Supplementary Material

Supplement 1

Characterization of the semi-automatic Matlab routine to quantify the size of proximal aponeurosis of biceps femoris long head size and interface to biceps femoris long head

The present document describes the structure of the semi-automatic Matlab routine to quantify the size of proximal aponeurosis of biceps femoris long head (Apo-BFLH) and interface to biceps femoris long head (BF_{LH}), as well the validation and reliability processes. Compared to the procedure described in literature, [7] which we noted very low inter-rater consistency in assessing the Apo-BF_{LH} volume [n = 10, male; ICC = 0.40 (−0.13–0.79)], this routine is based in an objective and consensual criteria (i.e. defined by 10 specialists in soft tissues identification based on magnetic resonance imaging data, i.e. MRI). Important to note that the previous procedure is based on a subjective criteria and highly examiner-dependent, whereas the Apo-BF_{LH} volume assessment depends on both the Apo-BF_{LH} volume length and cross-sectional areas (along its length) quantification.

The routine generally consists in identifying the aponeurosis cross-sectional area in each MRI slice, and using the inter-slice distance along the aponeurosis length, estimates the aponeurosis volume by quantifying the summation of the cross-sectional areas multiplied by the interslice distance. In addition, this routine allows researchers to determine the length of interface between the proximal aponeurosis and the biceps femoris long head, by manually selecting the most anterior and posterior point of the tissues interface. This routine was development in three steps. First, identification of an imaging criteria to detect the interface between the aponeurosis and the surrounding tissues. Second, in conceiving the Matlab routine. And, finally, the third step consisted in determining the routine intra- and intra-rater reliability in assessing the aponeurosis size parameters.

Step 1. Identification of a imaging threshold to determine the interface between aponeurosis and surrounding tissues

With the aim of having objective criteria to determine the interface (I) threshold between the aponeurosis cross-sectional area and the surrounding tissues, we have asked to 10 health professionals (i.e. 3 medical doctors, 3 physical therapist, and 4 doctorates in biomechanics) with experience in analyzing MRI data and identifying soft tissues structures, to draw the area of the biceps femoris long head proximal aponeurosis in 10 MRI slices (randomly selected from a male individual), and to select: i) an area within the biceps femoris long head belly (A) that would represent the skeletal muscle tissue; an area with the aponeurosis cross-sectional area (B) that would represent the aponeurosis tissue; and iii) an area in the interface (I).

Using the imageJ software (NIH, v1.47, USA), the average grayscale intensity of A, B, and areas, and the percentual pantone value between A and B (P) was determined in each MRI slice (i.e. total of 100 slices) using the following equation [1]:

\[ P = (I - A) / ((A-B)/100) \]  

From the 100 slices processed, the average P value found was 60%, and no significant differences were found for the P value among the examiners. Thus, the P = 60 was considered as the threshold criteria for the interface between the aponeurosis cross-sectional area and the surrounding tissues.

Step 2. Semiautomatic Matlab® routine development

After step 1, we proceeded to the conception of the semiautomatic Matlab® routine, whose script code can be found at the end of this document. Briefly, this routine requires the following procedures (Fig. 1):

1. Upload and read the MRI slices in .dicom format.
2. In each slice, zoom in on the region of interest.
3. Select representative areas of biceps femoris long head and proximal aponeurosis of biceps femoris long head.
4. Identify the most anterior and distal points at the interface between the biceps femoris long head and the proximal aponeurosis of biceps femoris long head.
5. Examine the aponeurosis thickness higher than 0.72 mm at the ends of aponeurosis.

By using the anatomical threshold criteria determined in step 1 (i.e. P = 60%), and the algorithm mentioned in equation [1], the routine allowed in each MRI slice the determination of: i) aponeurosis cross-sectional area; and ii) the length between the aponeurosis and the biceps femoris long head. Using these outcomes, and considering the interslice distance between MRI slices (i.e. 5mm), the aponeurosis volume and the area of interface between the proximal aponeurosis and the biceps femoris long head muscle belly was estimated using an excel sheet. The Matlab routine can be downloaded from the following website: http://cimt.uchile.cl/mcerra/, and its script can be found at the end of the supplement.

