006) 'Interplay of TNF- α and IL-10 in regulating oxidative stress in isolated adult cardiac myocytes', *Journal of Molecular and Cellular Cardiology*. J Mol Cell Cardiol, 41(6), pp. 1023–1030. doi: 10.1016/j.yjmcc.2006.08.005.
19. Kheniser, K. G., Kashyap, S. R. and Kasumov, T. (2019) 'A systematic review: the appraisal of the effects of metformin on lipoprotein modification and function', *Obesity Science and Practice*. Wiley-Blackwell, pp. 36–45. doi: 10.1002/osp4.309.
20. Ko, S. H. and Kim, H. S. (2020) 'Menopause-associated lipid metabolic disorders and foods beneficial for postmenopausal women', *Nutrients*. MDPI AG. doi: 10.3390/nu12010202.
21. Kuzkaya, N., Weissmann, N., Harrison, D. G. and Dikalov, S. (2003) 'Interactions of peroxynitrite, tetrahydrobiopterin, ascorbic acid, and thiols: Implications for uncoupling endothelial nitric-oxide synthase', *Journal of Biological Chemistry*. J Biol Chem, 278(25), pp. 22546–22554. doi: 10.1074/jbc.M302227200.
22. Lasota, A. and Danowska-Klonowska, D. (2004) 'Experimental osteoporosis—different methods of ovariectomy in female white rats.', *Roczniki Akademii Medycznej w Białymstoku (1995)*, 49 Suppl 1, pp. 129–131.

23. Lee, H., Jeon, H. L., Park, S. J. and Shin, J. Y. (2019) 'Effect of statins, metformin, angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers on age-related macular degeneration', *Yonsei Medical Journal*. Yonsei University College of Medicine, 60(7), pp. 679–686. doi: 10.3349/ymj.2019.60.7.679.
24. Levitan, I., Volkov, S. and Subbaiah, P. V. (2010) 'Oxidized LDL: Diversity, patterns of recognition, and pathophysiology', *Antioxidants and Redox Signaling*. Mary Ann Liebert, Inc., pp. 39–75. doi: 10.1089/ars.2009.2733.
25. Lin, S. H., Cheng, P. C., Tu, S. Te, Hsu, S. R., Cheng, Y. C. and Liu, Y. H. (2018) 'Effect of metformin monotherapy on serum lipid profile in statin-naïve individuals with newly diagnosed type 2 diabetes mellitus: A cohort study', *PeerJ*. PeerJ Inc., 2018(6), p. e4578. doi: 10.7717/peerj.4578.
26. Liu, M. L., Xu, X., Rang, W. Q., Li, Y. J. and Song, H. P. (2004) 'Influence of ovariectomy and 17 β -estradiol treatment on insulin sensitivity, lipid metabolism and post-ischemic cardiac function', *International Journal of Cardiology*. Int J Cardiol, 97(3), pp. 485–493. doi: 10.1016/j.ijcard.2003.11.046.
27. Lobato, N. S., Filgueira, F. P., Hagihara, G. N., Akamine, E. H., Pariz, J. R., Tostes, R. C., Carvalho, M. H. C. and Fortes, Z. B. (2012) 'Improvement of metabolic parameters and vascular function by metformin in obese non-diabetic rats', *Life Sciences*, 90(5–6), pp. 228–235. doi: 10.1016/j.lfs.2011.11.005.
28. Manabe, S., Okura, T., Watanabe, S., Fukuoka, T. and Higaki, J. (2005) 'Effects of Angiotensin II Receptor Blockade with Valsartan on Pro-Inflammatory Cytokines in Patients with Essential Hypertension', *Journal of Cardiovascular Pharmacology*, 46(6), pp. 735–739. doi: 10.1097/01.fjc.0000185783.00391.60.
29. Mangge, H. (2014) 'Antioxidants, inflammation and cardiovascular disease', *World Journal of Cardiology*. Baishideng Publishing Group Inc., 6(6), p. 462. doi: 10.4330/wjc.v6.i6.462.
30. Mendelsohn, M. E. and Karas, R. H. (1999) 'The protective effects of estrogen on the cardiovascular system', *New England Journal of Medicine*. Edited by F. H. Epstein. Massachusetts Medical Society, pp. 1801–1811. doi: 10.1056/NEJM199906103402306.
31. Mihara, M. and Uchiyama, M. (1978) 'Determination of malonaldehyde precursor in tissues by thiobarbituric acid test', *Analytical Biochemistry*, 86(1), pp. 271–278.
32. Mittal, M., Siddiqui, M. R., Tran, K., Reddy, S. P. and Malik, A. B. (2014) 'Reactive oxygen species in inflammation and tissue injury', *Antioxidants and Redox Signaling*. Mary Ann Liebert, Inc., pp. 1126–1167. doi: 10.1089/ars.2012.5149.
33. Münzel, T., Camici, G. G., Maack, C., Bonetti, N. R., Fuster, V. and Kovacic, J. C. (2017) 'Impact of Oxidative Stress on the Heart and Vasculature: Part 2 of a 3-Part Series', *Journal of the American College of Cardiology*. Elsevier USA, pp. 212–229. doi: 10.1016/j.jacc.2017.05.035.
34. Murr, C., Winklhofer-Roob, B. M., Schroecksnadel, K., Maritschnegg, M., Mangge, H., Böhm, B. O., Winkelmann, B. R., März, W. and Fuchs, D. (2009) 'Inverse association between serum concentrations

- of neopterin and antioxidants in patients with and without angiographic coronary artery disease', *Atherosclerosis*. *Atherosclerosis*, 202(2), pp. 543–549. doi: 10.1016/j.atherosclerosis.2008.04.047.
35. Nair, A. B. and Jacob, S. (2016) 'A simple practice guide for dose conversion between animals and human.', *Journal of basic and clinical pharmacy*. Wolters Kluwer – Medknow Publications, 7(2), pp. 27–31. doi: 10.4103/0976-0105.177703.
 36. Naseem, K. M. (2005) 'The role of nitric oxide in cardiovascular diseases', *Molecular Aspects of Medicine*. Elsevier Ltd, 26(1–2), pp. 33–65. doi: 10.1016/j.mam.2004.09.003.
 37. Nasri, H. and Rafieian-Kopaei, M. (2014) 'Metformin: Current knowledge', *Journal of Research in Medical Sciences*. Isfahan University of Medical Sciences(IUMS), pp. 658–664.
 38. Nomikos, G., Spyraiki, C., Kazandjian, A. and Sfikakis, A. (1987) 'Estrogen treatment to ovariectomized rats modifies morphine-induced behavior', *Pharmacology, Biochemistry and Behavior*. Elsevier, 27(4), pp. 611–617. doi: 10.1016/0091-3057(87)90182-1.
 39. Oliveira, P. W. C., de Sousa, G. J., Caliman, I. F., Lamas, A. Z., Santos de Medeiros, A. R., de Andrade, T. U., de Abreu, G. R., de Figueiredo, S. G. and Bissoli, N. S. (2014) 'Metformin ameliorates ovariectomy-induced vascular dysfunction in non-diabetic wistar rats', *Clinical Science*. Portland Press Ltd, 127(4), pp. 265–275. doi: 10.1042/CS20130553.
 40. Parthasarathy, S., Raghavamenon, A., Garelnabi, M. O. and Santanam, N. (2010) 'Oxidized low-density lipoprotein.', *Methods in molecular biology (Clifton, N.J.)*, 610, pp. 403–17. doi: 10.1007/978-1-60327-029-8_24.
 41. Persky, A. M., Green, P. S., Stublely, L., Howell, C. O., Zaulyanov, L., Brazeau, G. A. and Simpkins, J. W. (2000) 'Protective effect of estrogens against oxidative damage to heart and skeletal muscle in vivo and in vitro', *Proceedings of the Society for Experimental Biology and Medicine*. Blackwell Publishing Inc., 223(1), pp. 59–66. doi: 10.1046/j.1525-1373.2000.22308.x.
 42. Phan, B. A. P. and Toth, P. P. (2014) 'Dyslipidemia in women: Etiology and management', *International Journal of Women's Health*. Dove Press, pp. 185–194. doi: 10.2147/IJWH.S38133.
 43. Rezzani, R., Bonomini, F., Tengattini, S., Fabiano, A. and Bianchi, R. (2008) 'Atherosclerosis and oxidative stress', *Histology and Histopathology*. *Histol Histopathol*, 23(3), pp. 381–390. doi: 10.14670/HH-23.381.
 44. Ronchetti, S. A., Machiavelli, L. I., Quinteros, F. A., Duvilanski, B. H. and Cabilla, J. P. (2016) 'Nitric Oxide Plays a Key Role in Ovariectomy-Induced Apoptosis in Anterior Pituitary: Interplay between Nitric Oxide Pathway and Estrogen', *PLOS ONE*. Edited by M. Colasanti. Public Library of Science, 11(9), p. e0162455. doi: 10.1371/journal.pone.0162455.
 45. Roth, G. A., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C., Abbasi, N., Abbastabar, H., Abd-Allah, F., Abdela, J., Abdelalim, A., Abdollahpour, I., Abdulkader, R. S., Abebe, H. T., Abebe, M., Abebe, Z., Abejie, A. N., Abera, S. F., Abil, O. Z., Abraha, H. N., Abrham, A. R., Abu-Raddad, L. J., Accrombessi, M. M. K., Acharya, D., Adamu, A. A., Adebayo, O. M., Adedoyin, R. A., Adekanmbi, V., Adetokunboh, O. O., Adhena, B. M., Adib, M. G., Admasie, A., Afshin, A., Agarwal, G., Agesa, K. M., Agrawal, A., Agrawal, S., Ahmadi, A., Ahmadi, M., Ahmed, M. B., Ahmed, S., Aichour, A. N., Aichour, I., Aichour, M. T. E., Akbari, M. E.,

