Vaccinium macrocarpon extract restrains muscle wasting induced by Western diet-derived AGEs

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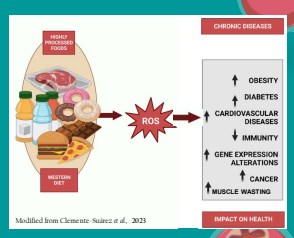
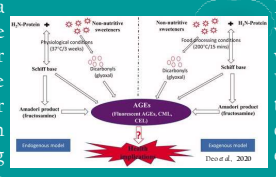
WESTERN DIET

Western diet (WD) is a modern dietary pattern characterized by high intake of processed and refined foods, red meats, sugars, cheese, and saturated fats containing elevated advanced glycation end-products (dietary AGEs; dAGEs), and low intake of fruit, vegetables, whole grains, and nuts [1]. Moreover, methods of preparation (heat, dehydration) and processing (grilling, frying) typical of WD induce further generation of dAGEs, which can be absorbed by the gastrointestinal tract and only partially excreted in the urine [2]. WD has been linked to a range of chronic diseases, including obesity, type 2 diabetes, cardiovascular disease, and muscle wasting (MW) [1].



ADVANCED GLYCATION END-PRODUCTS

Advanced glycation end-products (AGEs) represent a heterogeneous group of non-enzymatic adducts that can be endogenously formed in hyperglycemia conditions or exogenously sourced from diet (dAGEs). AGEs induce tissue damage by directly altering protein function or binding their receptor, RAGE, thus sustaining systemic/local inflammation and oxidative stress, as typically observed in subjects consuming WD [3]. dAGEs remain in contact with tissues for longer time than endogenous AGEs, strongly contributing to tissue damage [2]. dAGE accumulation in skeletal muscle, blood, and skin has been reported in sarcopenia conditions in the elderly, and in diabetic subjects, and RAGE signaling sustains MW in several conditions [4,5].

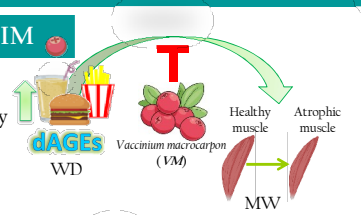


VACCINIUM MACROCARPON

Recently, phytotherapy products with traditional values have been tested *in vitro* and *in vivo*, demonstrating their medical properties [6]. *Vaccinium macrocarpon* (*VM*) is extensively studied owing to its chemical composition, *i. e.*, phenolic compounds, flavonols, procyanidins, and anthocyanins exerting antioxidant effects and interfering with different signaling pathways. *VM* is used in chronic kidney disease, in urinary tract infections, and gut microbiota modulation [7]. We have demonstrated that *VM* is able to restrain MW *in vitro* by reducing AGE formation/accumulation *(see POSTER #42)*.

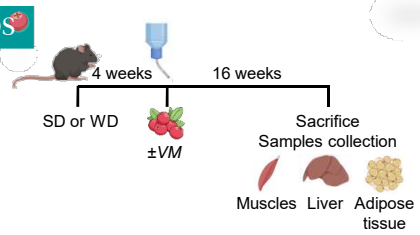
AIM

Might *Vaccinium macrocarpon* extract prevent/counteract WD-dependent MW by reducing dAGE accumulation/activity?

