

SUPPLEMENTARY MATERIAL

Skeletal muscle involvement in patients with truncations of titin and familial dilated
cardiomyopathy

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Supplementary methods S1

Matching of controls

Healthy control subjects were matched individually 1:1 to participants with TTNtv. All participants were scanned using the same magnetic resonance imaging equipment, using the same scanning protocol specified below. Controls were included from the general population in a research setting, and did not have a clinical indication for muscle testing. Patients and healthy controls were matched based on three factors: age (aiming at age within 2 years of the patient), sex, body-mass index (aiming at a maximal difference within 2 units). These three factors were chosen since they have previously been found to correlate with muscle fat fraction.

Magnetic Resonance Imaging Protocol

Subjects were scanned with a head, neck and spine coil, two body matrixes and a peripheral angio coil in a head first, supine position in a 3.0 T Siemens scanner (Magnetom Verio Tim System; Siemens AG, Erlangen, Germany). The MRI protocol included whole body three-planned localizers (echo time [TE] = 85 msec, repetition time = 1500 msec, slice thickness = 6.0 mm, distance factor = 100% between slices, and field of view was 480 mm.), axial T1-weighted imaging at C6, Th12, L4, S2 mid thigh and mid calf (TE = 25 msec, repetition time = 550 msec, slice thickness = 6.0 mm, distance factor = 20% between slices, and field of view was 400 to 500 mm.) and axial Dixon imaging at C6, TH12, L4, S2 mid thigh and mid calf ((TE = 2.45, repetition time = 5.59, slice thickness = 3.5 mm, distance factor = 20 %, field of view = 400 to 500 mm.)).