Akinyemi, R. O., Akseer, N., Al-Aly, Z., Al-Eyadhy, A., Al-Raddadi, R. M., Alahdab, F., Alam, K., Alam, T., Alebel, A., Alene, K. A., Alijanzadeh, M., Alizadeh-Navaei, R., Aljunid, S. M., Alkerwi, A., Alla, F., Allebeck, P., Alonso, J., Altirkawi, K., Alvis-Guzman, N., Amare, A. T., Aminde, L. N., Amini, E., Ammar, W., Amoako, Y. A., Anber, N. H., Andrei, C. L., Androudi, S., Anmut, M. D., Anjomshoa, M., Ansari, H., Ansha, M. G., Antonio, C. A. T., Anwari, P., Aremu, O., Arnlov, J., Arora, A., Arora, M., Artaman, A., Aryal, K. K., Asayesh, H., Asfaw, E. T., Ataro, Z., Atique, S., Atre, S. R., Ausloos, M., Avokpaho, E. F. G. A., Awasthi, A., Ayala Quintanilla, B. P., Ayele, Y., Ayer, R., Azzopardi, P. S., Babazadeh, A., Bacha, U., Badali, H., Badawi, A., Bali, A. G., Ballesteros, K. E., Banach, M., Banerjee, K., Bannick, M. S., Banoub, J. A. M., Barboza, M. A., Barker-Collo, S. L., Barnighausen, T. W., Barquera, S., Barrero, L. H., Bassat, Q., Basu, S., Baune, B. T., Baynes, H. W., Bazargan-Hejazi, S., Bedi, N., Beghi, E., Behzadifar, Masoud, Behzadifar, Meysam, Bejot, Y., Bekele, B. B., Belachew, A. B., Belay, E., Belay, Y. A., Bell, M. L., Bello, A. K., Bennett, D. A., Bensenor, I. M., Berman, A. E., Bernabe, E., Bernstein, R. S., Bertolacci, G. J., Beuran, M., Beyranvand, T., Bhalla, A., Bhattarai, S., Bhaumik, S., Bhutta, Z. A., Biadgo, B., Biehl, M. H., Bijani, A., Bikbov, B., Bilano, V., Bililign, N., Bin Sayeed, M. S., Bisanzio, D., Biswas, T., Blacker, B. F., Basara, B. B., Borschmann, R., Bosetti, C., Bozorgmehr, K., Brady, O. J., Brant, L. C., Brayne, C., Brazinova, A., Breitborde, N. J. K., Brenner, H., Briant, P. S., Britton, G., Brugha, T., Busse, R., Butt, Z. A., Callender, C. S. K. H., Campos-Nonato, I. R., Campuzano Rincon, J. C., Cano, J., Car, M., Cardenas, R., Carreras, G., Carrero, J. J., Carter, A., Carvalho, F., Castaneda-Orjuela, C. A., Castillo Rivas, J., Castle, C. D., Castro, C., Castro, F., Catala-Lopez, F., Cerin, E., Chaiah, Y., Chang, J. C., Charlson, F. J., Chaturvedi, P., Chiang, P. P. C., Chimed-Ochir, O., Chisumpa, V. H., Chitheer, A., Chowdhury, R., Christensen, H., Christopher, D. J., Chung, S. C., Cicuttini, F. M., Ciobanu, L. G., Cirillo, M., Cohen, A. J., Cooper, L. T., Cortesi, P. A., Cortinovis, M., Cousin, E., Cowie, B. C., Criqui, M. H., Cromwell, E. A., Crowe, C. S., Crump, J. A., Cunningham, M., Daba, A. K., Dadi, A. F., Dandona, L., Dandona, R., Dang, A. K., Dargan, P. I., Daryani, A., Das, S. K., Gupta, Rajat Das, Neves, J. Das, Dasa, T. T., Dash, A. P., Davis, A. C., Davis Weaver, N., Davitoiu, D. V., Davletov, K., De La Hoz, F. P., De Neve, J. W., Degefa, M. G., Degenhardt, L., Degfie, T. T., Deiparine, S., Demoz, G. T., Demtsu, B. B., Denova-Gutierrez, E., Deribe, K., Derveniz, N., Des Jarlais, D. C., Dessie, G. A., Dey, S., Dharmaratne, S. D., Dicker, D., Dinberu, M. T., Ding, E. L., Dirac, M. A., Djalalinia, S., Dokova, K., Doku, D. T., Donnelly, C. A., Dorsey, E. R., Doshi, P. P., Douwes-Schultz, D., Doyle, K. E., Driscoll, T. R., Dubey, M., Dubljanin, E., Duken, E. E., Duncan, B. B., Duraes, A. R., Ebrahimi, H., Ebrahimpour, S., Edessa, D., Edvardsson, D., Eggen, A. E., El Bcheraoui, C., El Sayed Zaki, M., El-Khatib, Z., Elkout, H., Ellingsen, C. L., Endres, M., Endries, A. Y., Er, B., Erskine, H. E., Eshrati, B., Eskandarieh, S., Esmaeili, R., Esteghamati, A., Fakhar, M., Fakhim, H., Faramarzi, M., Fareed, M., Farhadi, F., Farinha, C. S. E., Faro, A., Farvid, M. S., Farzadfar, F., Farzaei, M. H., Feigin, V. L., Feigl, A. B., Fentahun, N., Fereshtehnejad, S. M., Fernandes, E., Fernandes, J. C., Ferrari, A. J., Feyissa, G. T., Filip, I., Finegold, S., Fischer, F., Fitzmaurice, C., Foigt, N. A., Foreman, K. J., Fornari, C., Frank, T. D., Fukumoto, T., Fuller, J. E., Fullman, N., Furst, T., Furtado, J. M., Futran, N. D., Gallus, S., Garcia-Basteiro, A. L., Garcia-Gordillo, M. A., Gardner, W. M., Gebre, A. K., Gebrehiwot, T. T., Gebremedhin, A. T., Gebremichael, B., Gebremichael, T. G., Gelano, T. F., Geleijnse, J. M., Genova-Maleras, R., Geramo, Y. C. D., Gething, P. W., Gezae, K. E., Ghadami, M. R., Ghadimi, R., Ghasemi Falavarjani, K., Ghasemi-Kasman, M., Ghimire, M., Gibney, K. B., Gill, P. S., Gill, T. K., Gillum, R. F., Ginawi, I. A., Giroud, M.,

Giussani, G., Goenka, S., Goldberg, E. M., Goli, S., Gomez-Dantes, H., Gona, P. N., Gopalani, S. V., Gorman, T. M., Goto, A., Goulart, A. C., Gnedovskaya, E. V., Grada, A., Grosso, G., Gugni, H. C., Guimaraes, A. L. S., Guo, Y., Gupta, P. C., Gupta, Rahul, Gupta, Rajeev, Gupta, T., Gutierrez, R. A., Gyawali, B., Haagsma, J. A., Hafezi-Nejad, N., Hagos, T. B., Hailegiyorgis, T. T., Hailu, G. B., Haj-Mirzaian, Arvin, Haj-Mirzaian, Arya, Hamadeh, R. R., Hamidi, S., Handal, A. J., Hankey, G. J., Harb, H. L., Harikrishnan, S., Haro, J. M., Hasan, M., Hassankhani, H., Hassen, H. Y., Havmoeller, R., Hay, R. J., Hay, S. I., He, Y., Hedayatizadeh-Omran, A., Hegazy, M. I., Heibati, B., Heidari, M., Hendrie, D., Henok, A., Henry, N. J., Herteliu, C., Heydarpour, F., Heydarpour, P., Heydarpour, S., Hibstu, D. T., Hoek, H. W., Hole, M. K., Homaie Rad, E., Hoogar, P., Hosgood, H. D., Hosseini, S. M., Hosseinzadeh, M., Hostiuc, M., Hostiuc, S., Hotez, P. J., Hoy, D. G., Hsiao, T., Hu, G., Huang, J. J., Hussein, A., Hussien, M. M., Hutfless, S., Idrisov, B., Ilesanmi, O. S., Iqbal, U., Irvani, S. S. N., Irvine, C. M. S., Islam, N., Islam, S. M. S., Islami, F., Jacobsen, K. H., Jahangiry, L., Jahanmehr, N., Jain, S. K., Jakovljevic, M., Jalu, M. T., James, S. L., Javanbakht, M., Jayatilleke, A. U., Jeemon, P., Jenkins, K. J., Jha, R. P., Jha, V., Johnson, C. O., Johnson, S. C., Jonas, J. B., Joshi, A., Jozwiak, J. J., Jungari, S. B., Jurisson, M., Kabir, Z., Kadel, R., Kahsay, A., Kalani, R., Karami, M., Karami Matin, B., Karch, A., Karema, C., Karimi-Sari, H., Kasaeian, A., Kassa, D. H., Kassa, G. M., Kassa, T. D., Kassebaum, N. J., Katikireddi, S. V., Kaul, A., Kazemi, Z., Kazemi Karyani, A., Kazi, D. S., Kefale, A. T., Keiyoro, P. N., Kemp, G. R., Kengne, A. P., Keren, A., Kesavachandran, C. N., Khader, Y. S., Khafaei, B., Khafaei, M. A., Khajavi, A., Khalid, N., Khalil, I. A., Khan, E. A., Khan, M. S., Khan, M. A., Khang, Y. H., Khater, M. M., Khoja, A. T., Khosravi, A., Khosravi, M. H., Khubchandani, J., Kiadaliri, A. A., Kibret, G. D., Kidanemariam, Z. T., Kiirithio, D. N., Kim, D., Kim, Y. E., Kim, Y. J., Kimokoti, R. W., Kinfu, Y., Kisa, A., Kissimova-Skarbek, K., Kivimaki, M., Knudsen, A. K. S., Kocarnik, J. M., Kochhar, S., Kokubo, Y., Kolola, T., Kopec, J. A., Koul, P. A., Koyanagi, A., Kravchenko, M. A., Krishan, K., Kuate Defo, B., Kucuk Bicer, B., Kumar, G. A., Kumar, M., Kumar, P., Kutz, M. J., Kuzin, I., Kyu, H. H., Lad, D. P., Lad, S. D., Lafranconi, A., Lal, D. K., Lalloo, R., Lallukka, T., Lam, J. O., Lami, F. H., Lansingh, V. C., Lansky, S., Larson, H. J., Latifi, A., Lau, K. M. M., Lazarus, J. V., Lebedev, G., Lee, P. H., Leigh, J., Leili, M., Leshargie, C. T., Li, S., Li, Y., Liang, J., Lim, L. L., Lim, S. S., Limenih, M. A., Linn, S., Liu, S., Liu, Y., Lodha, R., Lonsdale, C., Lopez, A. D., Lorkowski, S., Lotufo, P. A., Lozano, R., Lunevicius, R., Ma, S., Macarayan, E. R. K., Mackay, M. T., MacLachlan, J. H., Maddison, E. R., Madotto, F., Magdy Abd El Razek, H., Magdy Abd El Razek, M., Maghavani, D. P., Majdan, M., Majdzadeh, R., Majeed, A., Malekzadeh, R., Malta, D. C., Manda, A. L., Mandarano-Filho, L. G., Manguerra, H., Mansournia, M. A., Mapoma, C. C., Marami, D., Maravilla, J. C., Marcenes, W., Marczak, L., Marks, A., Marks, G. B., Martinez, G., Martins-Melo, F. R., Martopullo, I., Marz, W., Marzan, M. B., Masci, J. R., Massenburg, B. B., Mathur, M. R., Mathur, P., Matzopoulos, R., Maulik, P. K., Mazidi, M., McAlinden, C., McGrath, J. J., McKee, M., McMahon, B. J., Mehata, S., Mehndiratta, M. M., Mehrotra, R., Mehta, K. M., Mehta, V., Mekonnen, T. C., Melese, A., Melku, M., Memiah, P. T. N., Memish, Z. A., Mendoza, W., Mengistu, D. T., Mengistu, G., Mensah, G. A., Mereta, S. T., Meretoja, A., Meretoja, T. J., Mestrovic, T., Mezgebe, H. B., Miazgowski, B., Miazgowski, T., Millea, A. I., Miller, T. R., Miller-Petrie, M. K., Mini, G. K., Mirabi, P., Mirarefin, M., Mirica, A., Mirakhimov, E. M., Misganaw, A. T., Mitiku, H., Moazen, B., Mohammad, K. A., Mohammadi, M., Mohammadifard, N., Mohammed, M. A., Mohammed, S., Mohan, V., Mokdad, A. H., Molokhia, M., Monasta, L., Moradi, G., Moradi-Lakeh, M.,