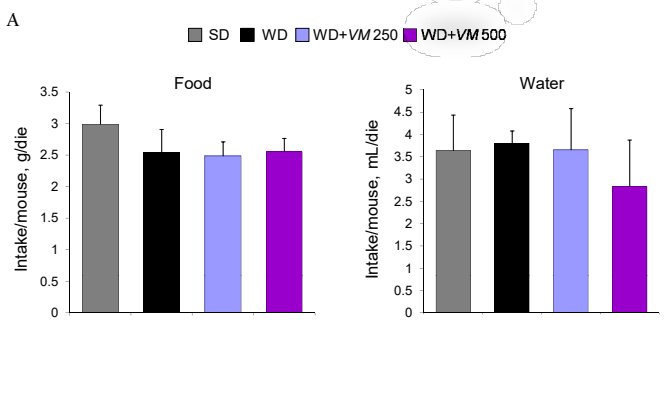


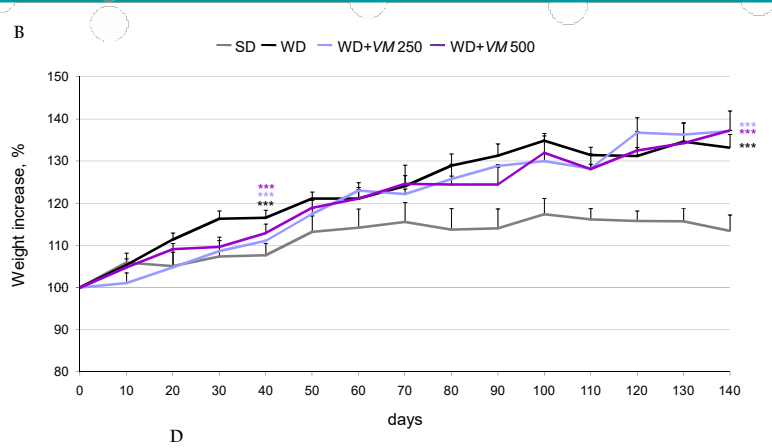
METHODS

Adult male mice were fed with standard diet (SD) or Western diet (WD; "WESTERN TYPE" 1.25% cholesterol, High Fat/High cholesterol diet) containing high dAGEs for 20 weeks, monitoring the food and water intake, body weight, and muscle performance by *Kondziela's Inverted Screen Test*. During the last 16 weeks, some WD-fed mice were administered with *VM* standardized extract (250 or 500 mg/kg/die) directly melted in the water.

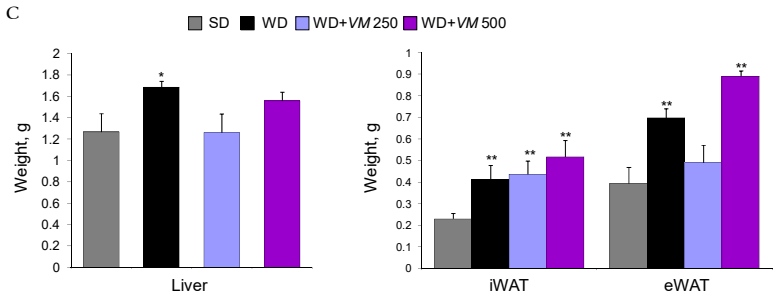


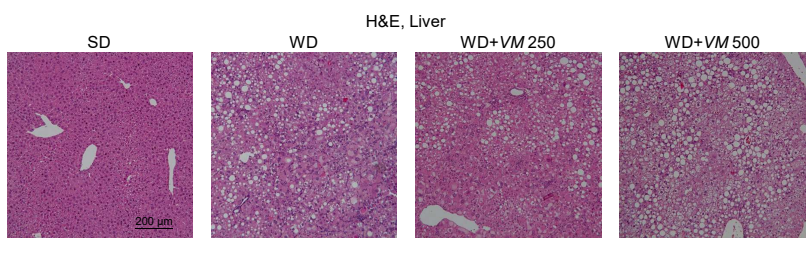
1. WD increases body, liver, and adipose tissue weights and induces liver steatosis. WD-dependent increase in liver and eWAT weights is counteracted by *VM* administration



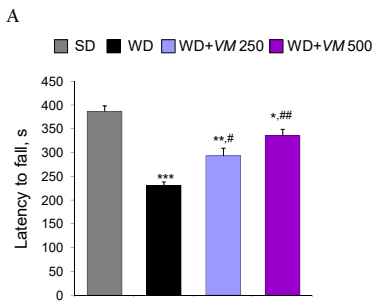


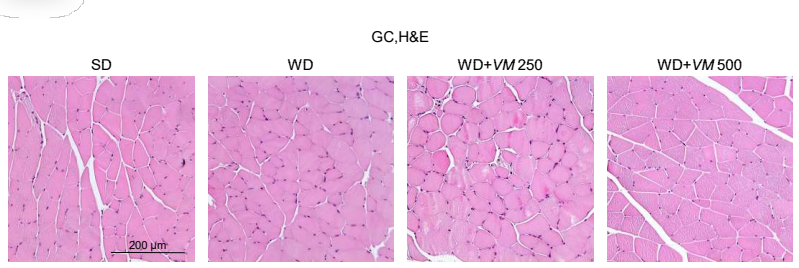
(A) Mice fed with SD or WD in the absence or presence of *VM* extract ate and drank the same quantity of food and water. (B) WD-fed mice increased their body weights starting from day 40, irrespective of the presence of *VM*. (C) At the sacrifice, mice fed with WD showed increased weights of livers, and inguinal (iWAT) and epididymal (eWAT) white adipose tissues. The administration of *VM* extract (250 mg/kg/die) significantly reduced the liver and eWAT weights. (D) Paraffin-embedded livers were stained with hematoxylin and eosin (H&E). Histology revealed that WD induces accumulation of adipose tissue in liver (steatosis), which was not reduced by *VM*. Reported are representative images. Values are means ± SEM. Statistical analysis was conducted using t-test *p<0.05, **p<0.01, and ***p<0.001 *vs* SD-fed mice.