Muscles investigated

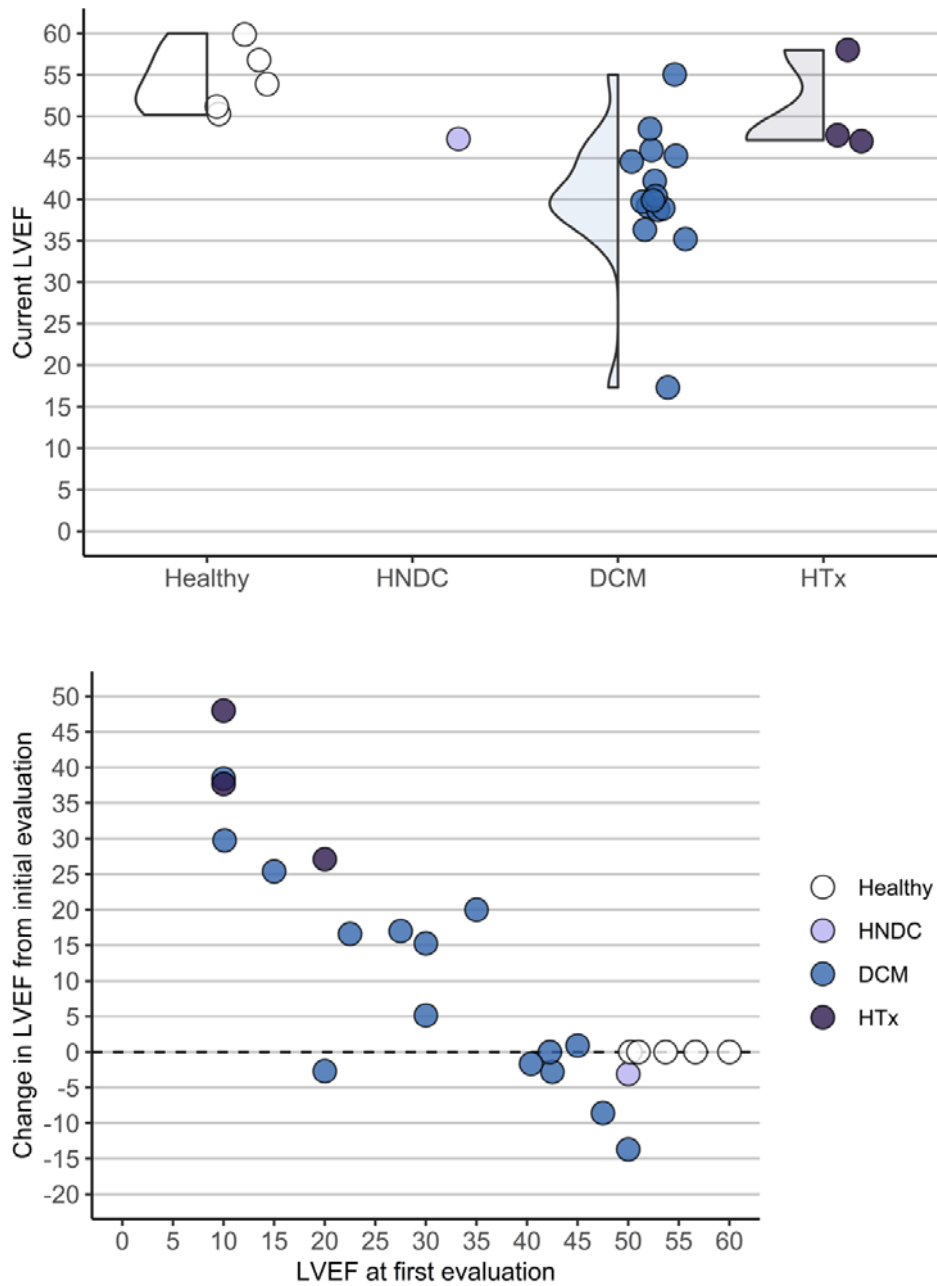
In total, we investigated 9 individual muscles of the back and 20 individual muscles of the legs. Fat fractions in muscles of the back were evaluated individually and included the deep back muscles at C6, erector spinae at Th12 and L4, multifidus at Th12 and L4, psoas major, and gluteus maximus, medius, and minimus. We evaluated 10 muscles at the midpoint of the thigh, grouped into three compartments: the anterior, medial, and posterior thigh compartments. Muscles of the anterior thigh included rectus femoris, sartorius and vastus lateralis, medialis, and intermedius muscles. Muscles of the medial thigh included the adductor and gracilis muscles. Muscles of the posterior thigh included biceps femoris, semitendinosus, and semimembranosus

muscles. At the thickest point of the calf, a third of the distance from the knee to the ankle, we evaluated a total of 10 muscles, grouped into five compartments: the anterior, lateral, medial superficial posterior, lateral superficial posterior and posterior compartments. Muscles in the anterior calf compartment included tibialis anterior and extensor digitorum longus muscles. Muscles of the lateral calf compartment included the peroneus longus and brevis muscles. The medial gastrocnemius muscle made up the medial superficial posterior calf compartment, while the lateral gastrocnemius and soleus muscles made up the lateral superior posterior compartment. Finally, the posterior calf compartment included the tibialis posterior, flexor digitorum longus and flexor hallucis longus muscles.

