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Chronic inorganic **nitrate administration** increases the expression of genes involved in the browning of gonadal adipose tissue in **ovariectomized** rats

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Introduction

Introduction

Menopause

- 10% of the world's adult population
- 1.2 billion by **2030**
- Increased risk of type 2 diabetes and obesity

Ng M, Fleming T et al. *The Lancet*, 2014

Flegal KM et al. *Jama*, 2016

Hales CM et al. *Jama*, 2018

Introduction

Menopause and Obesity

➤ Prevalence

- ✓ 857 million (1993)
- ✓ 2.1 billion (2013)
- ✓ 3.7 billion (2025)

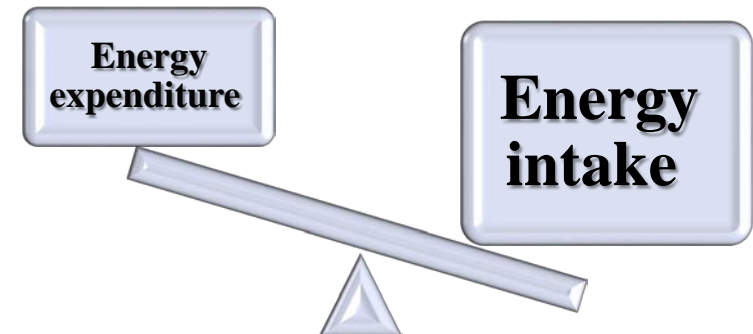
➤ Women are more susceptible to obesity

- ✓ 35.7% in 2006 to 40.8% in 2016
- ✓ increases from 12-17% in premenopausal women to 19-25% in postmenopausal women

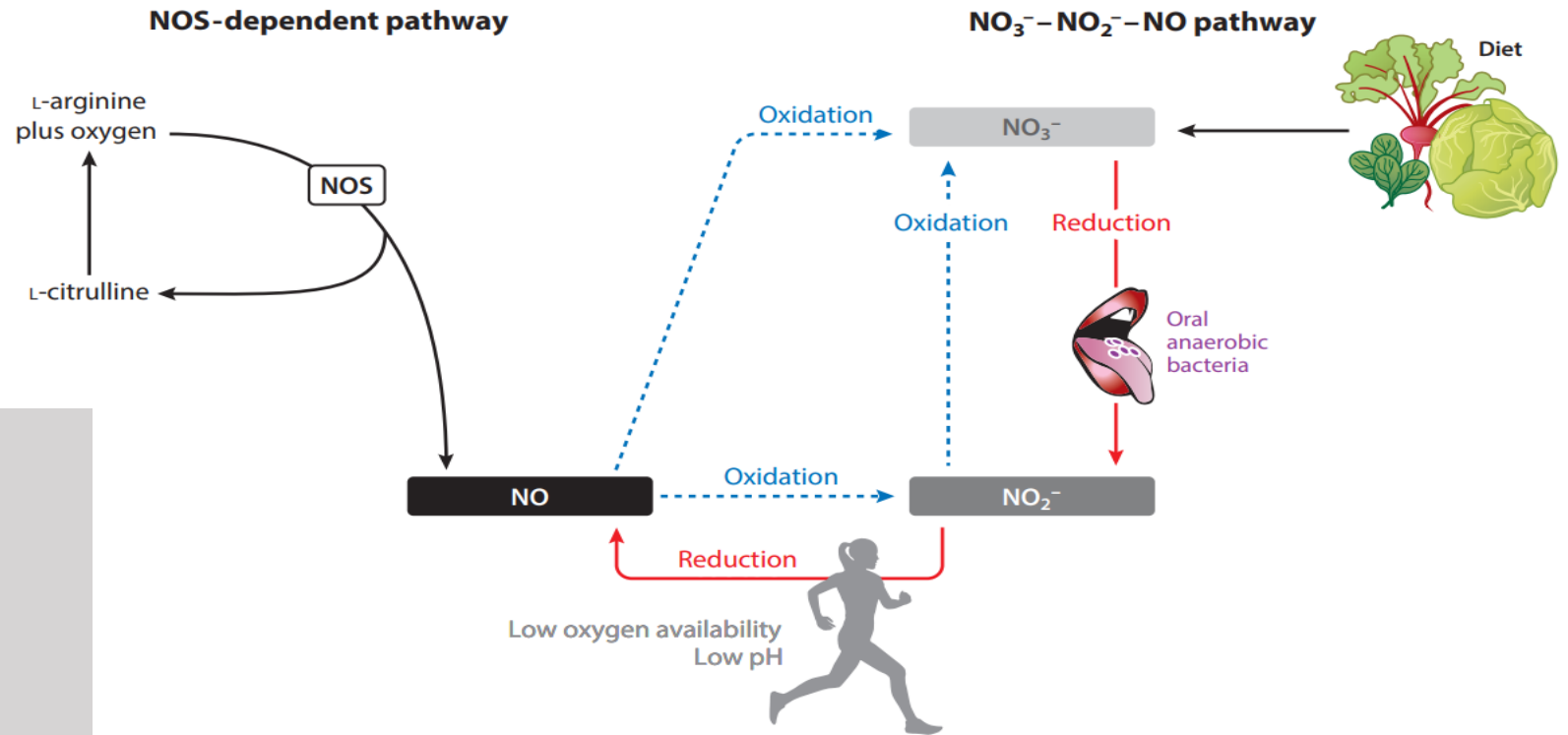
Ng M, Fleming T et al. *The Lancet*, 2014

