

Association of Computed Tomographic Leg Muscle Characteristics With Lower Limb and Cardiovascular Events in Patients With Peripheral Artery Disease

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Background—Poor lower extremity physical performance is an independent predictor of unfavorable outcome in patients with peripheral artery disease (PAD); however, few studies have assessed muscle characteristics on imaging directly.

Method and Results—A novel 3-dimensional semi-automated protocol was developed to estimate leg muscle volume and density (mean attenuation) from computed tomography images. Patients with PAD who underwent a lower extremity computed tomography scan at a tertiary vascular surgery center were included, and were followed up using hospital records and linked data as part of a retrospective cohort study. The primary outcomes were lower limb events (major amputation or peripheral revascularization) and cardiovascular events (myocardial infarction, stroke, or cardiovascular death). Two hundred and twenty-three patients with PAD were included (median age 69.0 years; 73% men) and followed for a median of 4.9 [2.6–7.0] years. During this time there were 99 index lower limb events and 97 cardiovascular events. Low leg muscle density was associated with increased risk of lower limb (rate ratio 1.41 [1.11–1.80] per SD reduction) and cardiovascular events (rate ratio 1.60 [1.29–1.99] per SD reduction). Low muscle density remained an independent predictor of cardiovascular (but not lower limb) events, after adjusting for age, sex, traditional cardiovascular risk factors, and angiographic PAD severity (rate ratio 1.39 [1.09–1.77] per lower SD). In contrast, leg muscle volume was not associated with outcomes after adjusting for risk factors and PAD severity.

Conclusions—Low leg muscle density, but not volume, is a strong, independent predictor of major cardiovascular events among people with PAD. Further research is needed to understand the mechanisms underlying these associations. (*J Am Heart Assoc.* 2018;7:e009943. DOI: 10.1161/JAHA.118.009943.)

Key Words: lower limb skeletal muscle • peripheral artery disease • prognosis

Peripheral artery disease (PAD) is an important cause of premature death and disability, affecting over 200 million people worldwide.^{1,2} Patients with PAD have increased risk of leg amputation and cardiovascular events, such as

myocardial infarction, stroke, and cardiovascular death.^{3,4} Even with current effective medical therapy, the risk of cardiovascular events is \approx 3 times higher in patients with PAD compared with age- and sex- matched controls.^{5,6} More effective treatments are needed to reduce the high risk of lower limb and cardiovascular events in this population.

People with PAD experience debilitating lower limb symptoms, including intermittent claudication, rest pain, arterial ulcers and gangrene, which severely limit their physical activity.⁷ Randomized clinical trials demonstrate that supervised exercise therapy and home-based exercise interventions improve walking performance in patients with PAD.^{8–11} However, PAD exercise programs are not widely available in most countries, possibly because of limited evidence on the long-term effects of exercise programs on lower limb and cardiovascular events.¹² Several studies suggest that lower limb strength is a strong predictor of clinical outcome in patients with PAD, but there is limited evidence on the direct associations of leg muscle characteristics and PAD outcomes.^{13–15} Better understanding of the prognostic significance of particular leg muscle characteristics might help

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Clinical Perspective

What Is New?

- Poor lower extremity physical performance is an independent predictor of cardiovascular events in patients with peripheral artery disease; however, few studies have assessed leg muscle imaging characteristics directly.
- In this study, a novel 3-dimensional semi-automated protocol was developed to measure leg muscle characteristics in patients with peripheral artery disease.
- Low leg muscle density was a strong predictor of both adverse lower limb and cardiovascular events.

What Are the Clinical Implications?

- Further research is needed to better understand the reasons for the association between low leg muscle density and cardiovascular events among patients with peripheral artery disease, and to identify interventions that uniquely improve leg muscle density.

guide the development of effective treatments, such as particular exercise modes, to improve PAD outcomes.¹⁶ The aim of this study was to assess the association of computed tomography (CT)-measured leg muscle characteristics with adverse lower limb and cardiovascular events in patients with PAD.

