

# Impact of long-Term Swimming Exercise on Rat Femur Bone Quality

## Biomedicines

**Laura Freitas** <sup>1,2,\*</sup>, **Andrea Bezerra** <sup>1,2</sup>, **Ana Resende-Coelho** <sup>1,2</sup>, **Maria Gomez-Lazaro** <sup>3</sup>,  
**Leonardo Maciel** <sup>1,2,4,5</sup>, **Tânia Amorim** <sup>6</sup>, **Ricardo J. Fernandes** <sup>7,8</sup> and **Hélder Fonseca** <sup>1,2</sup>

<sup>1</sup> Research Centre in Physical Activity, Health and Leisure (CIAFEL), Faculty of Sport, University of Porto, 4200-450 Porto, Portugal; dea.beatriz@hotmail.com (A.B.); ancatarina@gmail.com (A.R.-C.); yung\_maciel@hotmail.com (L.M.); hfonseca@fade.up.pt (H.F.)

<sup>2</sup> Laboratory for Integrative and Translational Research in Population Health (ITR), 4050-600 Porto, Portugal

<sup>3</sup> i3S – Institute for Research and Innovation in Health, University of Porto, 4200-135 Porto, Portugal; maria.glazaro@i3s.up.pt

<sup>4</sup> Postgraduate Nursing Program, Federal University of Sergipe, São Cristóvão 49100-000, Brazil

<sup>5</sup> Department of Physiotherapy, Federal University of Sergipe, Lagarto 49400-000, Brazil

<sup>6</sup> Fame Laboratory, Department of Physical Education and Sport Science, University of Thessaly, 421-00 Trikala, Greece; tania\_amorim@hotmail.com

<sup>7</sup> Centre of Research, Education, Innovation and Intervention in Sport (CIFIID), Faculty of Sport, University of Porto, 4200-450 Porto, Portugal; ricfer@fade.up.pt

<sup>8</sup> Porto Biomechanics Laboratory (LABIOMEP), Faculty of Sport, University of Porto, 4050-313 Porto, Portugal

