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DIETARY POMEGRANATE ALLEVIATES THE OXIDATIVE STRESS, ADIPOSITY AND FATTY LIVER CAUSED BY THE SUSTAINED INTAKE OF ULTRAPROCESED BEEF

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I. INTRODUCTION

The global rise in consumption of ultra-processed foods, particularly meat products, has been linked to metabolic disorders, including oxidative stress, obesity, and non-alcoholic fatty liver disease (NAFLD) [1]. These effects are attributed to the formation of pro-oxidant compounds (e.g., advanced glycation end-products, lipid peroxides) during thermal processing and reheating [2]. Pomegranate (*Punica granatum*), rich in polyphenols like punicalagins, has demonstrated antioxidant and anti-obesity properties in preclinical models after a continuous fructose consumption [3], but its potential to counteract the metabolic impact of processed meat remains underexplored. This study investigated whether dietary pomegranate supplementation alleviates metabolic disturbances induced by sustained intake of ultra-processed beef (UPB) in a *Wistar* rat model.

II. MATERIALS AND METHODS

Wistar rats (*Rattus norvegicus*) were used in this experiment in accordance with Spanish legal regulations (RD 53/2013), the bioethics committee of the University of Extremadura (approval code 137-2020), and the authorization from the Board of Extremadura (EXP20200904). Basal feeds were modified by including up to 30% of protein from whether roasted beef (250 ºC for 10 minutes) or UPB (roasted, chilled [10 days/4 ºC] and reheated in microwave [600 W/2 min]). An additional feed was designed by including a punicalagin-rich pomegranate dietary supplement (400 mg/kg). The three diets were isocaloric. The experiment lasted 10 weeks. Upon euthanasia, blood was collected and analyzed for markers liver function, oxidative stress and metabolomics [3]. Lipid deposits were dissected and weighed while liver tissue was also collected and analyzed for lipid content and microscopy analyses [3]. Parametric and non-parametric analyses of data were applied for styding differences between rats exposed to dietary treatments, namely, beef (B), ultraprocessed beef (UPB) and UPB with pomegranate (UPB-P).

III. RESULTS AND DISCUSSION

Table 1. Biochemical markers of oxidative stress in plasma and liver function analyzed in the study: Protein carbonyls, pentosidine, APOPs (advanced protein oxidation products), TBARS (thiobarbituric acid reactive substances), ALP (alkaline phosphatase), ALT (alanine aminotransferase), and GGT (γ-glutamyl transferase) in groups B, UPB and UPB-P.

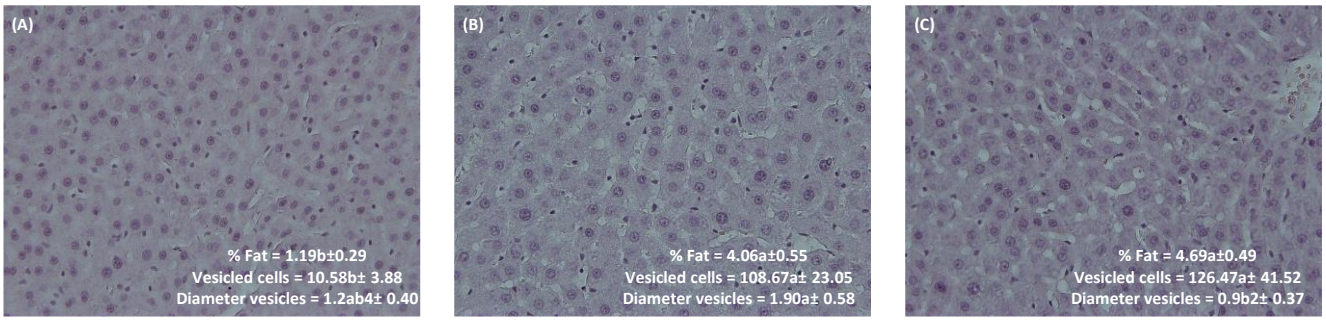
	B	UPB	UPB-P
Protein carbonyls (nmol/mg prot)	0.89b±0.22	1.88a±0.51	1.03ab±0.42
Pentosidine (FU)	1.14b±0.20	2.13a±0.29	0.89b±0.50
APOPs (FU)	289b±32	451a±55	240b±39
TBARS (mg/kg prot)	0.12c±0.03	0.39a±0.09	0.21b±0.08
ALP (U/L)	3.57b±1.12	6.29a±1.51	5.86a±1.41
ALT (U/L)	14.71b±2.09	17.00a±3.17	17.43a±3.20
GGT (U/L)	31.33b±3.07	49.17a±10.82	26.83b±7.78

Table 2. Adipose tissue weights in experimental groups: E-WAT (extracavital white adipose tissue), I-WAT (intracavital white adipose tissue), and WAT (total white adipose tissue) in rats fed from groups B, UPB and UPB-P. Values represent mean ± SD, with different superscript letters indicating statistically significant differences ($p<0.05$) between groups.