Flegal KM et al. *Jama*, 2016

Hales CM et al. *Jama*, 2018



The pathways of nitric oxide (NO) production

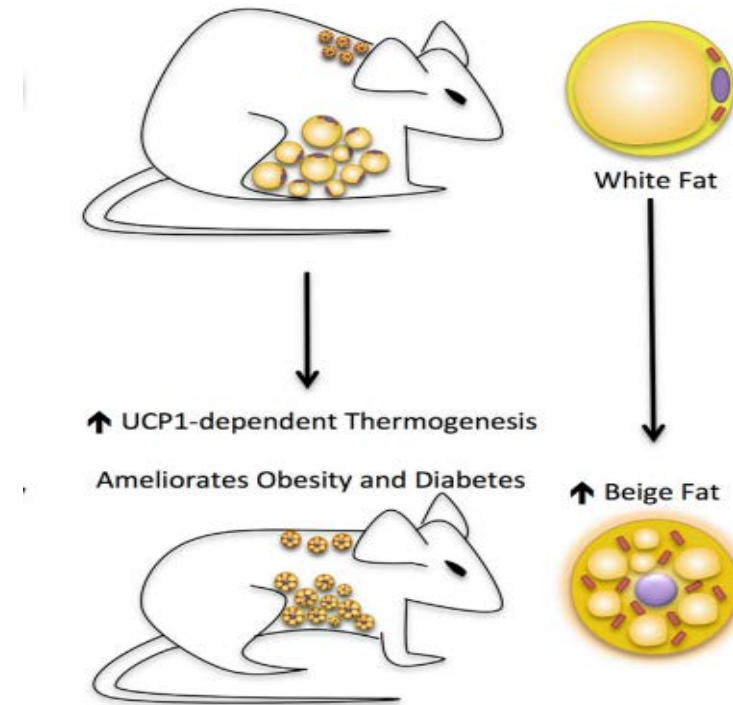
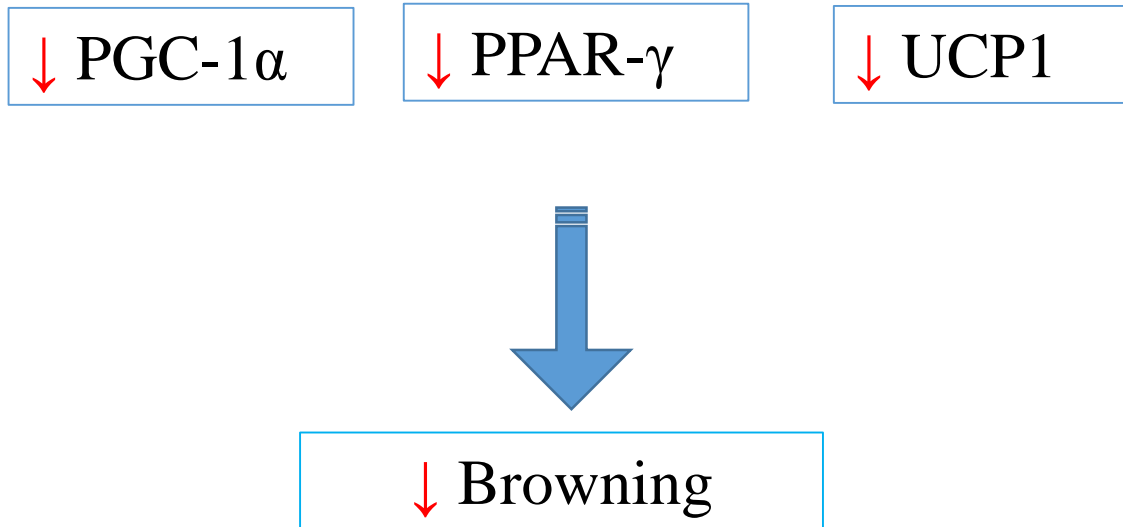


