## A punicalagin-rich pomegranate supplement counteracts the physiological impairments caused by the intake of ultra-processed beef in Wistar Rats

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**Introduction:** Punicalagin (PG), a major antioxidant bioactive ingredient in pomegranate juice, has been proven to have anti-oxidative stress properties and protective effects against several chronic diseases (Johanningsmeier and Harris, 2011). The intake of highly oxidized ultra-processed red meat and/or fructose-enriched diets are associated with higher incidence of non-communicable diseases (Dekker et al., 2010; Händel et al., 2019). The present study was carried out to analyse the protective effect of a punicalagin-rich pomegranate supplement on the liver function, serum biochemistry and lipid deposition in Wistar rats fed on ultra-processed beef and PG.

**Material and methods:** All protocols were performed according to Helsinki declaration and approved by Animal Experimentation Ethical panel; process n° EXP-20200904). 21 Male Wistar rats were divided in three groups and maintained for 10 weeks with different diets: regular chow with 14% non-animal protein content and water with 300 mg/l of fructose (FRUC), an experimental chow supplemented with ultra-processed beef (roasted [200°C/10 min], chilled [4°C/10 days] and microwaved reheated [650KW/2 min]) and 30% protein content (T-OX), and an experimental chow supplemented with the aforementioned ultra-processed beef and 200 ppm of a commercial (Granatum Plus®) punicalagin-rich pomegranate extract (T-OX+PG). Food and water were available ad libitum, except for FRU animals in which drinking water was replaced by the above-mentioned fructose drink. Animal weight, water and feed consumption were monitored throughout the feeding period. At the end of week 10, animals were anaesthetised (4% isoflurane), euthanized (exsanguination) and necropsied. Visceral adipose tissue (VAT) was dissected and weighted. Blood from cardiac puncture was stored into EDTA tubes. A serum chemistry panel and a lipid profile were done. All statistical analysis was done using R Statistical Software (R 3.0.0) (Core, 2020) and figures were produced using the package ggplot2 (Wickman, 2016). P was set at < 0.05.

**Results:** Higher water intake was observed in T-OX and T-OX+PG groups vs FRUC (33.94 vs 30.98 vs 23.34 g/ animal/day) as a likely consequence of the high-protein intake. A significant decrease in VAT weights was observed in T-OX+PG group vs T-OX and FRUC groups (60.05 vs 88.54 vs 92.08 mg/g life weight). These results are of clinical interest as VAT deposition is related to an enhanced secretion of proinflamatory cytokines and several diseases such as hypertension and dyslipidaemia (Kelley et al., 2000). Higher values of total protein and albumin were observed in T-OX group vs T-OX+PG and FRUC groups (4.95 vs 3.50 vs 3.35 and 2.98 vs 2.51 vs 2.34 g/ dl, respectively). Moreover, the albumin/globulin ratio value, an indicator of possible impairs in liver, kidneys or intestinal functions was improved in T-OX+PG group vs T-OX (0.51 vs 0.64 and 46.33 vs 77.47 mg/dl respectively). Similar results have been observed in diabetic mice model orally treated with PG (An et al., 2020). Moreover, AST (Serum aspartate aminotransferase) values were decreased in T-OX+PG group vs T-OX (26.83 vs 45.60 U/l respectively), which indicate a liver protective effect of PG. Total cholesterol, LDL-cholesterol were significantly decreased in T-OX+PG groups.

**Conclusion:** The intake of punicalagin as a diet supplement counteracted the physiological impairments caused by ultra-processed beef. These results could contribute to understanding the benefits of this bioactive supplement on humans' health.

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