\* Correspondence: laura\_c\_freitas@hotmail.com

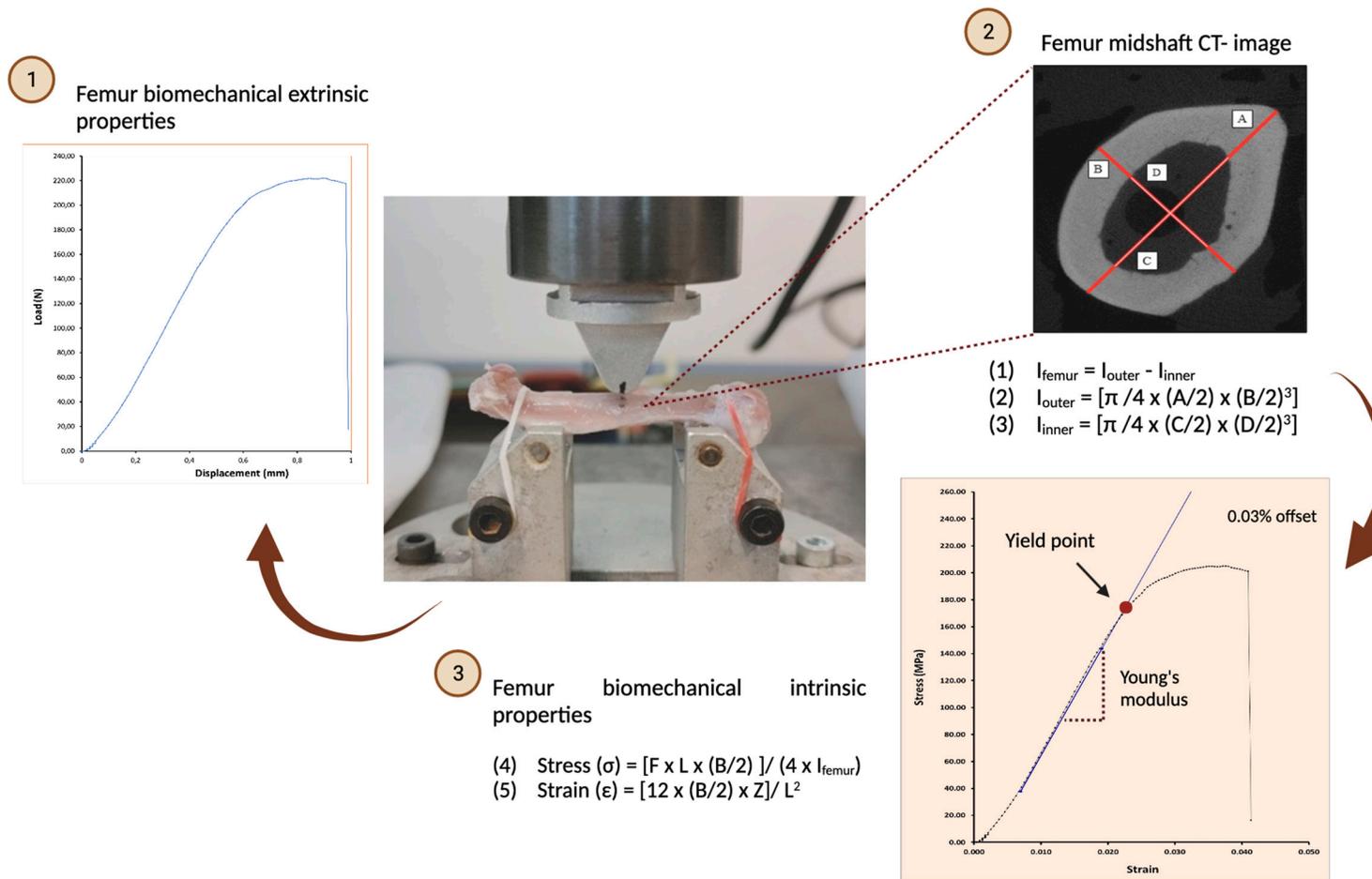
## Supplementary Materials

**Figure S1: Preparation of the right femur in antero-posterior position for the three-point bending test and the assessment of the load-displacement and stress-strain curves with respective equations.**

**1: The application of the three-point bending test to obtain load-displacement curve. 2: The calculation of moment of inertia through femoral midshaft dimensions from micro-computed tomography image to assess stress-strain curve. 3: The stress-strain curve measured by stress and strain respective equations. The illustration was developed using BioRender software. Lateromedial femur diameter (A); craniocaudal bone diameter (B); distance from the load application to the bone neutral axis (B/2); Lateromedial marrow diameter (C); craniocaudal marrow diameter (D); load applied (F); moment of inertia (I); distance between the supports (L); displacement (Z).....3**

**Equations S1 and S2 related to the calculation of uncoupling index and Z-score .....4**

**Figure S2: Fibre cross-sectional area of gastrocnemius red and white portions in growing rats between control (n= 10) and swimming (n= 9) groups after eight months of experiment (upper panel), with representative images of hematoxylin and eosin staining of transversal sections of previous muscles (lower panel). Data are displayed in n (absolute frequencies). Cross-sectional area (CSA); control group (CG); swimming group (SW). .....5**



**Figure S1: Preparation of the right femur in antero-posterior position for the three-point bending test and the assessment of the load-displacement and stress-strain curves with respective equations. 1: The application of the three-point bending test to obtain load-displacement curve. 2: The calculation of moment of inertia through femoral midshaft dimensions from micro-computed tomography image to assess stress-strain curve. 3: The stress-strain curve measured by stress and strain respective equations. The illustration was developed using BioRender software. Lateromedial femur diameter (A); craniocaudal bone diameter (B); distance from the load application to the bone neutral axis (B/2); Lateromedial marrow diameter (C); craniocaudal marrow diameter (D); load applied (F); moment of inertia (I); distance between the supports (L); displacement (Z).**