Moradinazar, M., Moraga, P., Morawska, L., Velasquez, I. M., Morgado-Da-Costa, J., Morrison, S. D., Moschos, M. M., Mouodi, S., Mousavi, S. M., Muchie, K. F., Mueller, U. O., Mukhopadhyay, S., Muller, K., Mumford, J. E., Musa, J., Musa, K. I., Mustafa, G., Muthupandian, S., Nachega, J. B., Nagel, G., Naheed, A., Nahvijou, A., Naik, G., Nair, S., Najafi, F., Naldi, L., Nam, H. S., Nangia, V., Nansseu, J. R., Nascimento, B. R., Natarajan, G., Neamati, N., Negoï, I., Negoï, R. I., Neupane, S., Newton, C. R. J., Ngalesoni, F. N., Ngunjiri, J. W., Nguyen, A. Q., Nguyen, G., Nguyen, Ha Thu, Nguyen, Huong Thanh, Nguyen, L. H., Nguyen, M., Nguyen, T. H., Nichols, E., Ningrum, D. N. A., Nirayo, Y. L., Nixon, M. R., Noluthungu, N., Nomura, S., Norheim, O. F., Noroozi, M., Norrving, B., Noubiap, J. J., Nouri, H. R., Shiadeh, M. N., Nowroozi, M. R., Nyasulu, P. S., Odell, C. M., Ofori-Asenso, R., Ogbo, F. A., Oh, I. H., Oladimeji, O., Olagunju, A. T., Olivares, P. R., Olsen, H. E., Olusanya, B. O., Olusanya, J. O., Ong, K. L., Ong, S. K. S., Oren, E., Orpana, H. M., Ortiz, A., Ortiz, J. R., Otstavnov, S. S., Overland, S., Owolabi, M. O., Ozdemir, R., Mahesh, P. A., Pacella, R., Pakhale, S., Pakhare, A. P., Pakpour, A. H., Pana, A., Panda-Jonas, S., Pandian, J. D., Parisi, A., Park, E. K., Parry, C. D. H., Parsian, H., Patel, S., Pati, S., Patton, G. C., Paturi, V. R., Paulson, K. R., Pereira, A., Pereira, D. M., Perico, N., Pesudovs, K., Petzold, M., Phillips, M. R., Piel, F. B., Pigott, D. M., Pillay, J. D., Pirsahab, M., Pishgar, F., Polinder, S., Postma, M. J., Pourshams, A., Poustchi, H., Pujar, A., Prakash, S., Prasad, N., Purcell, C. A., Qorbani, M., Quintana, H., Quistberg, D. A., Rade, K. W., Anwar Rafay, A. R., Rahim, F., Rahimi, K., Rahimi-Movaghar, A., Rahman, M., Rahman, M. H. U., Rahman, M. A., Rai, R. K., Rajsic, S., Ram, U., Ranabhat, C. L., Ranjan, P., Rao, P. C., Rawaf, D. L., Rawaf, S., Razo-Garcia, C., Reddy, K. S., Reiner, R. C., Reitsma, M. B., Remuzzi, G., Renzaho, A. M. N., Resnikoff, S., Rezaei, S., Rezaeian, S., Rezai, M. S., Riahi, S. M., Ribeiro, A. L. P., Rios-Blancas, M. J., Roba, K. T., Roberts, N. L. S., Robinson, S. R., Roeber, L., Ronfani, L., Roshandel, G., Rostami, A., Rothenbacher, D., Roy, A., Rubagotti, E., Sachdev, P. S., Saddik, B., Sadeghi, E., Safari, H., Safdarian, M., Safi, S., Safiri, S., Sagar, R., Sahebkar, A., Sahraian, M. A., Salam, N., Salama, J. S., Salamati, P., De Freitas Saldanha, R., Saleem, Z., Salimi, Y., Salvi, S. S., Salz, I., Sambala, E. Z., Samy, A. M., Sanabria, J., Dolores Sanchez-Nino, M., Santomauro, D. F., Santos, I. S., Santos, J. V., Santric Milicevic, M. M., Jose, B. P. S., Sarker, A. R., Sarmiento-Suarez, R., Sarrafzadegan, N., Sartorius, B., Sarvi, S., Sathian, B., Satpathy, M., Sawant, A. R., Sawhney, M., Saxena, S., Sayyah, M., Schaeffner, E., Schmidt, M. I., Schneider, I. J. C., Schottker, B., Schutte, A. E., Schwebel, D. C., Schwendicke, F., Scott, J. G., Sekerija, M., Sepanlou, S. G., Servan-Mori, E., Seyedmousavi, S., Shabaninejad, H., Shackelford, K. A., Shafieesabet, A., Shahbazi, M., Shaheen, A. A., Shaikh, M. A., Shams-Beyranvand, M., Shamsi, M., Shamsizadeh, M., Sharafi, K., Sharif, M., Sharif-Alhoseini, M., Sharma, R., She, J., Sheikh, A., Shi, P., Shiferaw, M. S., Shigematsu, M., Shiri, R., Shirkoobi, R., Shiue, I., Shokraneh, F., Shrimme, M. G., Si, S., Siabani, S., Siddiqi, T. J., Sigfusdottir, I. D., Sigurvinsdottir, R., Silberberg, D. H., Santos Silva, D. A., Silva, J. P., Da Silva, N. T., Silveira, D. G. A., Singh, J. A., Singh, N. P., Singh, P. K., Singh, V., Sinha, D. N., Sliwa, K., Smith, M., Sobaih, B. H., Sobhani, S., Sobngwi, E., Soneji, S. S., Soofi, M., Sorensen, R. J. D., Soriano, J. B., Soyiri, I. N., Sposato, L. A., Sreeramareddy, C. T., Srinivasan, V., Stanaway, J. D., Starodubov, V. I., Stathopoulou, V., Stein, D. J., Steiner, C., Stewart, L. G., Stokes, M. A., Subart, M. L., Sudaryanto, A., Sufiyan, M. B., Sur, P. J., Sutradhar, I., Sykes, B. L., Sylaja, P. N., Sylte, D. O., Szoeki, C. E. I., Tabares-Seisdedos, R., Tabuchi, T., Tadakamadla, S. K., Takahashi, K., Tandon, N., Tassew, S. G., Taveira, N., Tehrani-Banihashemi, A., Tekalign, T. G., Tekle, M. G., Temsah, M. H., Temsah, O., Terkawi,