Nitric oxide synthase (NOS)

1. Neural (nNOS/NOS1)
2. Inducible (iNOS/NOS2)
3. Endothelial (eNOS/NOS3)

Menopause and Obesity is associated with nitric oxide (NO) deficiency in both humans and animals

Introduction



➤ Knockout or inhibition of UCP1, PGC1- α , and PPAR- γ **increase risk of obesity**

PPAR- γ , peroxisome proliferator-activated receptor- γ ; PGC-1 α , PPAR- γ coactivator 1 α ; UCP1, uncoupling protein 1

Introduction

Despite the higher prevalence of obesity in **menopause women**, anti-obesity effects of inorganic **nitrate/nitrite** have mainly been studied in male animals.

Women vs. Men

Nitrate-nitrite-NO pathway

- ✓ Higher activity of the nitrate-nitrite-NO pathway
- ✓ Higher values of whole-body production and activity of NO
- ✓ Oral nitrate-reducing capacity
- ✓ Higher increases in nitrite level in serum (2-fold) following nitrate administration

Browning of WAT

- ✓ Higher expression of genes involved in browning of WAT
- ✓ Higher potential to induce browning of WAT
- ✓ Higher mass and activity of brown adipose tissue

Jackson JK et al. *Nutrition reviews* .2018

Kapil Vet al. *Hypertension*. 2010

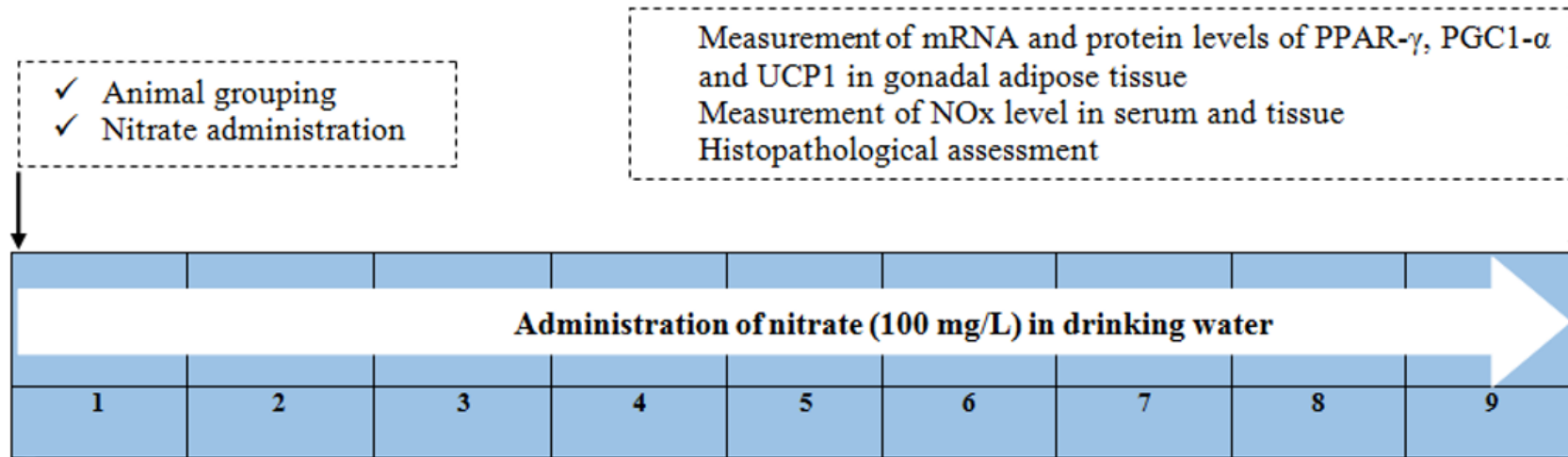
Forte P. *Hypertension*. 1998

Kapil V et al. *Free radical biology & medicine*. 2018

Material & methods

Methods

Experimental protocol and the timeline of the study



PPAR- γ , peroxisome proliferator-activated receptor- γ ; PGC-1 α , PPAR- γ coactivator 1 α ;
UCP1, uncoupling protein 1

- **NO metabolites (nitrite+nitrate; NO_x) concentration:** Griess method.
- **cGMP concentration:** Rat specific ELISA kit (ZellBioGmbH, Germany)