Skeletal muscle biopsy handling, staining and analyses

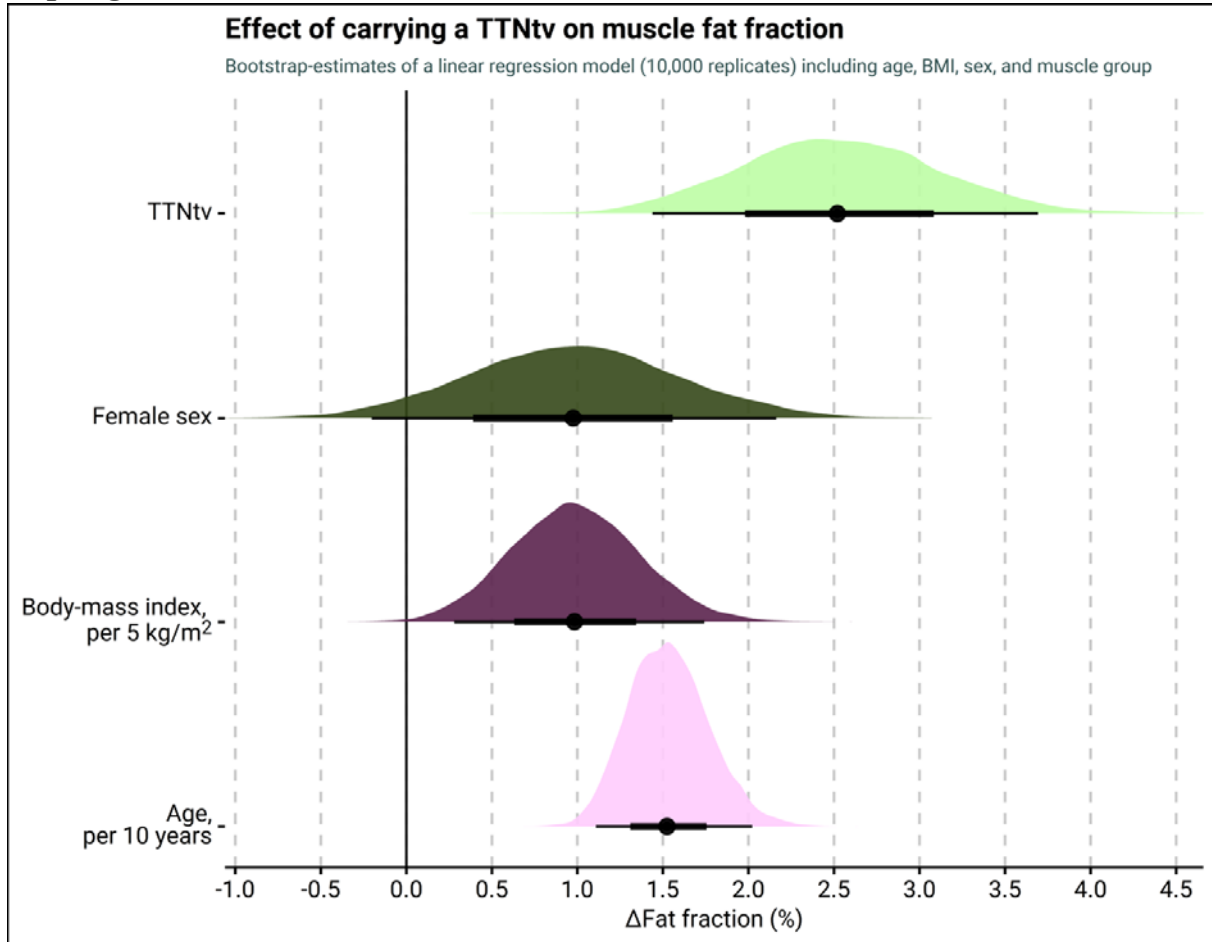
Muscle biopsies were taken from Vastus Lateralis using a Bergström needle. A piece was flash frozen in isopentane cooled in liquid nitrogen and stored at -80C and a piece was fixed in glutaraldehyde for a minimum of 48h in phosphatebuffer at 4C.

Sup Figure 1 – Cardiac phenotypes and systolic function and change in systolic function of included subjects with TTNtv