Step 3. Semiautomatic Matlab® routine reliability

With the purpose of testing the semiautomatic routine reliability in quantifying the aponeurosis size parameters by the same or a different examiner, two examiners were trained by the principal study researcher to process two times (in two different days), in a blinded manner, 10 MRI slices from one individual of the sample (randomly chosen from the study sample) by using the procedure described in step 2. After the processing, the intraclass correlation coefficient (ICC) was quantified to assess the intra- and inter-examiner reliabilities. A high reliability was observed at the intra-examiner level for quantifying the aponeurosis cross-sectional area [ICC = 0.88 (0.75–0.95)] and length of aponeurosis-biceps femoris long head interface [ICC = 0.82 (0.63–0.92)]; and at the inter-examiner level [aponeurosis cross-sectional area: 0.79 (0.35–0.94); length of aponeurosis-biceps femoris long head interface: 0.75 (0.28–0.93)].

Matlab routine script, used to quantify the cross-sectional area of the proximal aponeurosis of biceps femoris long head using MRI transverse slices.

[filename,user_canceled] = imgetfile;
info = dicominfo(filename);
pixelSpacing = info.PixelSpacing;
scale = pixelSpacing(1);
I = dicomread(filename);
imin = min(min(I));
imax = max(max(I));
I = uint8(255.0 * double(I-imin)/double(imax-imin));
factor = 6;
l = imresize(l, factor * size(l));
f = figure;
imshow(l, 'InitialMagnification', 250);
axis off; % Turn off axis numbering

% ask for aponeurosis
BW1 = roipoly;
[L,n] = bwlabel(BW1);
RGB = label2rgb(L, 'autumn', 'black', 'shuffle');
imshow(l, 'InitialMagnification', 250);
hold on;
himage = imshow(RGB);
himage.AlphaData = 0.3;
drawnow;

% ask for muscle
BW2 = roipoly;
mask = BW1;
mask(BW2) = 2;
[L,n] = bwlabel(mask);
RGB = label2rgb(L, 'autumn', 'black', 'shuffle');
imshow(l, 'InitialMagnification', 250);
hold on;
himage = imshow(RGB);
himage.AlphaData = 0.3;
drawnow;

meanApo = mean(I(BW1));
meanMus = mean(I(BW2));

thresholdPct = 60; % pct

threshold = meanApo + double(meanMus-meanApo) * thresholdPct/100.0;
aponeurosisTh = I<threshold;
global aponeurosis
aponeurosis = BW1 | aponeurosisTh ;
[L,n] = bwlabel(aponeurosis);
idx = find(BW1 == 1);
aponeurosis = L = = L(idx(1));
aponeurosis(BW2) = 2;
[L,n] = bwlabel(aponeurosis);
RGB = label2rgb(L, 'autumn', 'black', 'shuffle');

% Initial Image
hold on;
himage = imshow(RGB);
himage.AlphaData = 0.3;

% SLIDER
b = uicontrol('Parent',f,'Style','slider','Position',[81,54,419,23],... 'value',thresholdPct, 'min',0, 'max',100);
bgcolor = f.Color;
bl1 = uicontrol('Parent',f,'Style','text','Position',[23,50,54,23],... 'String','0','BackgroundColor',bgcolor);
bl2 = uicontrol('Parent',f,'Style','text','Position',[500,54,23,23],... 'String','100','BackgroundColor',bgcolor);
bl3 = uicontrol('Parent',f,'Style','text','Position',[240,25,100,23],... 'String',sprintf('Threshold %2.2f', thresholdPct), 'BackgroundColor',bgcolor);
b Callback = @(hObject, event) sliderCallback(hObject, event, meanMus, meanApo, I, BW1, bl3, BW2);

% QUANTIFY
btn = uicontrol('Style', 'pushbutton', 'String', 'Quantify',... 'Position', [20 600 50 20],... 'Callback', @(hObject, event) quantifyCallback(hObject, event, I, BW2, scale, factor, filename));