- **Protein levels of PPAR- γ , PGC-1 α , and UCP1:** Commercial ELISA kits (ZellBio GmbH, Germany).
- **mRNA expression of PPAR- γ , PGC-1 α , and UCP1 :** Real time PCR, (Corbett Rotor-Gene 6000, Germany)
- **Browning and size of adipocytes:** H&E staining and consequently stereology

Methods

Statistical analysis

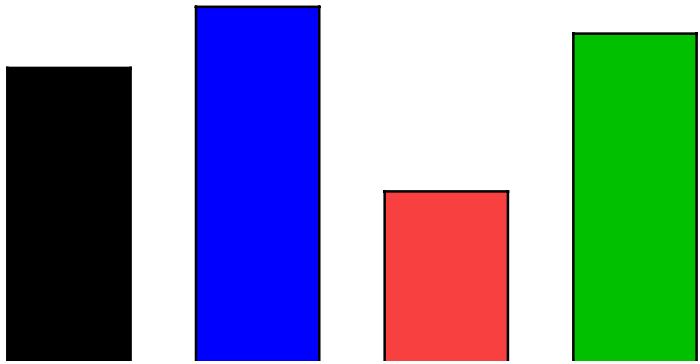
- ❑ Data were analyzed using the **GraphPad Prism** software (Version 6)
- ❑ Values are expressed as **mean ± SEM**.
- ❑ **Two-way mixed (betweenwithin)** analysis of variance (ANOVA) followed by the Bonferroni posthoc test was used for comparing mean values for verification
- ❑ **One-way ANOVA** followed by the Bonferroni post-hoc test was used to compare protein levels of UCP1, PPAR- γ , and PGC1- α , NO_x, and cGMP concentrations, as well as adipocyte density and area in gonadal adipose tissue samples between groups.
- ❑ Fold changes in mRNA expression for UCP1, PPAR- γ , and PGC1- α were calculated by the **$2^{-\Delta\Delta Ct}$ method** and the Mann-Whitney U test was used for comparing fold changes between groups.
- ❑ Two-sided **P-values < 0.05** were considered statistically significant.

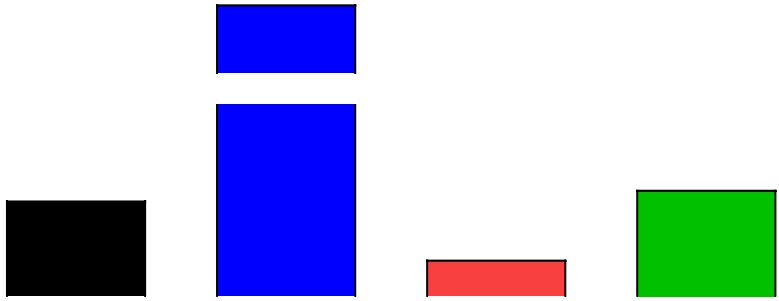
Results

Verification of ovariectomy in rats

Parameter	Before ovariectomy	After ovariectomy
Estradiol (pg/mL)	112.7 ± 10.8	41.12 ± 10.2*
Progesterone (ng/mL)	55.9 ± 7.2	14.2 ± 2.2*
Luteinizing hormone (mIU/mL)	1.3 ± 0.2	36.5 ± 10.4*
Follicle-stimulating hormone (mIU/mL)	42.2 ± 4.0	257.3 ± 60.3*
Body weight (g)	206.0 ± 2.3	250.1 ± 5.4*
Uterine weight (g)	1.27 ± 0.10	0.31 ± 0.03*

*P < 0.05; Results are mean±SEM (n=7/group).