- A. S., Teshale, M. Y., Tessema, B., Tessema, G. A., Thankappan, K. R., Thirunavukkarasu, S., Thomas, N., Thrift, A. G., Thurston, G. D., Tilahun, B., To, Q. G., Tobe-Gai, R., Tonelli, M., Topor-Madry, R., Torre, A. E., Tortajada-Girbes, M., Touver, M., Tovani-Palone, M. R., Tran, B. X., Tran, K. B., Tripathi, S., Troeger, C. E., Truelsen, T. C., Truong, N. T., Tsadik, A. G., Tsoi, D., Tudor Car, L., Murat Tuzcu, E., Tyrovolas, S., Ukwaja, K. N., Ullah, I., Undurraga, E. A., Updike, R. L., Usman, M. S., Uthman, O. A., Uzun, S. B., Vaduganathan, M., Vaezi, A., Vaidya, G., Valdez, P. R., Varavikova, E., Vasankari, T. J., Venketasubramanian, N., Villafaina, S., Violante, F. S., Vladimirov, S. K., Vlassov, V., Vollset, S. E., Vos, T., Wagner, G. R., Wagnew, F. S., Waheed, Y., Wallin, M. T., Walson, J. L., Wang, Y., Wang, Y. P., Wassie, M. M., Weiderpass, E., Weintraub, R. G., Weldegebreal, F., Weldegewergs, K. G., Werdecker, A., Werkneh, A. A., West, T. E., Westerman, R., Whiteford, H. A., Widecka, J., Wilner, L. B., Wilson, S., Winkler, A. S., Wiysonge, C. S., Wolfe, C. D. A., Wu, S., Wu, Y. C., Wyper, G. M. A., Xavier, D., Xu, G., Yadgir, S., Yadollahpour, A., Yahyazadeh Jabbari, S. H., Yakob, B., Yan, L. L., Yano, Y., Yaseri, M., Yasin, Y. J., Yentur, G. K., Yeshaneh, A., Yimer, E. M., Yip, P., Yirsaw, B. D., Yisma, E., Yonemoto, N., Yonga, G., Yoon, S. J., Yotebieng, M., Younis, M. Z., Yousefifard, M., Yu, C., Zadnik, V., Zaidi, Z., Zaman, S. Bin, Zamani, M., Zare, Z., Zeleke, A. J., Zenebe, Z. M., Zhang, A. L., Zhang, K., Zhou, M., Zodpey, S., Zuhlke, L. J., Naghavi, M. and Murray, C. J. L. (2018) 'Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017', *The Lancet*. Lancet Publishing Group, 392(10159), pp. 1736–1788. doi: 10.1016/S0140-6736(18)32203-7.
46. Stocker, R. and Keaney, J. F. (2004) 'Role of oxidative modifications in atherosclerosis', *Physiological Reviews*. doi: 10.1152/physrev.00047.2003.
47. Suzuki, S., Brown, C. M. and Wise, P. M. (2006) 'Mechanisms of neuroprotection by estrogen', *Endocrine*. Endocrine, pp. 209–215. doi: 10.1385/ENDO:29:2:209.
48. Torre-Amione, G. (2005) 'Immune activation in chronic heart failure', in *American Journal of Cardiology*. Elsevier Inc., pp. 3–8. doi: 10.1016/j.amjcard.2005.03.006.
49. Trott, D. W., Seawright, J. W., Luttrell, M. J. and Woodman, C. R. (2011) 'NAD(P)H oxidase-derived reactive oxygen species contribute to age-related impairments of endothelium-dependent dilation in rat soleus feed arteries', *Journal of Applied Physiology*. American Physiological Society, 110(5), pp. 1171–1180. doi: 10.1152/japplphysiol.01037.2010.
50. UK Prospective Diabetes Study (UKPDS) Group (1998) 'Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34)', *Lancet*. Elsevier Limited, 352(9131), pp. 854–865. doi: 10.1016/S0140-6736(98)07037-8.
51. Wang, X. F., Zhang, J. Y., Li, L., Zhao, X. Y., Tao, H. L. and Zhang, L. (2011) 'Metformin improves cardiac function in rats via activation of AMP-activated protein kinase', *Clinical and Experimental Pharmacology and Physiology*. Clin Exp Pharmacol Physiol, 38(2), pp. 94–101. doi: 10.1111/j.1440-1681.2010.05470.x.
52. Wu, T.-T., Gao, Y., Zheng, Y.-Y., Ma, Y.-T. and Xie, X. (2018) 'Atherogenic index of plasma (AIP): a novel predictive indicator for the coronary artery disease in postmenopausal women.', *Lipids in health and disease*. BioMed Central, 17(1), p. 197. doi: 10.1186/s12944-018-0828-z.

53. Xu, Y., Arenas, I. A., Armstrong, S. J., Plahta, W. C., Xu, H. and Davidge, S. T. (2006) 'Estrogen improves cardiac recovery after ischemia/reperfusion by decreasing tumor necrosis factor- α ', *Cardiovascular Research*. Cardiovasc Res, 69(4), pp. 836–844. doi: 10.1016/j.cardiores.2005.11.031.
54. Yamada, T., Ogawa, K., Tanaka, T. D., Nagoshi, T., Minai, K., Ogawa, T., Kawai, M. and Yoshimura, M. (2020) 'Increase in oxidized low-density lipoprotein level according to hyperglycemia in patients with cardiovascular disease: A study by structure equation modeling', *Diabetes Research and Clinical Practice*, 161, p. 108036. doi: 10.1016/j.diabres.2020.108036.
55. Yan, L., Kang, B., Li, G., Yin, Z. and Wang, Y. (2009) 'Effects of metformin on serum levels of sex hormone, leptin and insulin in ovariectomized Sprague-Dawley rats', *Pharmazie*, 64(12). doi: 10.1691/ph.2009.9646.
56. Yang, D., Elner, S. G., Bian, Z. M., Till, G. O., Petty, H. R. and Elner, V. M. (2007) 'Pro-inflammatory cytokines increase reactive oxygen species through mitochondria and NADPH oxidase in cultured RPE cells', *Experimental Eye Research*. NIH Public Access, 85(4), pp. 462–472. doi: 10.1016/j.exer.2007.06.013.
57. Yung, L. M., Wong, W. T., Tian, X. Y., Leung, F. P., Yung, L. H., Chen, Z. Y., Yao, X., Lau, C. W. and Huang, Y. (2011) 'Inhibition of Renin-Angiotensin System Reverses Endothelial Dysfunction and Oxidative Stress in Estrogen Deficient Rats', *PLoS ONE*. Edited by C. Borrás. Public Library of Science, 6(3), p. e17437. doi: 10.1371/journal.pone.0017437.
58. Zhang, F., Sun, W., Chen, J., Jiang, L., Yang, P., Huang, Y., Gong, A., Liu, S. and Ma, S. (2018) 'SREBP-2, a new target of metformin?', *Drug Design, Development and Therapy*. Dove Medical Press Ltd., 12, pp. 4163–4170. doi: 10.2147/DDDT.S190094.
59. Zhu, D., Chung, H. F., Dobson, A. J., Pandeya, N., Giles, G. G., Bruinsma, F., Brunner, E. J., Kuh, D., Hardy, R., Avis, N. E., Gold, E. B., Derby, C. A., Matthews, K. A., Cade, J. E., Greenwood, D. C., Demakakos, P., Brown, D. E., Sievert, L. L., Anderson, D., Hayashi, K., Lee, J. S., Mizunuma, H., Tillin, T., Simonsen, M. K., Adami, H. O., Weiderpass, E. and Mishra, G. D. (2019) 'Age at natural menopause and risk of incident cardiovascular disease: a pooled analysis of individual patient data', *The Lancet Public Health*. Elsevier Ltd, 4(11), pp. e553–e564. doi: 10.1016/S2468-2667(19)30155-0.
60. Zilinyi, R., Czompa, A., Czegledi, A., Gajtko, A., Pituk, D., Lekli, I. and Tosaki, A. (2018) 'The cardioprotective effect of metformin in doxorubicin-induced cardiotoxicity: The role of autophagy', *Molecules*. MDPI AG, 23(5). doi: 10.3390/molecules23051184.
61. Alzoubi, K., Khabour, O., Al-azzam, S., Tashtoush, M. and Mhaidat, N. (2014) 'Metformin Eased Cognitive Impairment Induced by Chronic L-methionine Administration: Potential Role of Oxidative Stress', *Current Neuropharmacology*. Bentham Science Publishers Ltd., 12(2), pp. 186–192. doi: 10.2174/1570159x11666131120223201.
62. Babaei, P., Mehdizadeh, R., Ansar, M. M. and Damirchi, A. (2010) 'Effects of ovariectomy and estrogen replacement therapy on visceral adipose tissue and serum adiponectin levels in rats', *Menopause International*. Menopause Int, 16(3), pp. 100–104. doi: 10.1258/mi.2010.010028.

63. Benigni, A., Cassis, P. and Remuzzi, G. (2010) 'Angiotensin II revisited: New roles in inflammation, immunology and aging', *EMBO Molecular Medicine*. Wiley-Blackwell, pp. 247–257. doi: 10.1002/emmm.201000080.
64. Brusselaers, N., Tamimi, R. M., Konings, P., Rosner, B., Adami, H. O. and Lagergren, J. (2018) 'Different menopausal hormone regimens and risk of Breast cancer', *Annals of Oncology*. Oxford University Press, 29(8), pp. 1771–1776. doi: 10.1093/annonc/mdy212.
65. Cai, G., Shi, G., Xue, S. and Lu, W. (2017) 'The atherogenic index of plasma is a strong and independent predictor for coronary artery disease in the Chinese Han population.', *Medicine*. Wolters Kluwer Health, 96(37), p. e8058. doi: 10.1097/MD.00000000000008058.
66. Ceccatelli, S. (1997) 'Expression and plasticity of NO synthase in the neuroendocrine system', *Brain Research Bulletin*. Brain Res Bull, 44(4), pp. 533–538. doi: 10.1016/S0361-9230(97)00239-6.
67. Chrysohoou, C., Panagiotakos, D. B., Pitsavos, C., Skoumas, J., Economou, M., Papadimitriou, L. and Stefanadis, C. (2007) 'The association between pre-hypertension status and oxidative stress markers related to atherosclerotic disease: The ATTICA study', *Atherosclerosis*. Atherosclerosis, 192(1), pp. 169–176. doi: 10.1016/j.atherosclerosis.2006.04.030.
68. Claiborne, A. (1985) 'Catalase activity In: Greenwald RA (ed) Handbook of methods for oxygen free radical research', in. CRC Press, Boca Raton, Florida, pp. 283–284.
69. Diaz-Morales, N., Rovira-Llopis, S., Bañuls, C., Lopez-Domenech, S., Escribano-Lopez, I., Veses, S., Jover, A., Rocha, M., Hernandez-Mijares, A. and Victor, V. M. (2017) 'Does Metformin Protect Diabetic Patients from Oxidative Stress and Leukocyte-Endothelium Interactions?', *Antioxidants and Redox Signaling*. Mary Ann Liebert Inc., 27(17), pp. 1439–1445. doi: 10.1089/ars.2017.7122.
70. Friedewald, W. T., Levy, R. I. and Fredrickson, D. S. (1972) 'Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge.', *Clinical chemistry*, 18(6), pp. 499–502.
71. Grant, P. J. (1996) 'The effects of high- and medium-dose metformin therapy on cardiovascular risk factors in patients with type II diabetes', *Diabetes Care*. American Diabetes Association Inc., 19(1), pp. 64–66. doi: 10.2337/diacare.19.1.64.
72. Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S. and Tannenbaum, S. R. (1982) 'Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids', *Analytical Biochemistry*. Academic Press, 126(1), pp. 131–138. doi: 10.1016/0003-2697(82)90118-X.
73. Gross, S. S., Jones, C. L., Hattori, Y. and Raman, C. S. (2000) 'Tetrahydrobiopterin: An essential cofactor of Nitric Oxide Synthase with an elusive role', in Loouis, I. J. (ed.) *Nitric Oxide: Biology and Pathobiology*. 1st edn. San Diego, CA: Academic Press, pp. 167–185.
74. Heitzer, T., Brockhoff, C., Mayer, B., Warnholtz, A., Mollnau, H., Henne, S., Meinertz, T. and Münzel, T. (2000) 'Tetrahydrobiopterin improves endothelium-dependent vasodilation in chronic smokers : evidence for a dysfunctional nitric oxide synthase.', *Circulation research*, 86(2), pp. E36–E41. doi: 10.1161/01.RES.86.2.e36.