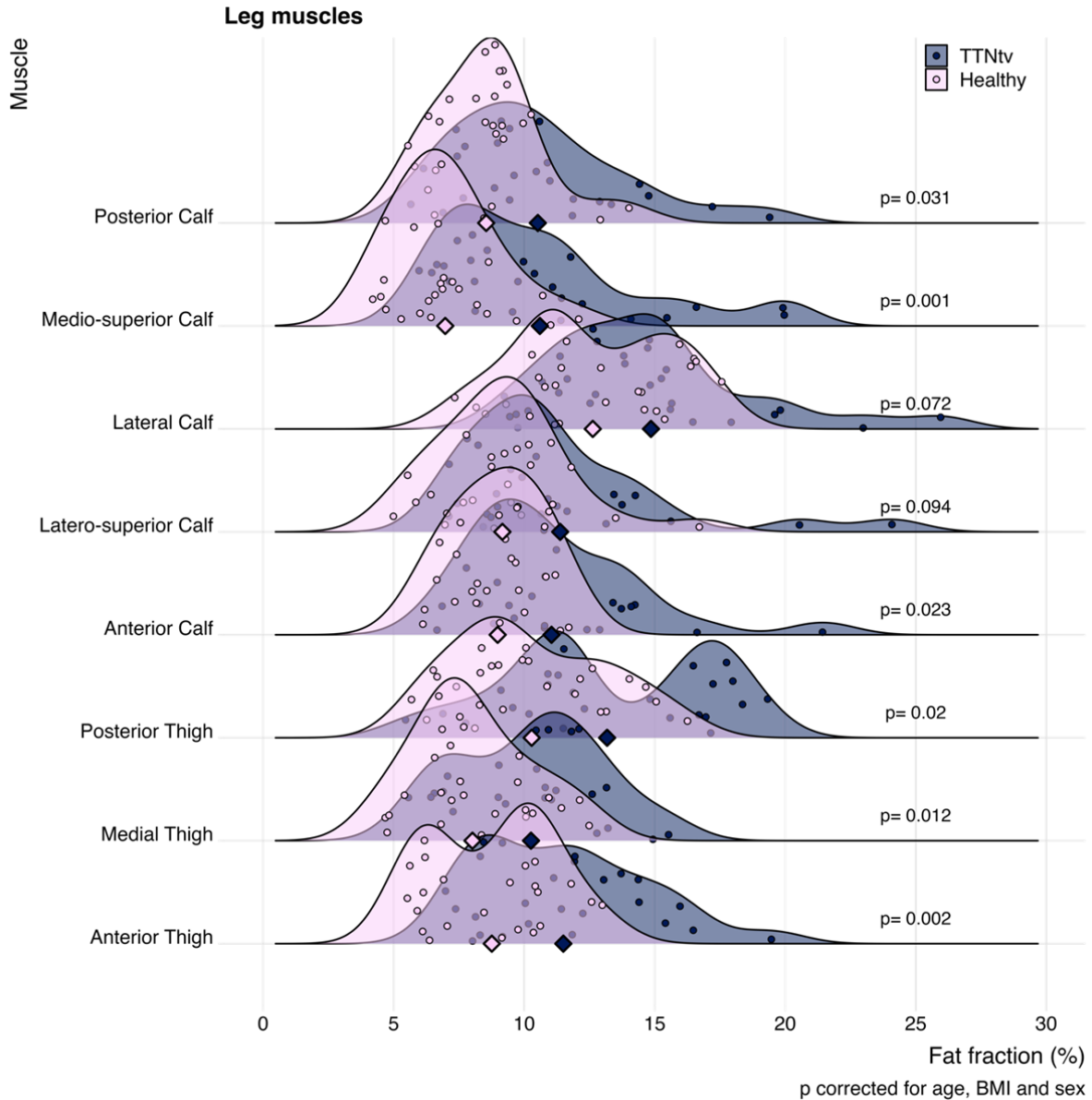
Legend: Systolic function in included subjects with TTNtv. The top graph shows the left ventricular ejection fraction (LVEF) at the time of the study, in subjects according to (worst diagnosed) cardiac phenotype. In the bottom graph, the change in LVEF from time of cardiomyopathy diagnosis (if relevant) is visualized according to LVEF at initial evaluation.