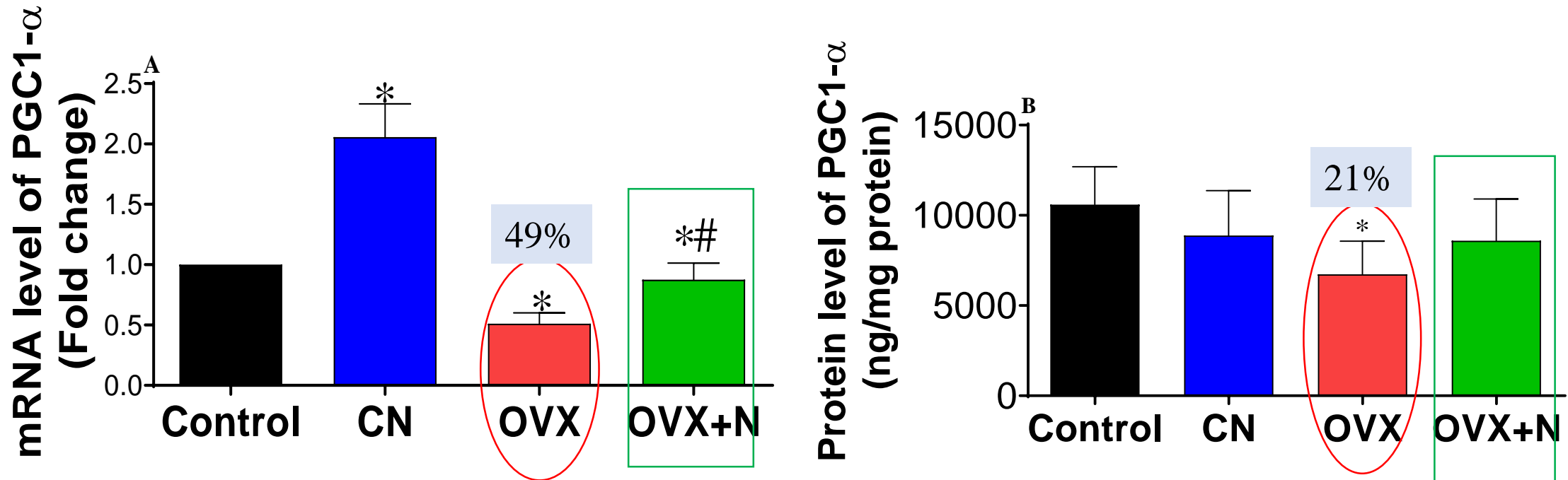




Results

Effect of sodium nitrate on mRNA (A) and protein (B) levels of PGC1- α in gonadal adipose tissue

