Quantitative evaluation of the saturation degree of adipocytes by means of contrast-enhanced computed tomography

Tim Balcaen[1,2], Andrea Benova[7], Michaela Tencerova[7], Wim De Borggraeve[1], Greet Kerckhofs[2,3,4,5,6]

[1]*Department of chemistry, KU Leuven, Belgium*
[2]*iMMC, UCLouvain, Belgium*
[3]*Pole of Morphology, Institute of Experimental and Clinical Research, UCLouvain, Brussels, Belgium*
[4]*Biomechanics lab, Institute of Mechanics, Materials and Civil Engineering, UCLouvain, Louvain-la-Neuve, Belgium*
[5] *Department Materials Engineering, KU Leuven, Leuven, Belgium*
[6]*Prometheus, Division of Skeletal Tissue Engineering, KU Leuven, Leuven, Belgium*
[7] *Molecular Physiology of Bone, Institute of Physiology of the Czech Academy of Sciences, Prague, Czechia.*

Since the invention of quantitative histomorphometrical techniques and magnetic resonance imaging (MRI), the research concerning bone marrow adipose tissue (BMAT) has exploded. Histomorphometry on the firsthand approaches visualization of adipocytes (Ads) in 2D in two different ways: staining the Ads by using a lipophilic stain (*e.g.* Oil Red O) or staining the tissue surrounding the Ads by using a hydrophilic stain (*e.g.* Haematoxylin & Eosin Y). Despite the high spatial resolution obtained and the high discriminative power, it is laborious and destructive for the tissue. MRI on the other hand is able to visualize bulk adipose tissue in 3D and to determine molecular properties-based chemical shifts of the molecules present in the matrix. However, due to limitations in terms of spatial resolution, MRI is not able to visualize individual Ads and hence is not able to compute their physical properties (*e.g.* volume, thickness, etc.) and only allows to give averaged information on lipid molecular structures. To complement the shortcomings of above-mentioned techniques, we have used contrast-enhanced X-ray microfocus computed tomography (CECT) imaging. This technique uses contrast-enhancing staining agents (CESAs) to increase the X-ray attenuation properties of soft tissues (*e.g.* muscle tissue, adipose tissue, vascular tissue, etc.). It has a higher spatial resolution (up to 0.2 μm^3) than can be obtained by MRI and delivers a 3D dataset of the tissue of interest.

The current standard CESA for visualization of Ads using CECT is osmium tetroxide (OsO_4).[1] This molecule readily reacts with double bonds in a (3+2) fashion, forming an osmate ester. As OsO_4 is a highly toxic and rather volatile (BP = 129.7 °C) compound, a non-toxic alternative with similar staining properties is desired. Moreover, decalcification of the tissue is necessary to obtain reliable results. To overcome the limitations of OsO_4, a novel CESA for 3D BMAT visualization, namely the 1:2 hafnium-substituted Wells-Dawson POM (Hf-WD 1:2 POM) was examined.[2] However, this stain is repelled by hydrophobic Ads and hence reveals them by staining the surrounding tissue. Limitations of this visualization method depend on the adipocyte density (Number of Adipocytes per Marrow Volume (N.Ad/Ma.V) and volume (Ad.V). Therefore, validating a non-toxic CESA that interacts strongly with adipocytes would be highly valuable to overcome the limitations inherent to the Hf-WD 1:2 POM. For this reason, three CESAs (Hexabrix, Hf-WD 1:2 POM and Lugol's iodine solution) have been evaluated, both individually and combined, for the quantitative structural analysis of BMAds in bovine muscle tissue (BMT), murine tibiae (MT) and murine caudal vertebrae (MCV). For this, we computed normalized grey values of the following tissue types (n = 5): muscle fiber, blood vessel, cortical bone, bone marrow, Ads in the center of the tissue and Ads at the extremities. Thereafter, volumetric analyses have been performed, where important bone parameters were computed combined with individual Ads parameters. The current results indicated that the grey values differ significantly between Ads in bovine muscle tissue (more saturated lipids) and in the murine caudal vertebrae (more unsaturated lipids). Moreover, volumetric analyses showed that not only the number of Ads is increased from normal diet to high fat diet, but also the Ad.V of these Ads.

Future objectives are completion of all the quantitative image analysis (grey values and volumetric analyses) and performing will involve (bio)chemical experiments on CESAs and the tissues in order to correlate CECT data with biological data concerning the saturation degree of the Ads. Since this technique could allow to determine bone parameters, relative saturation degree for each segmented Ad individually as well as Ad parameters within the same dataset, it will be highly beneficial for future research on BMAT.

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[2] G. Kerckhofs, S. Stegen, N. van Gastel, A. Sap, G. Falgayrac*, et al.*, *Biomaterials* **2018**, *159*, 1-12.