75. Ito, F., Sono, Y. and Ito, T. (2019) 'Measurement and clinical significance of lipid peroxidation as a biomarker of oxidative stress: Oxidative stress in diabetes, atherosclerosis, and chronic inflammation', *Antioxidants*. MDPI AG, 8(3). doi: 10.3390/antiox8030072.
76. Kattoor, A. J., Pothineni, N. V. K., Palagiri, D. and Mehta, J. L. (2017) 'Oxidative Stress in Atherosclerosis', *Current Atherosclerosis Reports*. Current Medicine Group LLC 1. doi: 10.1007/s11883-017-0678-6.
77. Katusic, Z. S. (2001) 'Vascular endothelial dysfunction: Does tetrahydrobiopterin play a role?', *American Journal of Physiology - Heart and Circulatory Physiology*. American Physiological Society. doi: 10.1152/ajpheart.2001.281.3.h981.
78. Kaur, K., Sharma, A. K., Dhingra, S. and Singal, P. K. (2006) 'Interplay of TNF- α and IL-10 in regulating oxidative stress in isolated adult cardiac myocytes', *Journal of Molecular and Cellular Cardiology*. J Mol Cell Cardiol, 41(6), pp. 1023–1030. doi: 10.1016/j.yjmcc.2006.08.005.
79. Kheniser, K. G., Kashyap, S. R. and Kasumov, T. (2019) 'A systematic review: the appraisal of the effects of metformin on lipoprotein modification and function', *Obesity Science and Practice*. Wiley-Blackwell, pp. 36–45. doi: 10.1002/osp4.309.
80. Ko, S. H. and Kim, H. S. (2020) 'Menopause-associated lipid metabolic disorders and foods beneficial for postmenopausal women', *Nutrients*. MDPI AG. doi: 10.3390/nu12010202.
81. Kuzkaya, N., Weissmann, N., Harrison, D. G. and Dikalov, S. (2003) 'Interactions of peroxynitrite, tetrahydrobiopterin, ascorbic acid, and thiols: Implications for uncoupling endothelial nitric-oxide synthase', *Journal of Biological Chemistry*. J Biol Chem, 278(25), pp. 22546–22554. doi: 10.1074/jbc.M302227200.
82. Lasota, A. and Danowska-Klonowska, D. (2004) 'Experimental osteoporosis—different methods of ovariectomy in female white rats.', *Roczniki Akademii Medycznej w Białymstoku (1995)*, 49 Suppl 1, pp. 129–131.
83. Lee, H., Jeon, H. L., Park, S. J. and Shin, J. Y. (2019) 'Effect of statins, metformin, angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers on age-related macular degeneration', *Yonsei Medical Journal*. Yonsei University College of Medicine, 60(7), pp. 679–686. doi: 10.3349/ymj.2019.60.7.679.
84. Levitan, I., Volkov, S. and Subbaiah, P. V. (2010) 'Oxidized LDL: Diversity, patterns of recognition, and pathophysiology', *Antioxidants and Redox Signaling*. Mary Ann Liebert, Inc., pp. 39–75. doi: 10.1089/ars.2009.2733.
85. Lin, S. H., Cheng, P. C., Tu, S. Te, Hsu, S. R., Cheng, Y. C. and Liu, Y. H. (2018) 'Effect of metformin monotherapy on serum lipid profile in statin-naïve individuals with newly diagnosed type 2 diabetes mellitus: A cohort study', *PeerJ*. PeerJ Inc., 2018(6), p. e4578. doi: 10.7717/peerj.4578.
86. Liu, M. L., Xu, X., Rang, W. Q., Li, Y. J. and Song, H. P. (2004) 'Influence of ovariectomy and 17 β -estradiol treatment on insulin sensitivity, lipid metabolism and post-ischemic cardiac function', *International Journal of Cardiology*. Int J Cardiol, 97(3), pp. 485–493. doi: 10.1016/j.ijcard.2003.11.046.

87. Lobato, N. S., Filgueira, F. P., Hagihara, G. N., Akamine, E. H., Pariz, J. R., Tostes, R. C., Carvalho, M. H. C. and Fortes, Z. B. (2012) 'Improvement of metabolic parameters and vascular function by metformin in obese non-diabetic rats', *Life Sciences*, 90(5–6), pp. 228–235. doi: 10.1016/j.lfs.2011.11.005.
88. Manabe, S., Okura, T., Watanabe, S., Fukuoka, T. and Higaki, J. (2005) 'Effects of Angiotensin II Receptor Blockade with Valsartan on Pro-Inflammatory Cytokines in Patients with Essential Hypertension', *Journal of Cardiovascular Pharmacology*, 46(6), pp. 735–739. doi: 10.1097/01.fjc.0000185783.00391.60.
89. Mangge, H. (2014) 'Antioxidants, inflammation and cardiovascular disease', *World Journal of Cardiology*. Baishideng Publishing Group Inc., 6(6), p. 462. doi: 10.4330/wjc.v6.i6.462.
90. Mendelsohn, M. E. and Karas, R. H. (1999) 'The protective effects of estrogen on the cardiovascular system', *New England Journal of Medicine*. Edited by F. H. Epstein. Massachusetts Medical Society, pp. 1801–1811. doi: 10.1056/NEJM199906103402306.
91. Mihara, M. and Uchiyama, M. (1978) 'Determination of malonaldehyde precursor in tissues by thiobarbituric acid test', *Analytical Biochemistry*, 86(1), pp. 271–278.
92. Mittal, M., Siddiqui, M. R., Tran, K., Reddy, S. P. and Malik, A. B. (2014) 'Reactive oxygen species in inflammation and tissue injury', *Antioxidants and Redox Signaling*. Mary Ann Liebert, Inc., pp. 1126–1167. doi: 10.1089/ars.2012.5149.
93. Münzel, T., Camici, G. G., Maack, C., Bonetti, N. R., Fuster, V. and Kovacic, J. C. (2017) 'Impact of Oxidative Stress on the Heart and Vasculature: Part 2 of a 3-Part Series', *Journal of the American College of Cardiology*. Elsevier USA, pp. 212–229. doi: 10.1016/j.jacc.2017.05.035.
94. Murr, C., Winklhofer-Roob, B. M., Schroecksnadel, K., Maritschnegg, M., Mangge, H., Böhm, B. O., Winkelmann, B. R., März, W. and Fuchs, D. (2009) 'Inverse association between serum concentrations of neopterin and antioxidants in patients with and without angiographic coronary artery disease', *Atherosclerosis*. Atherosclerosis, 202(2), pp. 543–549. doi: 10.1016/j.atherosclerosis.2008.04.047.
95. Nair, A. B. and Jacob, S. (2016) 'A simple practice guide for dose conversion between animals and human.', *Journal of basic and clinical pharmacy*. Wolters Kluwer – Medknow Publications, 7(2), pp. 27–31. doi: 10.4103/0976-0105.177703.
96. Naseem, K. M. (2005) 'The role of nitric oxide in cardiovascular diseases', *Molecular Aspects of Medicine*. Elsevier Ltd, 26(1–2), pp. 33–65. doi: 10.1016/j.mam.2004.09.003.
97. Nasri, H. and Rafieian-Kopaei, M. (2014) 'Metformin: Current knowledge', *Journal of Research in Medical Sciences*. Isfahan University of Medical Sciences(IUMS), pp. 658–664.
98. Nomikos, G., Spyraiki, C., Kazandjian, A. and Sfikakis, A. (1987) 'Estrogen treatment to ovariectomized rats modifies morphine-induced behavior', *Pharmacology, Biochemistry and Behavior*. Elsevier, 27(4), pp. 611–617. doi: 10.1016/0091-3057(87)90182-1.
99. Oliveira, P. W. C., de Sousa, G. J., Caliman, I. F., Lamas, A. Z., Santos de Medeiros, A. R., de Andrade, T. U., de Abreu, G. R., de Figueiredo, S. G. and Bissoli, N. S. (2014) 'Metformin ameliorates ovariectomy-