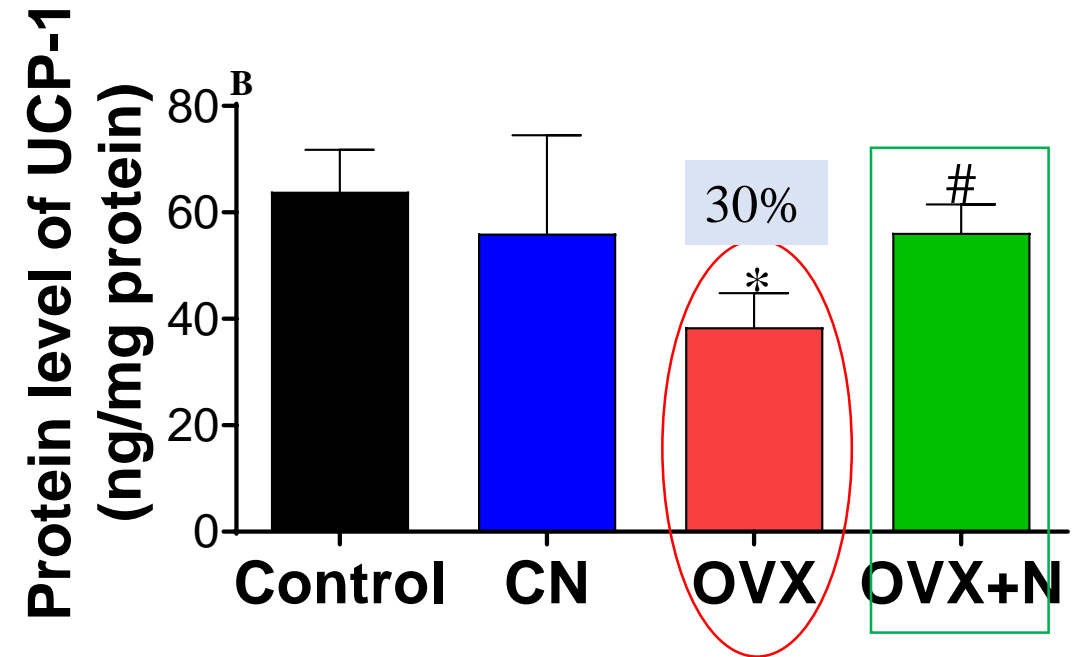
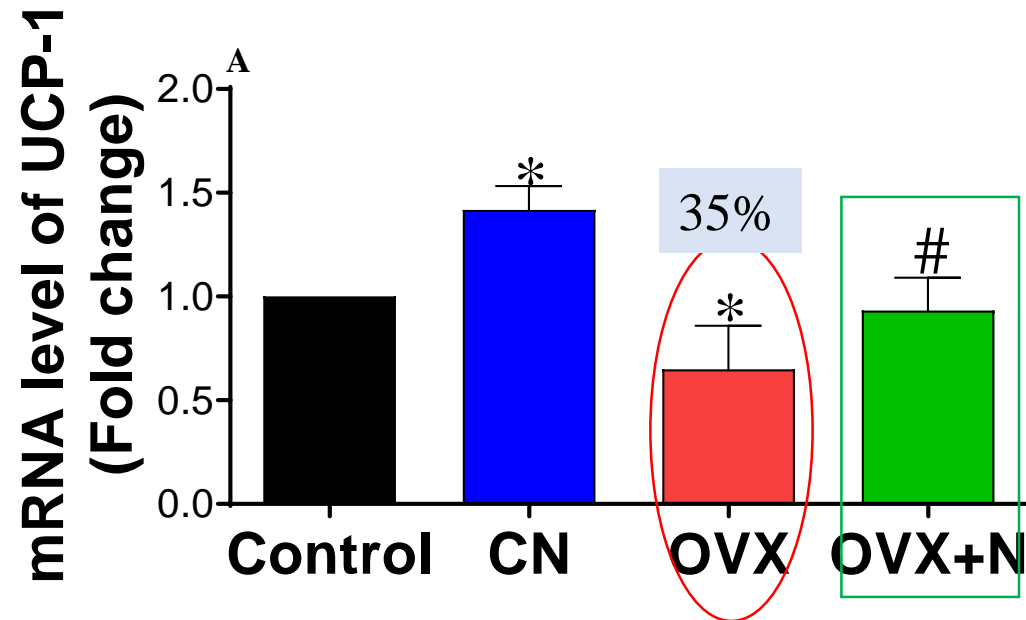
Results are mean \pm SEM (n = 7/group). * and # p < 0.05 compared to control and OVX groups, respectively. CN, control + nitrate; OVX+N, OVX+ nitrate.



