Have you been to the gym lately?

Micro-CT-based study of skeletal muscle and fat characteristics in a mouse model of chronic liver disease.

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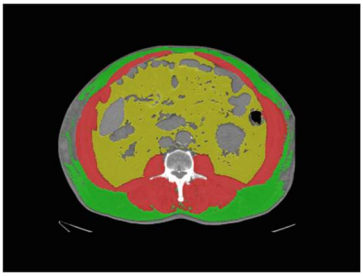
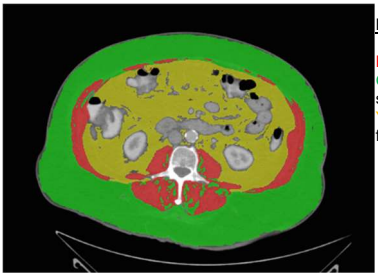
Aims

During the past century, medicine has made tremendous progress in curing once-fatal infectious disease. However, we are now facing a new challenge with the rise of obesity and "non-communicable disease" that accompagnies this pandemic. [1] Many obesity-related complications are already well known from the public, such as cardiovascular diseases and diabetes. Nonetheless, another "silent killer" has been under the radar for many years. Recently named "soda disease" by journalists, non-alcoholic fatty liver disease (NAFLD) is emerging as a real threat for the world population. The basic paradigm is very simple: we eat more calories and we spend less. NAFLD is seen as the hepatic complication of obesity and metabolic syndrome. Most recent estimate of NAFLD prevalence in the world is 25% [2], making it one of the most common disease in the world and the most common liver disease.
NAFLD encompasses a wide spectrum of disease ranging from simple accumulation of fat within the liver (steatosis) to non-alcoholic steatohepatitis (NASH), the progressive form. NASH associates with fibrosis, which may eventually lead to liver cirrhosis and liver failure. [3] Hence, NASH is anticipated to be the 1st cause of liver transplantation within the next few years. [2] Despite intense interest for the pathology during the last three decades, pathophysiology of NASH remains poorly understood and efficient FDA-approved treatment options are lacking. [4]

Recently, an interesting association between skeletal muscle mass and NAFLD has emerged: sarcopenia, a term often referred to as low muscle mass (the exact definition being still debated), has been linked to the presence of NAFLD. Patients with low skeletal muscle mass have a 3 to 5-fold increased risk of having NAFLD [5], and the prevalence of sarcopenia increases with the severity of NASH-associated fibrosis. [6,7]
However, notably due to the lack of prospective studies, no causal relationship has been demonstrated yet. Does sarcopenia cause liver disease progression or is it merely a by-stander reflecting the poor metabolic condition? To further investigate the role of skeletal muscle in NAFLD, we need a reliable tool to assess skeletal muscle quantity and quality in animal models, both invasively and non-invasively.

Dynamic evolution of muscle strength and endurance is usually easily obtained *in vivo,* while analysis of muscle composition requires muscle tissue harvested at the time of sacrifice in animal models or through muscular biopsy in human. Non-invasive measurements of muscle mass and quality remain however very challenging. In human, computed tomography (CT) is the current gold standard technique to assess muscle quantity and quality. [8] Indeed, a strong correlation exists between whole body composition and measures on abdominal CT (at third lumbar vertebrae or L3) as well as between whole body muscle volume and L3 muscle area (fig. 1). [9,10]





Legend:

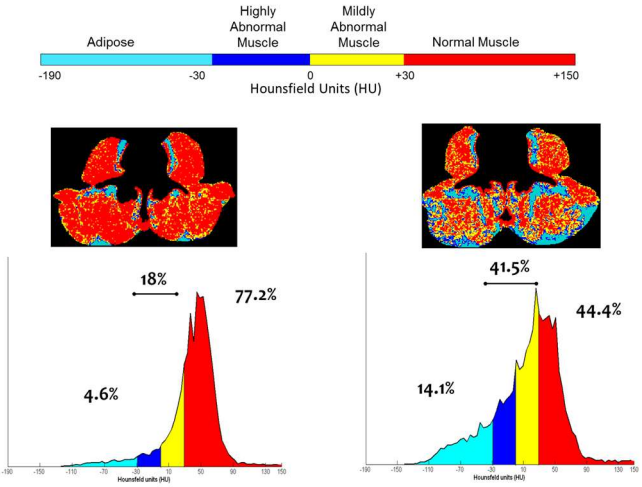
Red = muscle
Green = subcutaneous fat
Yellow = visceral fat

BMI: 34,8kg/m²
L3 Muscular surface: 249,3cm²

BMI: 34,2 kg/m²
L3 Muscular surface: 103,4 cm²

Despite having the same Body Mass index (most widely used body composition surrogate), these two patients (personal data) exhibit very different muscle areas

In addition, it is generally accepted that muscle density (in Hounsfield units) is directly correlated to muscle quality (fig. 2), albeit that there is still no clear consensus of what should be defined as muscle quality. Briefly, muscle density decreases when there is fatty infiltration inside muscle cells (intra-myocellular) and/or outside muscle cells (extra-myocellular). [11]



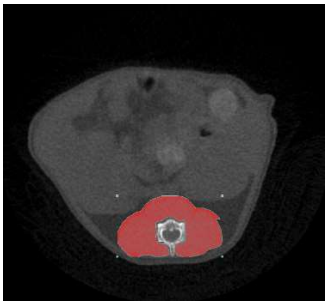
Aubrey et. Al; *Acta Physiologica* (2014)

To investigate whether there is a causal link between NAFLD and sarcopenia, multiple timepoints longitudinal studies investigating liver and muscle compartment during disease initiation and progression are needed. Therefore, we sought to determine whether micro-CT is a suitable technique to study body composition in an animal model of progressive obesity and NAFLD and whether it might detect early changes in body composition that conventional methods could not capture.