- induced vascular dysfunction in non-diabetic wistar rats', *Clinical Science*. Portland Press Ltd, 127(4), pp. 265–275. doi: 10.1042/CS20130553.
100. Parthasarathy, S., Raghavamenon, A., Garelnabi, M. O. and Santanam, N. (2010) 'Oxidized low-density lipoprotein.', *Methods in molecular biology (Clifton, N.J.)*, 610, pp. 403–17. doi: 10.1007/978-1-60327-029-8_24.
 101. Persky, A. M., Green, P. S., Stublely, L., Howell, C. O., Zaulyanov, L., Brazeau, G. A. and Simpkins, J. W. (2000) 'Protective effect of estrogens against oxidative damage to heart and skeletal muscle in vivo and in vitro', *Proceedings of the Society for Experimental Biology and Medicine*. Blackwell Publishing Inc., 223(1), pp. 59–66. doi: 10.1046/j.1525-1373.2000.22308.x.
 102. Phan, B. A. P. and Toth, P. P. (2014) 'Dyslipidemia in women: Etiology and management', *International Journal of Women's Health*. Dove Press, pp. 185–194. doi: 10.2147/IJWH.S38133.
 103. Rezzani, R., Bonomini, F., Tengattini, S., Fabiano, A. and Bianchi, R. (2008) 'Atherosclerosis and oxidative stress', *Histology and Histopathology*. Histol Histopathol, 23(3), pp. 381–390. doi: 10.14670/HH-23.381.
 104. Ronchetti, S. A., Machiavelli, L. I., Quinteros, F. A., Duvilanski, B. H. and Cabilla, J. P. (2016) 'Nitric Oxide Plays a Key Role in Ovariectomy-Induced Apoptosis in Anterior Pituitary: Interplay between Nitric Oxide Pathway and Estrogen', *PLOS ONE*. Edited by M. Colasanti. Public Library of Science, 11(9), p. e0162455. doi: 10.1371/journal.pone.0162455.
 105. Roth, G. A., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C., Abbasi, N., Abbastabar, H., Abd-Allah, F., Abdela, J., Abdelalim, A., Abdollahpour, I., Abdulkader, R. S., Abebe, H. T., Abebe, M., Abebe, Z., Abejie, A. N., Abera, S. F., Abil, O. Z., Abraha, H. N., Abrham, A. R., Abu-Raddad, L. J., Accrombessi, M. M. K., Acharya, D., Adamu, A. A., Adebayo, O. M., Adedoyin, R. A., Adekanmbi, V., Adetokunboh, O. O., Adhena, B. M., Adib, M. G., Admasie, A., Afshin, A., Agarwal, G., Agesa, K. M., Agrawal, A., Agrawal, S., Ahmadi, A., Ahmadi, M., Ahmed, M. B., Ahmed, S., Aichour, A. N., Aichour, I., Aichour, M. T. E., Akbari, M. E., Akinyemi, R. O., Akseer, N., Al-Aly, Z., Al-Eyadhy, A., Al-Raddadi, R. M., Alahdab, F., Alam, K., Alam, T., Alebel, A., Alene, K. A., Alijanzadeh, M., Alizadeh-Navaei, R., Aljunid, S. M., Alkerwi, A., Alla, F., Allebeck, P., Alonso, J., Altirkawi, K., Alvis-Guzman, N., Amare, A. T., Aminde, L. N., Amini, E., Ammar, W., Amoako, Y. A., Anber, N. H., Andrei, C. L., Androudi, S., Animut, M. D., Anjomshoa, M., Ansari, H., Ansha, M. G., Antonio, C. A. T., Anwari, P., Aremu, O., Arnlov, J., Arora, A., Arora, M., Artaman, A., Aryal, K. K., Asayesh, H., Asfaw, E. T., Ataro, Z., Atique, S., Atre, S. R., Ausloos, M., Avokpaho, E. F. G. A., Awasthi, A., Ayala Quintanilla, B. P., Ayele, Y., Ayer, R., Azzopardi, P. S., Babazadeh, A., Bacha, U., Badali, H., Badawi, A., Bali, A. G., Ballesteros, K. E., Banach, M., Banerjee, K., Bannick, M. S., Banoub, J. A. M., Barboza, M. A., Barker-Collo, S. L., Barnighausen, T. W., Barquera, S., Barrero, L. H., Bassat, Q., Basu, S., Baune, B. T., Baynes, H. W., Bazargan-Hejazi, S., Bedi, N., Beghi, E., Behzadifar, Masoud, Behzadifar, Meysam, Bejot, Y., Bekele, B. B., Belachew, A. B., Belay, E., Belay, Y. A., Bell, M. L., Bello, A. K., Bennett, D. A., Bensenor, I. M., Berman, A. E., Bernabe, E., Bernstein, R. S., Bertolacci, G. J., Beuran, M., Beyranvand, T., Bhalla, A., Bhattarai, S., Bhaumik, S., Bhutta, Z. A., Biadgo, B., Biehl, M. H., Bijani, A., Bikbov, B., Bilano, V., Bililign, N., Bin Sayeed, M. S., Bisanzio, D., Biswas, T., Blacker, B. F., Basara, B. B., Borschmann, R., Bosetti, C., Bozorgmehr, K., Brady, O. J., Brant, L. C., Brayne, C., Brazinova, A.,

Breitborde, N. J. K., Brenner, H., Briant, P. S., Britton, G., Brugha, T., Busse, R., Butt, Z. A., Callender, C. S. K. H., Campos-Nonato, I. R., Campuzano Rincon, J. C., Cano, J., Car, M., Cardenas, R., Carreras, G., Carrero, J. J., Carter, A., Carvalho, F., Castaneda-Orjuela, C. A., Castillo Rivas, J., Castle, C. D., Castro, C., Castro, F., Catala-Lopez, F., Cerin, E., Chaiah, Y., Chang, J. C., Charlson, F. J., Chaturvedi, P., Chiang, P. P. C., Chimed-Ochir, O., Chisumpa, V. H., Chitheer, A., Chowdhury, R., Christensen, H., Christopher, D. J., Chung, S. C., Cicuttini, F. M., Ciobanu, L. G., Cirillo, M., Cohen, A. J., Cooper, L. T., Cortesi, P. A., Cortinovis, M., Cousin, E., Cowie, B. C., Criqui, M. H., Cromwell, E. A., Crowe, C. S., Crump, J. A., Cunningham, M., Daba, A. K., Dadi, A. F., Dandona, L., Dandona, R., Dang, A. K., Dargan, P. I., Daryani, A., Das, S. K., Gupta, Rajat Das, Neves, J. Das, Dasa, T. T., Dash, A. P., Davis, A. C., Davis Weaver, N., Davitoiu, D. V., Davletov, K., De La Hoz, F. P., De Neve, J. W., Degefa, M. G., Degenhardt, L., Degfie, T. T., Deiparine, S., Demoz, G. T., Demtsu, B. B., Denova-Gutierrez, E., Deribe, K., Dervenis, N., Des Jarlais, D. C., Dessie, G. A., Dey, S., Dharmaratne, S. D., Dicker, D., Dinberu, M. T., Ding, E. L., Dirac, M. A., Djalalinia, S., Dokova, K., Doku, D. T., Donnelly, C. A., Dorsey, E. R., Doshi, P. P., Douwes-Schultz, D., Doyle, K. E., Driscoll, T. R., Dubey, M., Dubljanin, E., Duken, E. E., Duncan, B. B., Duraes, A. R., Ebrahimi, H., Ebrahimpour, S., Edessa, D., Edvardsson, D., Eggen, A. E., El Bcheraoui, C., El Sayed Zaki, M., El-Khatib, Z., Elkout, H., Ellingsen, C. L., Endres, M., Endries, A. Y., Er, B., Erskine, H. E., Eshrati, B., Eskandarieh, S., Esmaeili, R., Esteghamati, A., Fakhar, M., Fakhim, H., Faramarzi, M., Fareed, M., Farhadi, F., Farinha, C. S. E., Faro, A., Farvid, M. S., Farzadfar, F., Farzaei, M. H., Feigin, V. L., Feigl, A. B., Fentahun, N., Fereshtehnejad, S. M., Fernandes, E., Fernandes, J. C., Ferrari, A. J., Feyissa, G. T., Filip, I., Finegold, S., Fischer, F., Fitzmaurice, C., Foigt, N. A., Foreman, K. J., Fornari, C., Frank, T. D., Fukumoto, T., Fuller, J. E., Fullman, N., Furst, T., Furtado, J. M., Futran, N. D., Gallus, S., Garcia-Basteiro, A. L., Garcia-Gordillo, M. A., Gardner, W. M., Gebre, A. K., Gebrehiwot, T. T., Gebremedhin, A. T., Gebremichael, B., Gebremichael, T. G., Gelano, T. F., Geleijnse, J. M., Genova-Maleras, R., Geramo, Y. C. D., Gething, P. W., Gezae, K. E., Ghadami, M. R., Ghadimi, R., Ghasemi Falavarjani, K., Ghasemi-Kasman, M., Ghimire, M., Gibney, K. B., Gill, P. S., Gill, T. K., Gillum, R. F., Ginawi, I. A., Giroud, M., Giussani, G., Goenka, S., Goldberg, E. M., Goli, S., Gomez-Dantes, H., Gona, P. N., Gopalani, S. V., Gorman, T. M., Goto, A., Goulart, A. C., Gnedovskaya, E. V., Grada, A., Grosso, G., Gughani, H. C., Guimaraes, A. L. S., Guo, Y., Gupta, P. C., Gupta, Rahul, Gupta, Rajeev, Gupta, T., Gutierrez, R. A., Gyawali, B., Haagsma, J. A., Hafezi-Nejad, N., Hagos, T. B., Hailegiyorgis, T. T., Hailu, G. B., Haj-Mirzaian, Arvin, Haj-Mirzaian, Arya, Hamadeh, R. R., Hamidi, S., Handal, A. J., Hankey, G. J., Harb, H. L., Harikrishnan, S., Haro, J. M., Hasan, M., Hassankhani, H., Hassen, H. Y., Havmoeller, R., Hay, R. J., Hay, S. I., He, Y., Hedayatizadeh-Omran, A., Hegazy, M. I., Heibati, B., Heidari, M., Hendrie, D., Henok, A., Henry, N. J., Herteliu, C., Heydarpour, F., Heydarpour, P., Heydarpour, S., Hibstu, D. T., Hoek, H. W., Hole, M. K., Homaie Rad, E., Hoogar, P., Hosgood, H. D., Hosseini, S. M., Hosseinzadeh, M., Hostiuc, M., Hostiuc, S., Hotez, P. J., Hoy, D. G., Hsiao, T., Hu, G., Huang, J. J., Husseini, A., Hussen, M. M., Hutfless, S., Idrisov, B., Ilesanmi, O. S., Iqbal, U., Irvani, S. S. N., Irvine, C. M. S., Islam, N., Islam, S. M. S., Islami, F., Jacobsen, K. H., Jahangiry, L., Jahanmehr, N., Jain, S. K., Jakovljevic, M., Jalu, M. T., James, S. L., Javanbakht, M., Jayatilleke, A. U., Jeemon, P., Jenkins, K. J., Jha, R. P., Jha, V., Johnson, C. O., Johnson, S. C., Jonas, J. B., Joshi, A., Jozwiak, J. J., Jungari, S. B., Jurisson, M., Kabir, Z., Kadel, R., Kahsay, A., Kalani, R., Karami, M., Karami Matin, B., Karch, A., Karema, C., Karimi-Sari, H., Kasaeian, A.,