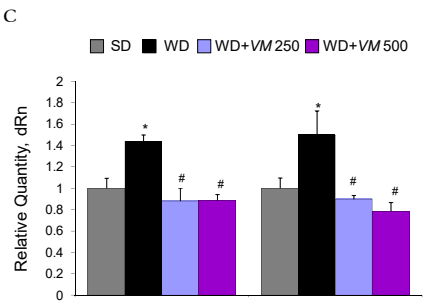


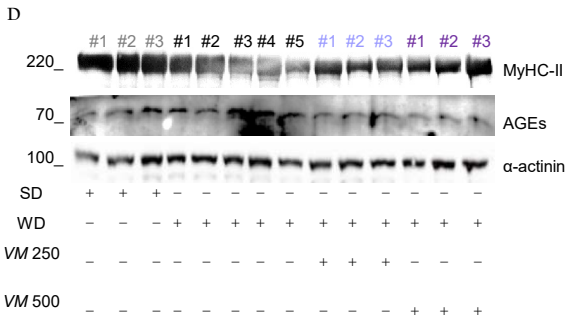


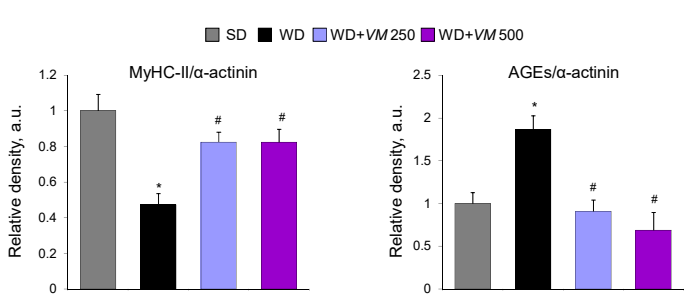
2. *VM* restrains WD-dependent muscle wasting (i.e., loss of muscle mass and functionality, and UPS-dependent MyHC degradation) by reducing AGE accumulation in muscles

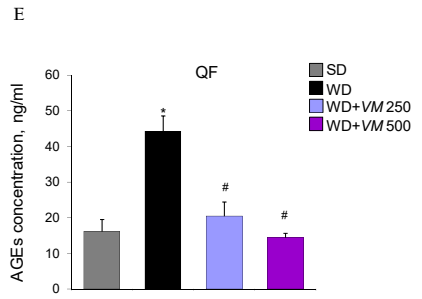












(A) During the 20-week experimentation, muscle performance was evaluated weekly by the *Kondziela's Inverted Screen Test*. Compared to SD, WD consumption reduced muscle functionality, which was rescued by the administration of *VM* (250 or 500 mg/kg/die). (B) WD *vs* SD-fed mice showed a reduced (-10%) average myofiber area in gastrocnemius (GC) muscles after staining with H&E, and *VM* consumption maintained the myofiber areas of WD-fed mice similar to those of SD-fed ones. Myofiber area was evaluated by using the *ImageJ* software. Reported are the percentages of myofiber area changes with respect to SD-fed mice. (C) Real-time PCR analysis of GC muscles revealed that WD activated the ubiquitin-proteasome system (UPS) inducing *Fbxo32* and *Trim63* gene expressions, which were completely counteracted by administration of *VM* extract. (D) Myosin heavy chain (MyHC)-II and AGEs amounts in GC muscles were evaluated by Western Blotting (WB) analysis and the relative densities were calculated with respect to α-actinin. A reduction in MyHC-II content and accumulation of AGEs were observed in WD-fed mice compared to SD-fed ones. WD-fed mice administered with *VM* maintained the amounts of MyHC-II and AGEs at similar levels to those of control (SD-fed) mice. (E) In accordance, AGE levels were strongly increased in *Quadriceps femoris* (QF) muscles of WD- *vs* SD-fed mice, and administration of *VM* counteracted AGE accumulation, as evaluated by ELISA dosage. Reported are representative images (B,D). Values are means ± SEM. Statistical analysis was conducted using t-test. *p<0.05, **p<0.01, and ***p<0.001 *vs* SD-fed mice. #p<0.05 and ##p<0.01, *vs* WD-fed mice.

CONCLUSIONS

WD consumption induced :

- Increase in body, liver, and adipose tissue weights;
- Liver steatosis;
- Reduction of muscle performance;
- Reduction of MyHC-II in muscles;
- Reduction of myofiber area;
- Activation of the ubiquitin-proteasome system;
- AGE accumulation in muscles.

VM administration maintained:

- ✓Physiological weights of liver and eWAT;
- ✓ Muscle performance;
- ✓MyHC-II amounts in muscles;
- ✓Myofiber area;
- ✓Low levels of *Fbxo32* and *Trim63* genes;
- ✓Low levels of AGEs in muscles.

Acknowledgments:

Project title: Fighting WESTERN diet-derived AGEs (advanced glycation end products) with natural compounds to mitigate muscle wasting in sarcobesity (WESTERNAGE) #P2022Z4EB5

References: *1.* Clemente-Suárez *et al., Nutrients.* 2023; 15:2749; *2.* Peppa *et al., Nutrients* 2021;13:3467; *3.* Salazar *et al., Int J Environ Res Public Health* 2021;18:7236; *4.* Salazar *et al., Int J Environ Res Public Health.* 2021; 18:7236; **5**. Chiappalupi *et al., J Cachexia Sarcopenia Muscle.* 2020; 11:929-946; **6**. Vijaykrishnaraj and Wang *Biomed Pharmacother.* 2021;144:112336; **7**. de Souza Gouveia Moreira *et al., J Nutr Metab* 2024;2024:9590066.