Results

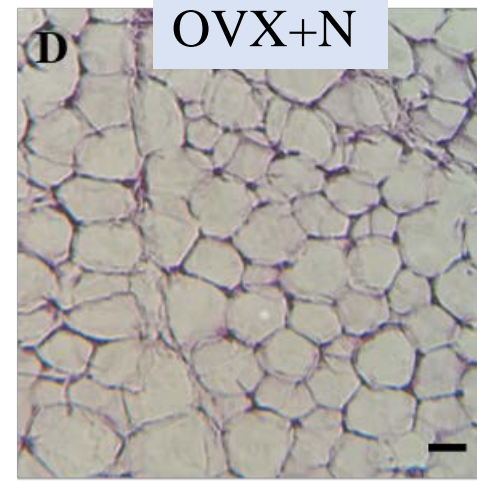
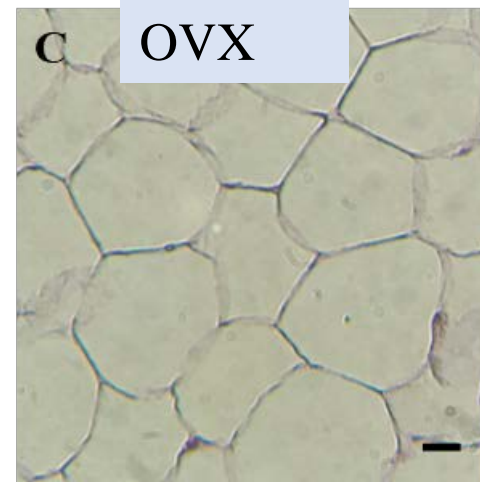
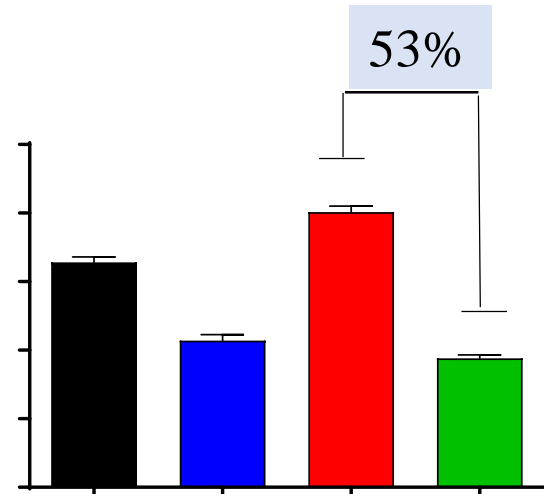
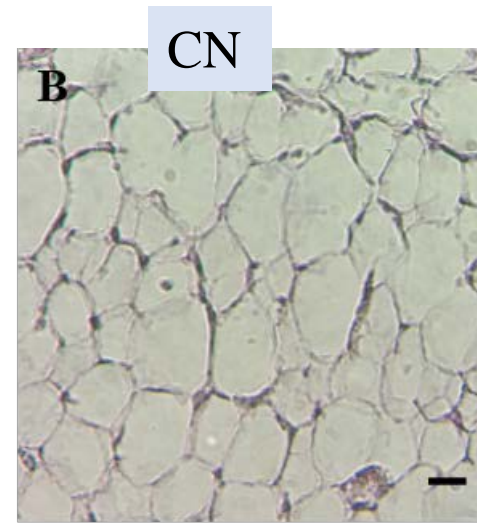
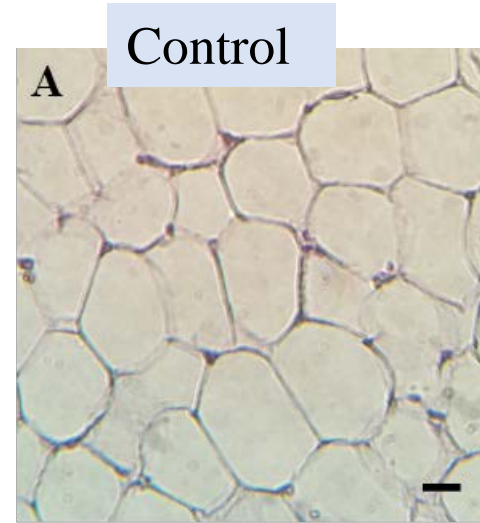
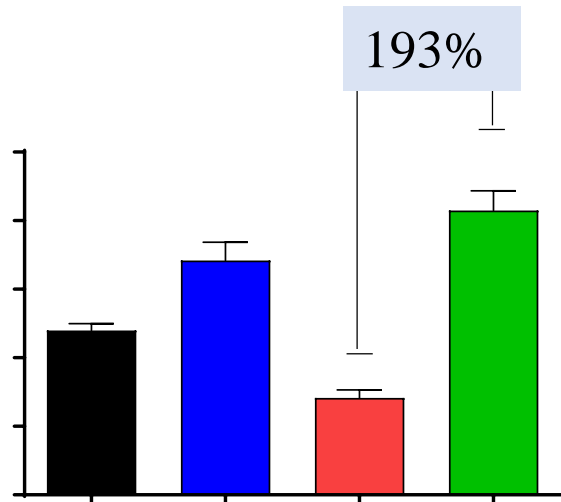
Effect of nitrate on mRNA (A) and protein (B) levels of UCP-1 in gonadal adipose tissue

Results are mean \pm SEM (n = 7/group). * and # p < 0.05 compared to control and OVX groups, respectively. CN, control + nitrate; OVX+N, OVX+ nitrate.