Kassa, D. H., Kassa, G. M., Kassa, T. D., Kassebaum, N. J., Katikireddi, S. V., Kaul, A., Kazemi, Z., Kazemi Karyani, A., Kazi, D. S., Kefale, A. T., Keiyoro, P. N., Kemp, G. R., Kengne, A. P., Keren, A., Kesavachandran, C. N., Khader, Y. S., Khafaei, B., Khafaie, M. A., Khajavi, A., Khalid, N., Khalil, I. A., Khan, E. A., Khan, M. S., Khan, M. A., Khang, Y. H., Khater, M. M., Khoja, A. T., Khosravi, A., Khosravi, M. H., Khubchandani, J., Kiadaliri, A. A., Kibret, G. D., Kidanemariam, Z. T., Kiirithio, D. N., Kim, D., Kim, Y. E., Kim, Y. J., Kimokoti, R. W., Kinfu, Y., Kisa, A., Kissimova-Skarbek, K., Kivimaki, M., Knudsen, A. K. S., Kocarnik, J. M., Kochhar, S., Kokubo, Y., Kolola, T., Kopec, J. A., Koul, P. A., Koyanagi, A., Kravchenko, M. A., Krishan, K., Kuate Defo, B., Kucuk Bicer, B., Kumar, G. A., Kumar, M., Kumar, P., Kutz, M. J., Kuzin, I., Kyu, H. H., Lad, D. P., Lad, S. D., Lafranconi, A., Lal, D. K., Lalloo, R., Lallukka, T., Lam, J. O., Lami, F. H., Lansingh, V. C., Lansky, S., Larson, H. J., Latifi, A., Lau, K. M. M., Lazarus, J. V., Lebedev, G., Lee, P. H., Leigh, J., Leili, M., Leshargie, C. T., Li, S., Li, Y., Liang, J., Lim, L. L., Lim, S. S., Limenih, M. A., Linn, S., Liu, S., Liu, Y., Lodha, R., Lonsdale, C., Lopez, A. D., Lorkowski, S., Lotufo, P. A., Lozano, R., Lunevicius, R., Ma, S., Macarayan, E. R. K., Mackay, M. T., MacLachlan, J. H., Maddison, E. R., Madotto, F., Magdy Abd El Razek, H., Magdy Abd El Razek, M., Maghavani, D. P., Majdan, M., Majdzadeh, R., Majeed, A., Malekzadeh, R., Malta, D. C., Manda, A. L., Mandarano-Filho, L. G., Manguerra, H., Mansournia, M. A., Mapoma, C. C., Marami, D., Maravilla, J. C., Marcenes, W., Marczak, L., Marks, A., Marks, G. B., Martinez, G., Martins-Melo, F. R., Martopullo, I., Marz, W., Marzan, M. B., Masci, J. R., Massenburg, B. B., Mathur, M. R., Mathur, P., Matzopoulos, R., Maulik, P. K., Mazidi, M., McAlinden, C., McGrath, J. J., McKee, M., McMahon, B. J., Mehata, S., Mehndiratta, M. M., Mehrotra, R., Mehta, K. M., Mehta, V., Mekonnen, T. C., Melese, A., Melku, M., Memiah, P. T. N., Memish, Z. A., Mendoza, W., Mengistu, D. T., Mengistu, G., Mensah, G. A., Mereta, S. T., Meretoja, A., Meretoja, T. J., Mestrovic, T., Mezgebe, H. B., Miazgowski, B., Miazgowski, T., Millea, A. I., Miller, T. R., Miller-Petrie, M. K., Mini, G. K., Mirabi, P., Mirarefin, M., Mirica, A., Mirakhimov, E. M., Misganaw, A. T., Mitiku, H., Moazen, B., Mohammad, K. A., Mohammadi, M., Mohammadifard, N., Mohammed, M. A., Mohammed, S., Mohan, V., Mokdad, A. H., Molokhia, M., Monasta, L., Moradi, G., Moradi-Lakeh, M., Moradinazar, M., Moraga, P., Morawska, L., Velasquez, I. M., Morgado-Da-Costa, J., Morrison, S. D., Moschos, M. M., Mouodi, S., Mousavi, S. M., Muchie, K. F., Mueller, U. O., Mukhopadhyay, S., Muller, K., Mumford, J. E., Musa, J., Musa, K. I., Mustafa, G., Muthupandian, S., Nachega, J. B., Nagel, G., Naheed, A., Nahvijou, A., Naik, G., Nair, S., Najafi, F., Naldi, L., Nam, H. S., Nangia, V., Nansseu, J. R., Nascimento, B. R., Natarajan, G., Neamati, N., Nego, I., Nego, R. I., Neupane, S., Newton, C. R. J., Ngalesoni, F. N., Ngunjiri, J. W., Nguyen, A. Q., Nguyen, G., Nguyen, Ha Thu, Nguyen, Huong Thanh, Nguyen, L. H., Nguyen, M., Nguyen, T. H., Nichols, E., Ningrum, D. N. A., Nirayo, Y. L., Nixon, M. R., Nolutshungu, N., Nomura, S., Norheim, O. F., Noroozi, M., Norrving, B., Noubiap, J. J., Nouri, H. R., Shiadeh, M. N., Nowroozi, M. R., Nyasulu, P. S., Odell, C. M., Ofori-Asenso, R., Ogbo, F. A., Oh, I. H., Oladimeji, O., Olagunju, A. T., Olivares, P. R., Olsen, H. E., Olusanya, B. O., Olusanya, J. O., Ong, K. L., Ong, S. K. S., Oren, E., Orpana, H. M., Ortiz, A., Ortiz, J. R., Otstavnov, S. S., Overland, S., Owolabi, M. O., Ozdemir, R., Mahesh, P. A., Pacella, R., Pakhale, S., Pakhare, A. P., Pakpour, A. H., Pana, A., Panda-Jonas, S., Pandian, J. D., Parisi, A., Park, E. K., Parry, C. D. H., Parsian, H., Patel, S., Pati, S., Patton, G. C., Paturi, V. R., Paulson, K. R., Pereira, A., Pereira, D. M., Perico, N., Pesudovs, K., Petzold, M., Phillips, M. R., Piel, F. B., Pigott, D. M., Pillay, J. D., Pirsheh, M., Pishgar, F., Polinder, S., Postma, M. J.,