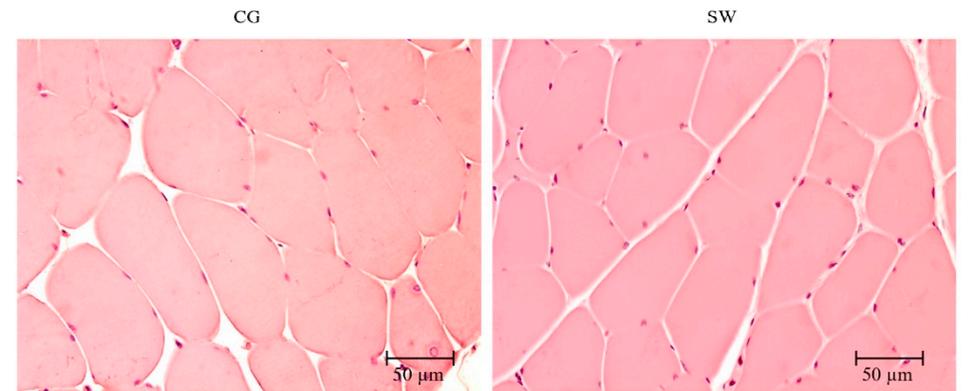
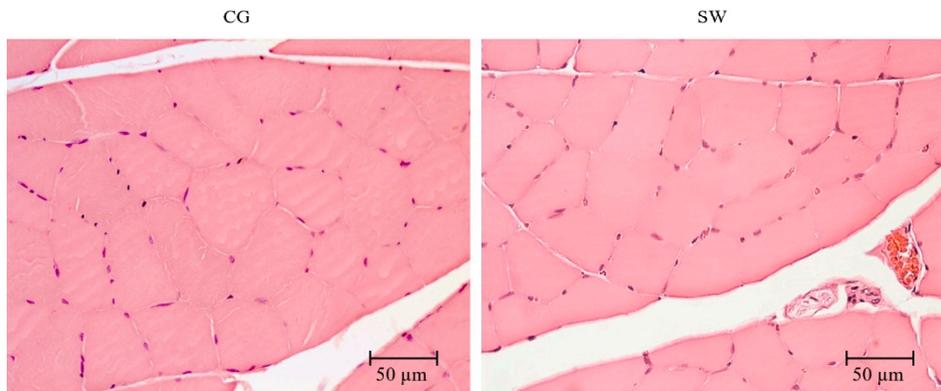
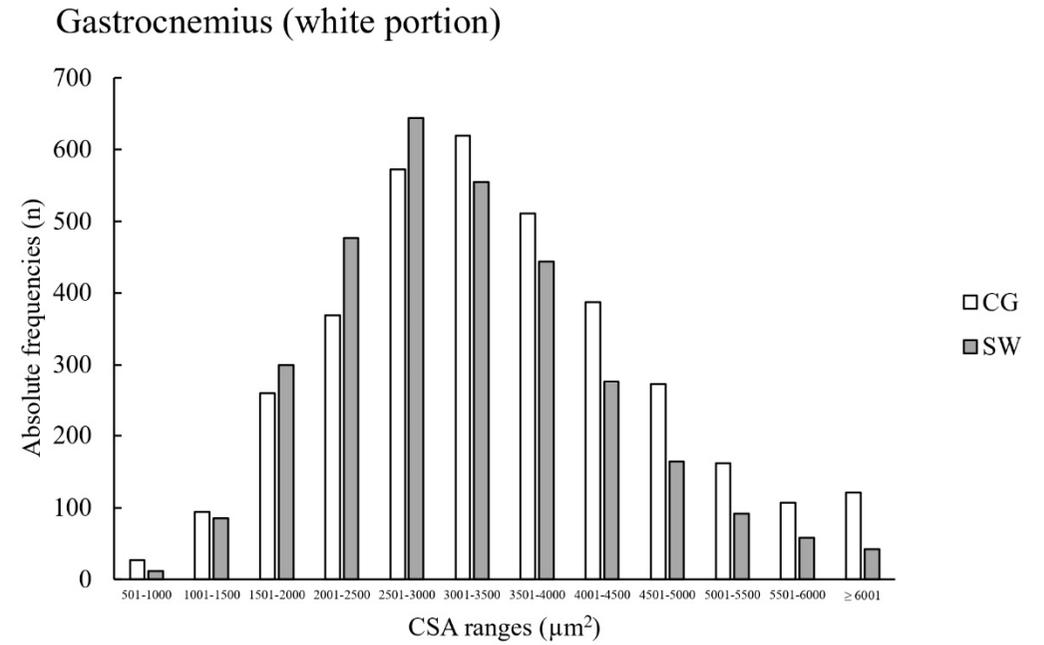
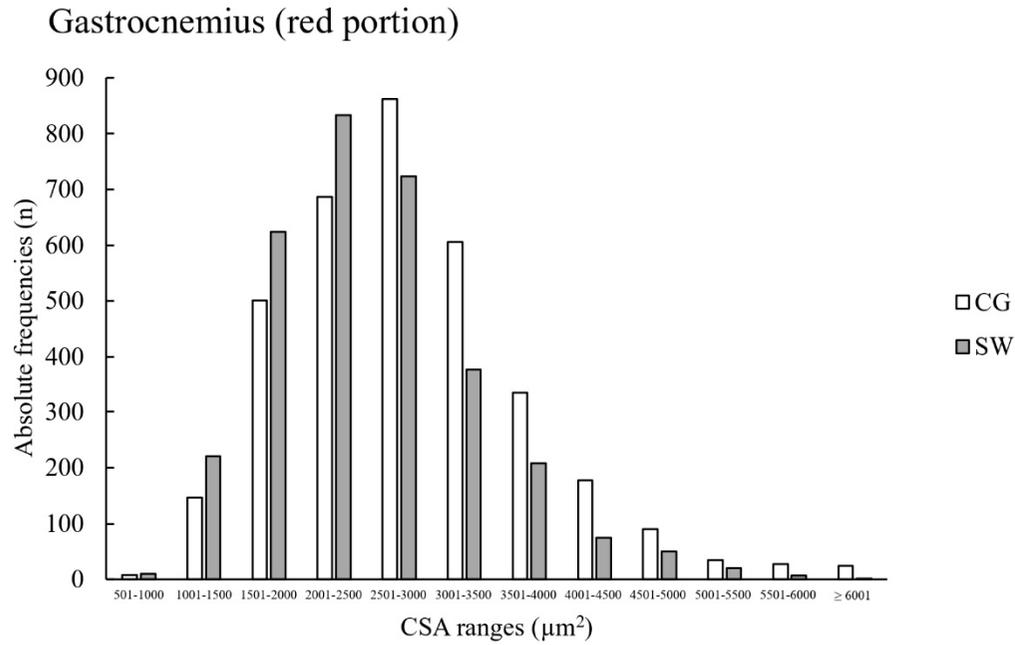
**Equations S1 and S2 related to the calculation of uncoupling index and Z-score**

**Equation (S1):**

$$\text{Uncoupling index} = Z\text{-score}_{\text{Osteocalcin}} / Z\text{-score}_{\text{CTX}}$$

**Equation (S2):**

$$Z\text{-score} = (\text{Animal value} - \text{Mean}_{\text{baseline}}) / \text{Standard Deviation}_{\text{baseline}}$$



**Figure S2: Fibre cross-sectional area of gastrocnemius red and white portions in growing rats between control (n= 10) and swimming (n= 9) groups after eight months of experiment (upper panel), with representative images of hematoxylin and eosin staining of transversal sections of previous muscles (lower panel). Data are displayed in n (absolute frequencies). Cross-sectional area (CSA); control group (CG); swimming group (SW).**