Sup Figure 2 – Overall effect of carrying a TTNtv on muscle fat replacement



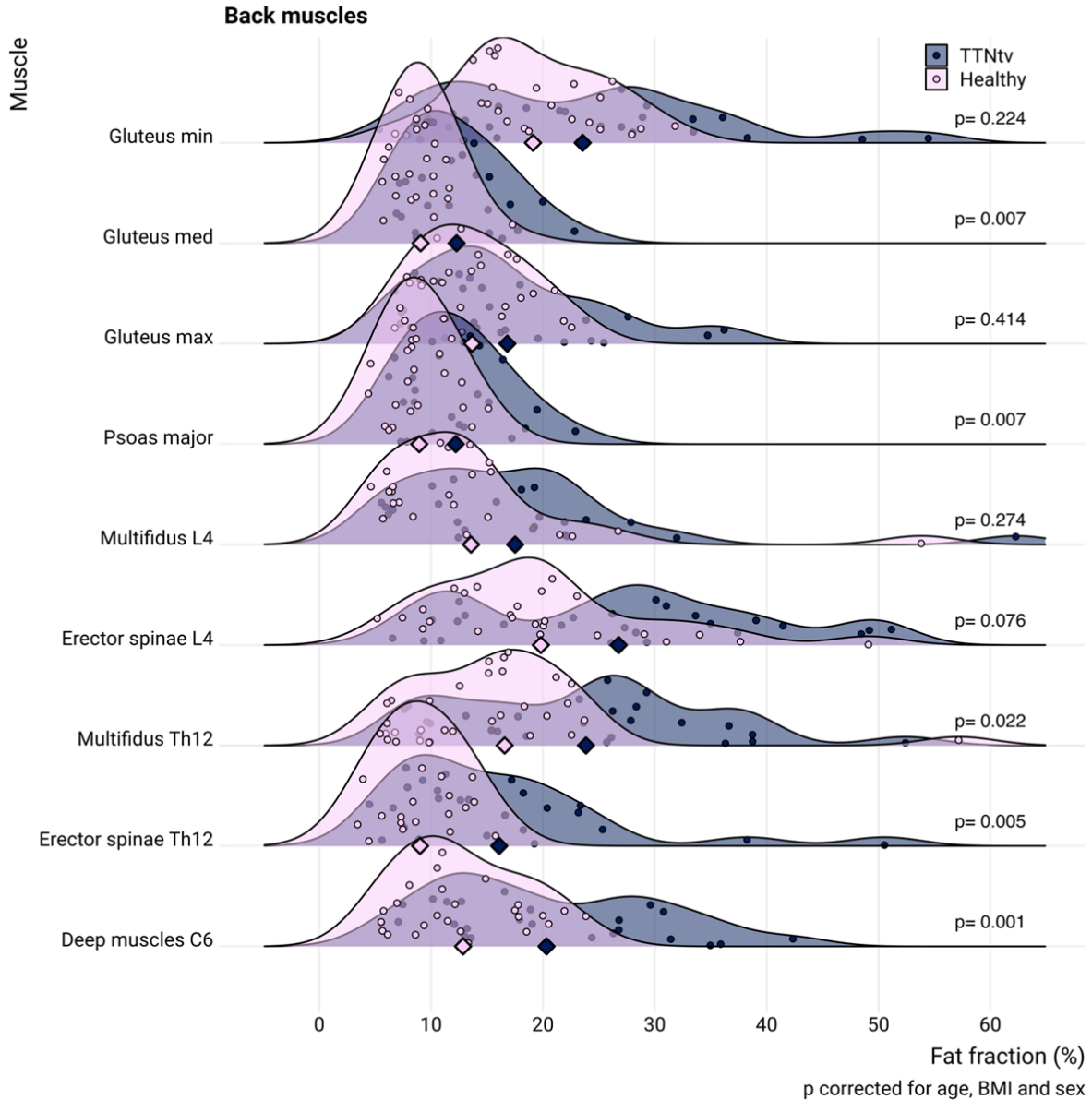
Legend: Results from ordinary least square regression of the fat fraction in skeletal muscle across all muscle groups. The effect estimates and confidence intervals are calculated from 10,000 multiple linear regression models performed on datasets created by bootstrap resampling with replacement of our study cohort (stratified by group). The colors denote the density function of point-estimates from the model, while the slab provides the point estimate (point) and the 66% (thick part) and 95% (thin part) uncertainty intervals from the probability density function.