Pourshams, A., Poustchi, H., Pujar, A., Prakash, S., Prasad, N., Purcell, C. A., Qorbani, M., Quintana, H., Quistberg, D. A., Rade, K. W., Anwar Rafay, A. R., Rahim, F., Rahimi, K., Rahimi-Movaghar, A., Rahman, M., Rahman, M. H. U., Rahman, M. A., Rai, R. K., Rajsic, S., Ram, U., Ranabhat, C. L., Ranjan, P., Rao, P. C., Rawaf, D. L., Rawaf, S., Razo-Garcia, C., Reddy, K. S., Reiner, R. C., Reitsma, M. B., Remuzzi, G., Renzaho, A. M. N., Resnikoff, S., Rezaei, S., Rezaeian, S., Rezai, M. S., Riahi, S. M., Ribeiro, A. L. P., Rios-Blancas, M. J., Roba, K. T., Roberts, N. L. S., Robinson, S. R., Roeber, L., Ronfani, L., Roshandel, G., Rostami, A., Rothenbacher, D., Roy, A., Rubagotti, E., Sachdev, P. S., Saddik, B., Sadeghi, E., Safari, H., Safdarian, M., Safi, S., Safiri, S., Sagar, R., Sahebkar, A., Sahraian, M. A., Salam, N., Salama, J. S., Salamati, P., De Freitas Saldanha, R., Saleem, Z., Salimi, Y., Salvi, S. S., Salz, I., Sambala, E. Z., Samy, A. M., Sanabria, J., Dolores Sanchez-Nino, M., Santomauro, D. F., Santos, I. S., Santos, J. V., Santric Milicevic, M. M., Jose, B. P. S., Sarker, A. R., Sarmiento-Suarez, R., Sarrafzadegan, N., Sartorius, B., Sarvi, S., Sathian, B., Satpathy, M., Sawant, A. R., Sawhney, M., Saxena, S., Sayyah, M., Schaeffner, E., Schmidt, M. I., Schneider, I. J. C., Schottker, B., Schutte, A. E., Schwebel, D. C., Schwendicke, F., Scott, J. G., Sekerija, M., Sepanlou, S. G., Servan-Mori, E., Seyedmousavi, S., Shabaninejad, H., Shackelford, K. A., Shafieesabet, A., Shahbazi, M., Shaheen, A. A., Shaikh, M. A., Shams-Beyranvand, M., Shamsi, M., Shamsizadeh, M., Sharafi, K., Sharif, M., Sharif-Alhoseini, M., Sharma, R., She, J., Sheikh, A., Shi, P., Shiferaw, M. S., Shigematsu, M., Shiri, R., Shirkoobi, R., Shiue, I., Shokraneh, F., Shrim, M. G., Si, S., Siabani, S., Siddiqi, T. J., Sigfusdottir, I. D., Sigurvinsdottir, R., Silberberg, D. H., Santos Silva, D. A., Silva, J. P., Da Silva, N. T., Silveira, D. G. A., Singh, J. A., Singh, N. P., Singh, P. K., Singh, V., Sinha, D. N., Sliwa, K., Smith, M., Sobaih, B. H., Sobhani, S., Sobngwi, E., Soneji, S. S., Soofi, M., Sorensen, R. J. D., Soriano, J. B., Soyiri, I. N., Sposato, L. A., Sreeramareddy, C. T., Srinivasan, V., Stanaway, J. D., Starodubov, V. I., Stathopoulou, V., Stein, D. J., Steiner, C., Stewart, L. G., Stokes, M. A., Subart, M. L., Sudaryanto, A., Sufiyan, M. B., Sur, P. J., Sutradhar, I., Sykes, B. L., Sylaja, P. N., Sylte, D. O., Szoek, C. E. I., Tabares-Seisdedos, R., Tabuchi, T., Tadakamadla, S. K., Takahashi, K., Tandon, N., Tassew, S. G., Taveira, N., Tehrani-Banihashemi, A., Tekalign, T. G., Tekle, M. G., Temsah, M. H., Temsah, O., Terkawi, A. S., Teshale, M. Y., Tessema, B., Tessema, G. A., Thankappan, K. R., Thirunavukkarasu, S., Thomas, N., Thrift, A. G., Thurston, G. D., Tilahun, B., To, Q. G., Tobe-Gai, R., Tonelli, M., Topor-Madry, R., Torre, A. E., Tortajada-Girbes, M., Tournier, M., Tovani-Palone, M. R., Tran, B. X., Tran, K. B., Tripathi, S., Troeger, C. E., Truelsen, T. C., Truong, N. T., Tsadik, A. G., Tsoi, D., Tudor Car, L., Murat Tuzcu, E., Tyrovolas, S., Ukwaja, K. N., Ullah, I., Undurraga, E. A., Updike, R. L., Usman, M. S., Uthman, O. A., Uzun, S. B., Vaduganathan, M., Vaezi, A., Vaidya, G., Valdez, P. R., Varavikova, E., Vasankari, T. J., Venketasubramanian, N., Villafaina, S., Violante, F. S., Vladimirov, S. K., Vlassov, V., Vollset, S. E., Vos, T., Wagner, G. R., Wagnew, F. S., Waheed, Y., Wallin, M. T., Walson, J. L., Wang, Y., Wang, Y. P., Wassie, M. M., Weidpass, E., Weintraub, R. G., Weldegebreal, F., Weldegewergs, K. G., Werdecker, A., Werkneh, A. A., West, T. E., Westerman, R., Whiteford, H. A., Widecka, J., Wilner, L. B., Wilson, S., Winkler, A. S., Wiysonge, C. S., Wolfe, C. D. A., Wu, S., Wu, Y. C., Wyper, G. M. A., Xavier, D., Xu, G., Yadgir, S., Yadollahpour, A., Yahyazadeh Jabbari, S. H., Yakob, B., Yan, L. L., Yano, Y., Yaseri, M., Yasin, Y. J., Yentur, G. K., Yeshaneh, A., Yimer, E. M., Yip, P., Yirsaw, B. D., Yisma, E., Yonemoto, N., Yonga, G., Yoon, S. J., Yotebieng, M., Younis, M. Z., Yousefifard, M., Yu, C., Zadnik, V., Zaidi, Z., Zaman, S. Bin, Zamani, M., Zare, Z., Zeleke, A. J., Zenebe, Z. M., Zhang, A. L., Zhang, K., Zhou, M., Zodpey, S., Zuhlke, L. J.,

- Naghavi, M. and Murray, C. J. L. (2018) 'Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017', *The Lancet*. Lancet Publishing Group, 392(10159), pp. 1736–1788. doi: 10.1016/S0140-6736(18)32203-7.
106. Stocker, R. and Keaney, J. F. (2004) 'Role of oxidative modifications in atherosclerosis', *Physiological Reviews*. doi: 10.1152/physrev.00047.2003.
 107. Suzuki, S., Brown, C. M. and Wise, P. M. (2006) 'Mechanisms of neuroprotection by estrogen', *Endocrine*. Endocrine, pp. 209–215. doi: 10.1385/ENDO:29:2:209.
 108. Torre-Amione, G. (2005) 'Immune activation in chronic heart failure', in *American Journal of Cardiology*. Elsevier Inc., pp. 3–8. doi: 10.1016/j.amjcard.2005.03.006.
 109. Trott, D. W., Seawright, J. W., Luttrell, M. J. and Woodman, C. R. (2011) 'NAD(P)H oxidase-derived reactive oxygen species contribute to age-related impairments of endothelium-dependent dilation in rat soleus feed arteries', *Journal of Applied Physiology*. American Physiological Society, 110(5), pp. 1171–1180. doi: 10.1152/japplphysiol.01037.2010.
 110. UK Prospective Diabetes Study (UKPDS) Group (1998) 'Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34)', *Lancet*. Elsevier Limited, 352(9131), pp. 854–865. doi: 10.1016/S0140-6736(98)07037-8.
 111. Wang, X. F., Zhang, J. Y., Li, L., Zhao, X. Y., Tao, H. L. and Zhang, L. (2011) 'Metformin improves cardiac function in rats via activation of AMP-activated protein kinase', *Clinical and Experimental Pharmacology and Physiology*. Clin Exp Pharmacol Physiol, 38(2), pp. 94–101. doi: 10.1111/j.1440-1681.2010.05470.x.
 112. Wu, T.-T., Gao, Y., Zheng, Y.-Y., Ma, Y.-T. and Xie, X. (2018) 'Atherogenic index of plasma (AIP): a novel predictive indicator for the coronary artery disease in postmenopausal women.', *Lipids in health and disease*. BioMed Central, 17(1), p. 197. doi: 10.1186/s12944-018-0828-z.
 113. Xu, Y., Arenas, I. A., Armstrong, S. J., Plahta, W. C., Xu, H. and Davidge, S. T. (2006) 'Estrogen improves cardiac recovery after ischemia/reperfusion by decreasing tumor necrosis factor- α ', *Cardiovascular Research*. Cardiovasc Res, 69(4), pp. 836–844. doi: 10.1016/j.cardiores.2005.11.031.
 114. Yamada, T., Ogawa, K., Tanaka, T. D., Nagoshi, T., Minai, K., Ogawa, T., Kawai, M. and Yoshimura, M. (2020) 'Increase in oxidized low-density lipoprotein level according to hyperglycemia in patients with cardiovascular disease: A study by structure equation modeling', *Diabetes Research and Clinical Practice*, 161, p. 108036. doi: 10.1016/j.diabres.2020.108036.
 115. Yan, L., Kang, B., Li, G., Yin, Z. and Wang, Y. (2009) 'Effects of metformin on serum levels of sex hormone, leptin and insulin in ovariectomized Sprague-Dawley rats', *Pharmazie*, 64(12). doi: 10.1691/ph.2009.9646.
 116. Yang, D., Elner, S. G., Bian, Z. M., Till, G. O., Petty, H. R. and Elner, V. M. (2007) 'Pro-inflammatory cytokines increase reactive oxygen species through mitochondria and NADPH oxidase in cultured RPE cells', *Experimental Eye Research*. NIH Public Access, 85(4), pp. 462–472. doi: 10.1016/j.exer.2007.06.013.

117. Yung, L. M., Wong, W. T., Tian, X. Y., Leung, F. P., Yung, L. H., Chen, Z. Y., Yao, X., Lau, C. W. and Huang, Y. (2011) 'Inhibition of Renin-Angiotensin System Reverses Endothelial Dysfunction and Oxidative Stress in Estrogen Deficient Rats', *PLoS ONE*. Edited by C. Borras. Public Library of Science, 6(3), p. e17437. doi: 10.1371/journal.pone.0017437.
118. Zhang, F., Sun, W., Chen, J., Jiang, L., Yang, P., Huang, Y., Gong, A., Liu, S. and Ma, S. (2018) 'SREBP-2, a new target of metformin?', *Drug Design, Development and Therapy*. Dove Medical Press Ltd., 12, pp. 4163–4170. doi: 10.2147/DDDT.S190094.
119. Zhu, D., Chung, H. F., Dobson, A. J., Pandeya, N., Giles, G. G., Bruinsma, F., Brunner, E. J., Kuh, D., Hardy, R., Avis, N. E., Gold, E. B., Derby, C. A., Matthews, K. A., Cade, J. E., Greenwood, D. C., Demakakos, P., Brown, D. E., Sievert, L. L., Anderson, D., Hayashi, K., Lee, J. S., Mizunuma, H., Tillin, T., Simonsen, M. K., Adami, H. O., Weiderpass, E. and Mishra, G. D. (2019) 'Age at natural menopause and risk of incident cardiovascular disease: a pooled analysis of individual patient data', *The Lancet Public Health*. Elsevier Ltd, 4(11), pp. e553–e564. doi: 10.1016/S2468-2667(19)30155-0.
120. Zilinyi, R., Czompa, A., Czegledi, A., Gajtko, A., Pituk, D., Lekli, I. and Tosaki, A. (2018) 'The cardioprotective effect of metformin in doxorubicin-induced cardiotoxicity: The role of autophagy', *Molecules*. MDPI AG, 23(5). doi: 10.3390/molecules23051184.

Figures

Figure 1

Significant effects of ovariectomy (OVX), estradiol (OVX + E2) and metformin (OVX + MF) on A: tumor necrosis factor- α (TNF α), B: Interleukin 6 (IL-6) and C: Total nitrite and nitrate (NOx). Data are presented as means $\pm$ SD, n = 10. a - significant difference versus sham (SH) control animals; b - significant difference versus ovariectomized (OVX) animals and c - significant difference versus estradiol treatment. * = $p < 0.05$, ** = $p < 0.01$, and*** = $p < 0.001$.