Results

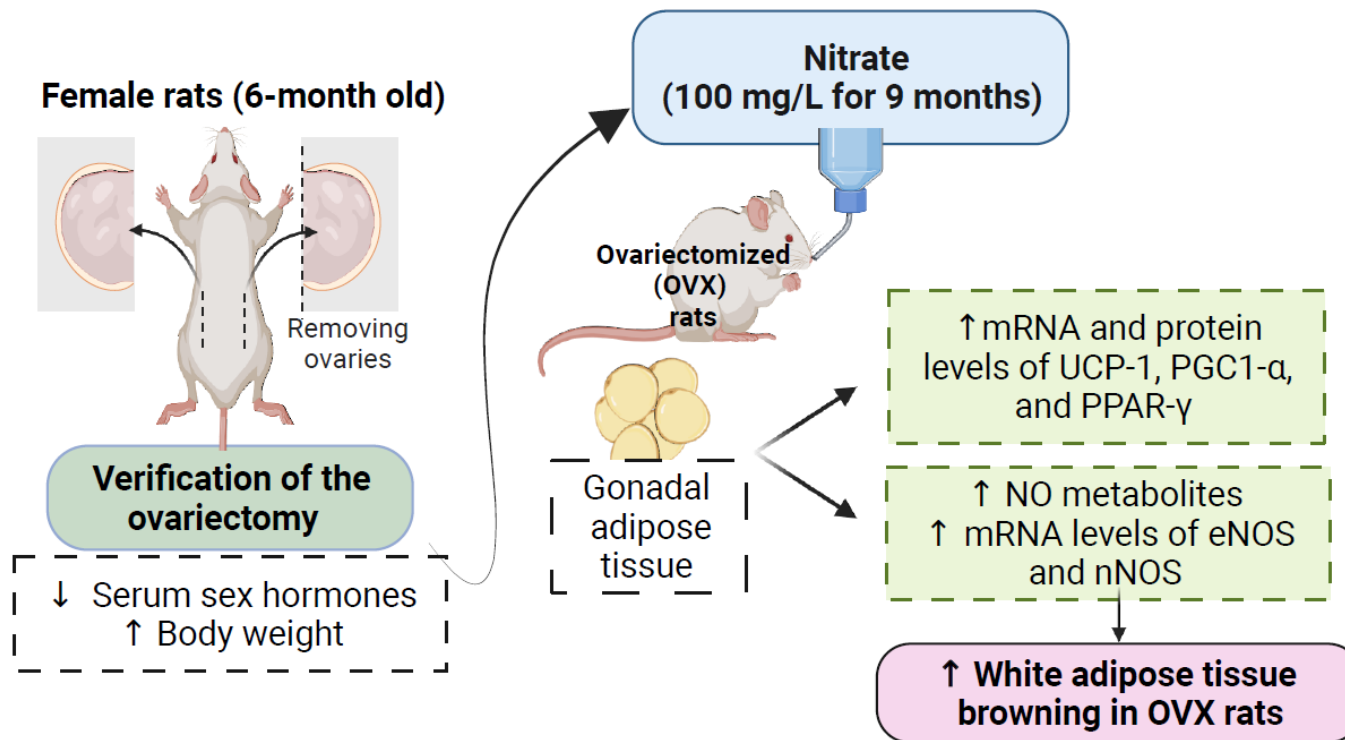
Effect of nitrate on adipocyte density (A) and adipocyte area (B) in gonadal adipose tissue



Results are mean \pm SEM (n = 7/group). * and # p < 0.05 compared to control and OVX groups, respectively. CN, control + nitrate; OVX+N, OVX+ nitrate. (400x magnification, scale bar = 10 μ m).

Conclusion

Favorable effects of long-term nitrate administration in OVX rats is, at least in part, due to browning of WAT and also associated with increased PPAR- γ , PGC-1 α , and UCP1 level in adipose tissue.





Thankyou...