Sup Figure 3- Ridge plot comparing fat fractions in individual leg-muscles



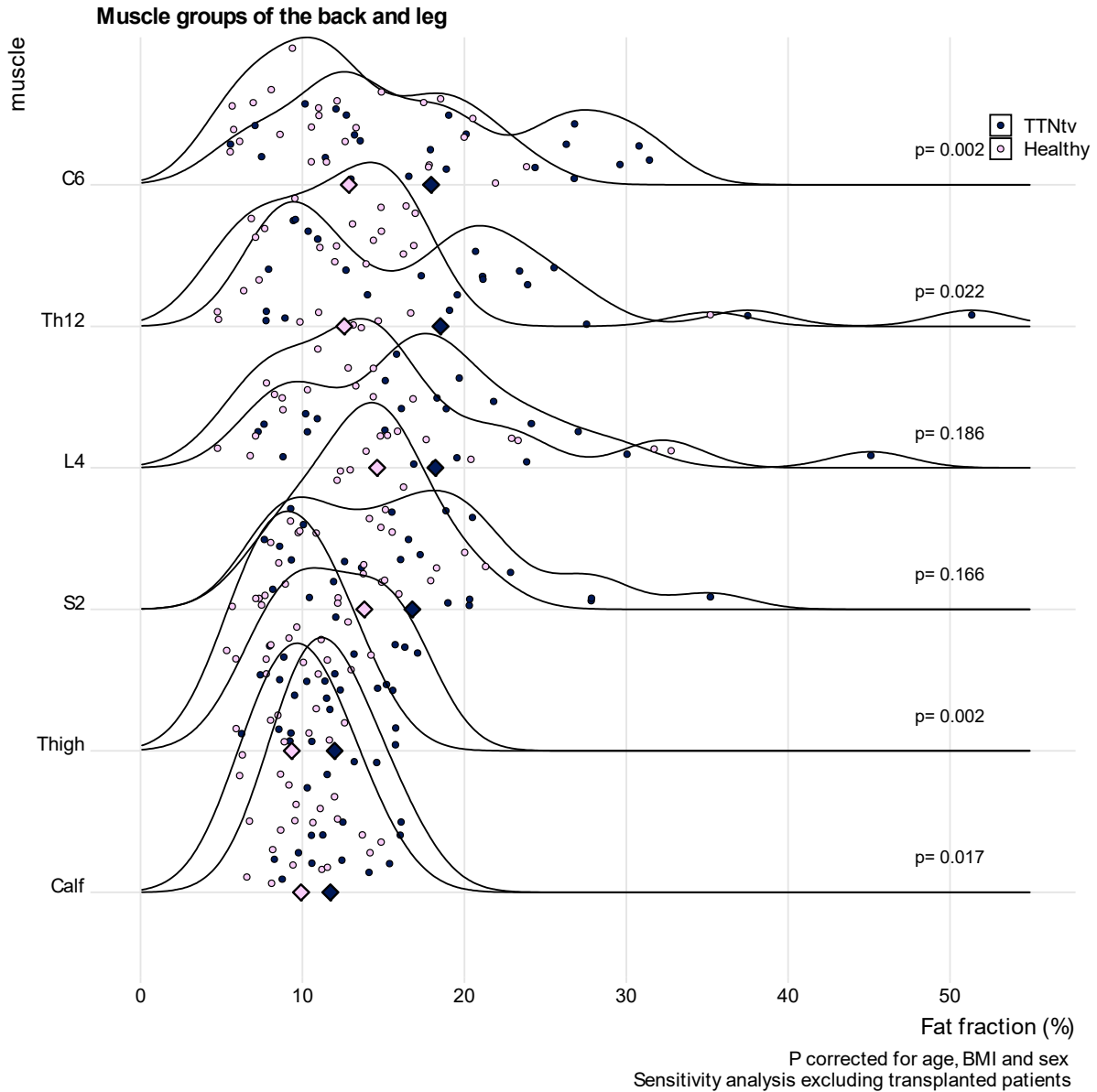
Legend: Ridgeline plot, showing the overall distribution and individually measured fat fractions of muscle-groups in the legs of participants with TTNtv (blue) and healthy controls (pink). The observed fat fraction was nominally higher in the TTNtv group in all muscles, of which the between group difference was statistically significant in 6 of 8 muscles.