Method

We longitudinally follow body weight gain and monitor body composition using whole body acquisition with micro-CT[12,13] (total lean and fat mass, visceral/sub-cutaneous fat mass, specific muscle area and density) in control mice (CTL) fed with normal diet, mice fed with high fat diet (NAFLD) and mutant mice (foz) fed with high fat diet (NAFLD-NASH).
Scanning was performed with Skyscan 1278 (Skyscan, Kontich, Belgium) at 50 µm voxel resolution using a source voltage of 65 kV and a current of 770 uA. Aluminum filter was set on 1 mm to optimize contrast. Rotation step for the X-ray source was set on 0.5°. Raw images were then reconstructed with NRecon to 3-D cross-sectional image data sets. Following parameters were set for the reconstruction: beam hardening to 10%, smoothing to 2, minimum for CS to Image Conversion to 0, maximum for CS to Image Conversion to 0.02. Analyses of reconstructed images were performed using SkyScan software (CTan) and segmentation of different tissue compartments were based on specific tissue density in Hounsfield units (HU). Two types of analysis were performed on CT reconstructed datasets :

Whole body analysis: whole body fat volume (visceral, subcutaneous and intra-organ lipids), whole body lean volume (muscle and organ) and whole body bone volume in cm^3.
Single slice based analysis: visceral area (mm^2) at L4 and dorsal muscle at L3 were selected to assess muscular area (mm^2) and quality (H.U).



Region of interest : dorsal muscle

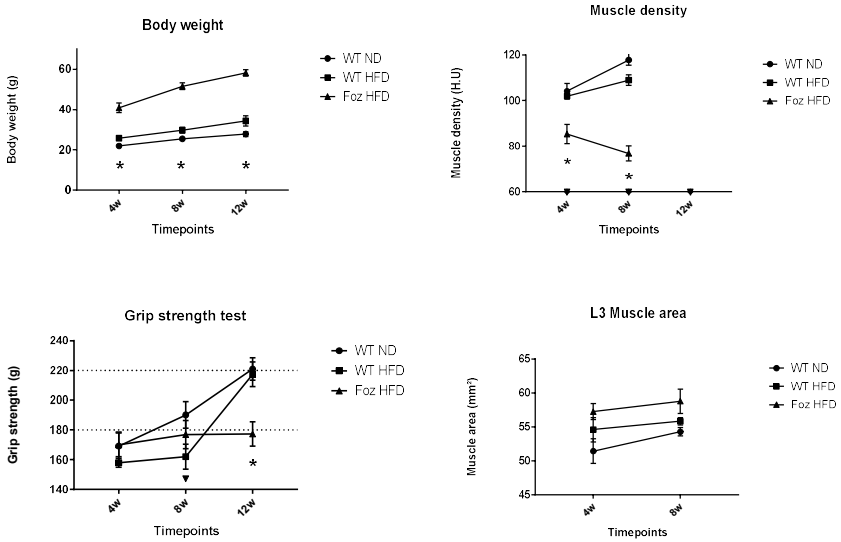
Region of interest : visceral fat

The imaging-based findings will be correlated with functional assessment of muscle strength (grip test) and, at selected time point, with histological analysis of muscle fibers and composition. Liver and adipose tissue will be analysed concomitantly.

Results

Three timepoints are shown for each group of mice: 4, 8 and 12 weeks. Body weight raised during the study, but more so in NAFLD-NASH mice ($p<0,0001$ at 4W). There is no significant difference in body weight between CTL and NAFLD model. We can already appreciate that muscle density, measured with a ROI on para-spinal muscle, is significantly lower in the NAFLD/NASH group as early as 4W compared to simple NAFLD and CTL ($p<0,01$ and $p<0,005$ respectively). There is no significant difference in grip strength at 4W and 8W between the three groups and strength is progressively increasing in the CTL and NAFLD group, with a noticeable delay (albeit non-significant) of strength gain in NAFLD group. Conversely to the other groups, muscle strength does not significantly increase over time in NAFLD-NASH and we can appreciate a very significant difference in strength between CTL and NAFLD vs NAFLD-NASH group at 12W ($p<0,004$ and $p<0,008$ respectively).

Interestingly, 12W is the known timepoint where NASH begins in this model. There is no significant difference in muscle area at 4W, 8W and 12W.



Legend:

WT ND = Wild-type mice fed with normal diet (CTL)
WT HFD = Wild-type mice fed with high fat diet (NAFLD)
Foz HFD = Mutant mice fed with high fat diet (NAFLD-NASH)

Conclusion

In summary, the loss of muscle density seems to be an early marker of poor muscle condition, preceding loss of strength and possibly preceding loss of muscle quantity in later timepoints. The latest needs to be confirmed and these preliminary results will be soon completed by body composition/muscle study at 20W/32W. We will further correlate body composition changes to liver disease progression.

Micro-CT is a powerful tool to analyse muscle quality/quantity non-invasively and can help deciphering the links between sarcopenia and ongoing chronic disease, by identifying the temporal relationship between loss of muscle mass, quality and functionality to the stage of disease.

Taken together, the combination of non-invasive based assessment (micro-CT-based body composition, weight, grip strength, etc.) and invasive techniques (immuno-histology, PCR, western blot) can provide a global frame of ongoing changes and help understand the natural history of the disease. Specific period where major changes occur can be better identified and further explored, aiming to find out relevant biomarkers and therapeutic targets for this devastating condition.

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