	B	UPB	UPB-P
E-WAT (g fat/kg)	21.71b±2.25	30.25a±3.15	25.38ab±3.71
I-WAT (g fat/kg)	54.86b±3.25	93.12a±6.96	61.19b±5.04
WAT (g fat/kg)	75.89b±5.09	122.67a±7.48	85.96b±7.99

Table 1 shows that the sustained consumption of ultra-processed beef significantly increased oxidative stress markers (protein carbonyls, pentosidine, APOPs and TBARS, $p < 0.05$) and hepatic damage (ALP, ALT and GGT, $p<0.05$). These results are likely consequence of the delivery and the toxicological effects of lipid and protein oxidation products accumulated in highly processed beef (data not shown). Moreover, UPB induced systemic adiposity (Table 2), particularly in visceral depots (1.7-fold change intracavitary WAT vs control, $p < 0.001$), which likely contributed to hepatic lipid accumulation through elevated fatty acid transportation. Figure 1 shows histological sections from livers of the three group tested. Interestingly, pomegranate supplementation (UPB-P) reversed these effects, reducing oxidative stress (protein carbonyls 1.8-fold, pentosidine and APOPs restored to sub-baseline levels, $p < 0.05$ vs UPB), normalizing visceral adiposity (I-WAT no difference vs B, $p > 0.05$), and improving hepatic lipid metabolism.

Figure1. H&E microscopic images of livers from experimental animals fed B (A), UPB (B) and UPB-P (C).



Yet, the lipid degeneration observed in the liver was not counteracted by pomegranate. TBARS remained elevated in the UPB-P group compared to B (p<0.05) suggesting that pomegranate's polyphenols may scavenge free radicals but cannot fully reverse membrane damage caused by pre-formed lipid peroxides or already formed advanced glycation-end products. In addition, ALP and ALT, indicators of biliary dysfuction and hepatic damage, were not affected by pomegranate. To understand further the implication of UPB on adiposity, gut microbiota and blood metabolomics were assesed.

The gut microbiome underwent significant modifications following consumption of ultra-processed beef (UPB) and UPB supplemented with pomegranate extract (UPB-P). At the phylum level, UPB consumption reduced *Bacteroidota* (38.1% vs 42.4% in controls) and *Firmicutes* (38.6% vs 49.8%), while UPB-P partially restored *Bacteroidota* dominance (44.3%), a shift that may explain the improved saccharolytic activity observed through metabolites like 3-carboxyindole. The dramatic *Verrucomicrobiota* increase (19.8% in UPB vs 3.2% in controls), potentially indicating mucin layer disruption, was partially reversed in UPB-P (15.8%), suggesting pomegranate polyphenols may help protect intestinal barrier function. Notably, *Desulfobacterota* (sulfate-reducing bacteria associated with inflammation) decreased in UPB (0.55% vs 1.40% in controls) but rebounded in UPB-P (1.50%), possibly reflecting polyphenol-mediated modulation of redox balance. However, persistent higher levels proteolytic markers like p-cresol in both UPB groups (7.78 and 9.08 × 10^5) suggest that irreversible oxidative damage to meat proteins occurred before pomegranate polyphenols could exert protective effects. The antioxidant limitations were further highlighted by the complete loss of linoleyl carnitine in UPB groups and progressive increase in inflammatory platelet-activating factor (from 4.72 × 10^7 in controls to 17.4 × 10^7 in UPB-P).

IV. CONCLUSION

The results demonstrate that sustained consumption of ultra-processed beef (UPB) significantly increased oxidative stress, hepatic damage, and visceral adiposity, likely due to disturbed energetic metabolism owing to the toxicological effects of lipid and protein oxidation species in UPB. Pomegranate supplementation (UPB-P) partially mitigated these effects by reducing oxidative stress markers, normalizing adiposity, and improving gut microbiota composition (e.g., restoring *Bacteroidota* dominance and reducing *Verrucomicrobiota*). However, its protective effects were limited against pre-formed lipid peroxides and advanced protein oxidation and glycation end-products.

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