Sup Figure 4 – Ridge plot comparing fat fractions in individual back-muscles



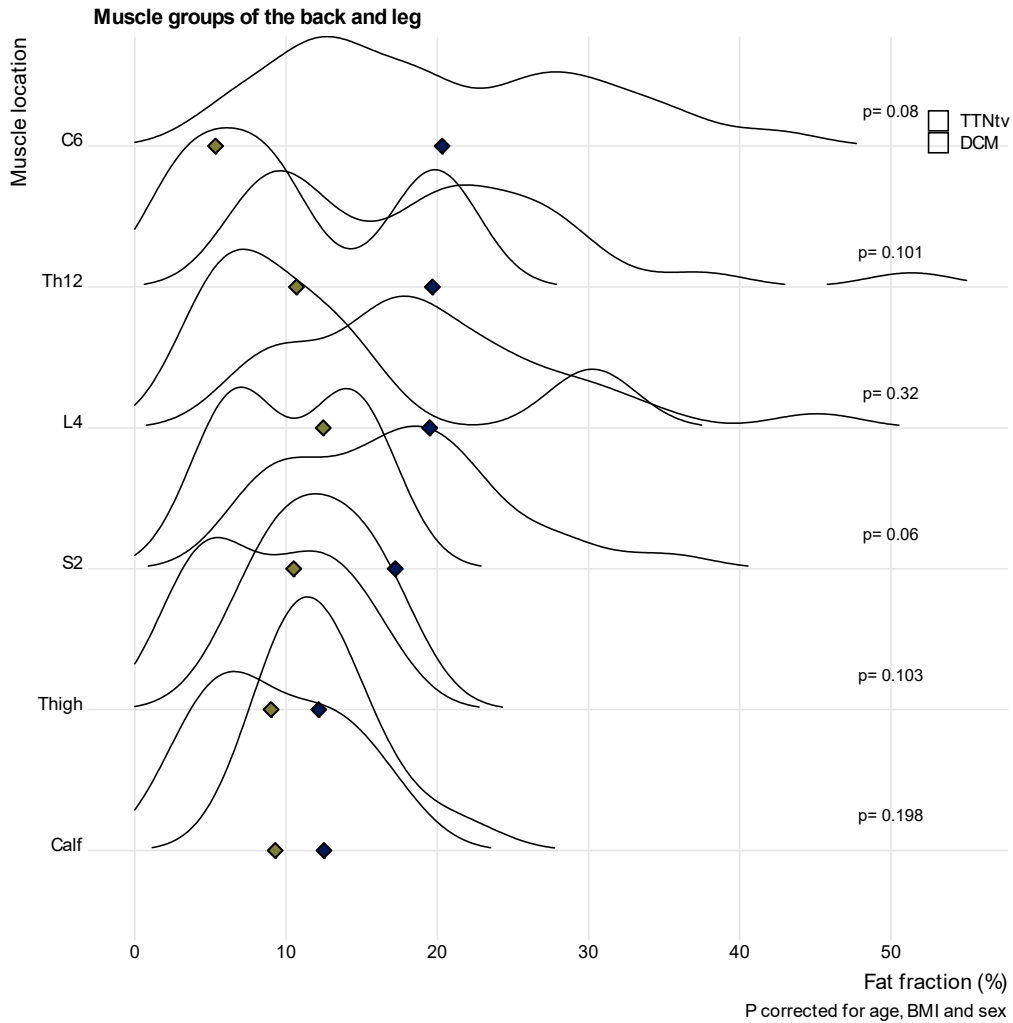
Legend: Ridgeline plot, showing the overall distribution and individually measured fat fractions of paraspinal and lower back muscles in participants with TTNtv (blue) and healthy controls (pink). The observed fat fraction was nominally higher in the TTNtv group in all muscles of which the between group difference was statistically significant in 5 of 9 muscles.

Sup Figure 5 – Fat fractions in major muscle groups at six scan-positions, excluding transplanted patients



Legend: Ridgeline plot showing the distribution of fat fraction values from magnetic resonance imaging in participants carrying TTNtv (blue, n=22) vs healthy matched controls (pink, n=25). Values from individual participants are represented by points inside the density function. Diamond-shaped symbols denote mean values. P-values are corrected for age, sex, and body-mass index (BMI).

Sup Figure 6 – Fat fraction at six scan positions in participants with TTNtv versus participants with non-TTNtv cardiomyopathy



Legend: Ridgeline plot showing the distribution of fat fraction values from magnetic resonance imaging in participants carrying TTNtv (blue, n=22) vs healthy matched controls (pink, n=25). Values from individual participants are represented by points inside the density function. Diamond-shaped symbols denote mean values. P-values are corrected for age, sex, and body-mass index (BMI).

Sup Figure 7 – Linear correlations of muscle fat fractions and age

Legend: Relationship between fat fraction of muscles and age in subjects carrying TTNtv, DCM of other genetic causes and healthy controls grouped together. Values from individual patients are represented by points and are colored according to group. The correlation between age and fat fraction was significant in all muscle groups although the correlations was markedly stronger for back- compared to thigh- or calf muscles.

Sup Table 1 – Clinical characteristics

Characteristic	TTNtv, N = 25	Controls, N = 25	p-value ²
Females	11 (44%)	12 (48%)	0.8
Body-mass index	26.6 (IQR: 24.8 to 34.0)	25.4 (IQR: 24.0 to 28.2)	0.2
Age, years	51 (15)	48 (13)	0.6
Cardiac medications			
RAAS	15 (60%)	NA	-
Betablockers	11 (44%)	NA	-
MRA	4 (16%)	NA	-
Diuretics	9 (36%)	NA	-
Amiodarone	2 (8%)	NA	-
DOAC	3 (12%)	NA	-
Prednisolone	3 (12%)	NA	-
Everolimus	2 (8%)	NA	-
Ciclosporin	2 (8%)	NA	-
Co-morbidities			
Diabetes	3 (12%)	NA	-
Hypertension	4 (16 %)	NA	-
Atrial fibrillation	4 (16%)	NA	-
Reported symptoms			
Muscle soreness	6 (24%)	NA	-
Muscle weakness	5 (20%)	NA	-
Muscle wasting	2 (8%)	NA	-
Fatigue	6 (24%)	NA	-
Syncope (any cause)	5 (20%)	NA	-
Palpitations	9 (36%)	NA	-

Characteristic	TTNtv, N = 25	Controls, N = 25	p-value ²
Imaging and plasma biomarkers of cardiac and muscle function			
Thigh muscles CSA, cm ²	109 (20)	129 (34)	0.039
Calf muscles CSA, cm ²	52 (13)	58 (12)	0.11
Plasma creatine kinase, U/l	109 (IQR: 85 to 195)	NA	-
Plasma myoglobin, µg/L	51 (IQR: 39 to 68)	NA	-
Plasma NT-proBNP, pmol/L	11 (IQR: 6 to 40)	NA	-
Plasma creatinine, µmol/L	82 (IQR: 71 to 104)	NA	-

Abbreviations: CSA = cross-sectional area, DOAC = direct-acting oral anticoagulants, NT-proBNP = N-terminal prohormone of brain natriuretic peptide, MRA = mineralo-receptor antagonist, RAAS = renin-angiotensin-aldosterone system inhibitors.