Methods

Study Population

This was a retrospective study of individuals presenting to a vascular surgery department at a single tertiary hospital in Queensland, Australia, between May 2004 and September 2012. Inclusion criteria for the study were the following: (1) lower extremity CT angiogram conducted as part of routine clinical assessment; (2) evidence of PAD on the CT angiogram defined as $\geq 50\%$ stenosis in at least 1 lower limb artery; and (3) at least 1 follow-up assessment as an inpatient or outpatient. Patients were excluded if they had not received CT, had undergone a major lower-limb amputation before CT, or had an angiogram that was of poor quality or included imaging artifact (for example, from prosthetic implants). The study, including a waiver of consent, was approved by the Townsville Hospital and Health Service Human Research Ethics Committee (HREC/12/QTHS/75; SA/12/QTHS/131) and James Cook University (H4947). Public Health Act approval was obtained, including approval to obtain data from the Queensland Health Admitted Patient Data Collection and the Queensland death register (RD004829; RD005150). The data that support the findings of this study are available

from the corresponding author upon reasonable request by bona fide academic researchers.

Clinical characteristics collected included presenting symptoms, age, sex, diabetes mellitus, hypertension, smoking history, ischemic heart disease, stroke, and current medications. Definitions for each of these variables are included in the Supplementary Methods (Data S1). Intermittent claudication was defined as exertional lower-extremity muscle pain that caused the participant to stop walking and resolved with rest. Critical limb ischemia was defined as rest pain, arterial ulcers or gangrene, attributable to objectively confirmed occlusive PAD in line with international guidelines.¹⁷ Patients who did not have classical symptoms of PAD (intermittent claudication, rest pain, tissue loss) were categorized into a group termed atypical symptoms. This group also included patients who had imaging evidence of PAD (stenosis $\geq 50\%$ in 1 major lower limb artery) and underwent CT angiography for assessment of peripheral aneurysms.

Ankle–Brachial Pressure Index Measurement

Ankle–brachial pressure index (ABI) was assessed by qualified vascular sonographers in a subgroup of the total sample, using previously validated methods.¹⁸ Measurements were performed within 12 months of the date of the CT scan and before any revascularization procedure (further details in the Supplementary Methods).

CT Image Acquisition

Lower-limb CT scans were requested as part of the clinical care of the patients when peripheral revascularization was being considered, or as part of investigation of concurrent abdominal aortic aneurysm or lower-limb arterial aneurysm in patients with concurrent PAD. CT scans were performed using a 64-slice multislice scanner (Philips, North Ryde, New South Wales, Australia) with 3-mm axial slice thickness under a set acquisition protocol.¹⁹ Images were transferred to a dedicated workstation with OsiriX 64-bit software (version 5.8.1, Pixmeo, Geneva, Switzerland) for analysis.

Assessment of PAD Severity From CT Angiograms

PAD severity was assessed using the ANGIO Score as previously reported.¹⁹ In brief, the ANGIO Score represents a novel method to quantify PAD severity through analysis of CT angiogram images. A higher ANGIO Score indicates more severe PAD (see Supplementary Methods). Patients were also classified according to whether or not they had an occlusion of the aorta or iliac arteries (defined as occlusion of aorto-iliac arteries), common femoral, superficial femoral, or popliteal arteries (defined as occlusion of the femoro-popliteal arteries)

or anterior tibial, posterior tibial, or peroneal arteries (defined as occlusion of the infragenicular arteries) in the study leg. For the purposes of statistical analysis, the study leg was chosen as the side with the highest severity of PAD as judged by the unilateral ANGIO Score. If both lower limbs had the same ANGIO Score values or ANGIO Score measurements were not undertaken because of artifact, then the study side was assigned to the right leg.

Leg Muscle Assessment From CT Images

A protocol was developed to measure leg muscle volume and radiological density (mean attenuation). Muscles imaged within axial CT slices between the tibial tuberosity and the ankle joint were included. For each of these slices, regions of interest (ROIs) were constructed around the outside of visible muscle tissue on the axial view. The ROI was defined as the area from the deep fascia superficially to (but not including) the periosteum of the relevant bones. The appropriate positioning of the ROI was checked, and if required adjusted, by examining multiple axial slices from the CT images. All tissue not included within the ROI was excluded. Finally, muscle within the ROI was isolated by applying a previously defined radiological threshold (window level, 50 Hounsfield units [HU]; window width, 100 HU) within the attenuation range for skeletal muscle.^{20,21} The inbuilt “Calculate Volume” tool was applied to estimate the volume and average radiological density of the muscle within the 3-dimensional reconstruction (Figure 1). Leg muscle volumes were standardized to the total volume of leg tissue between the tibial

tuberosity and ankle joint to adjust for differences in body size, and reported as percentage muscle volume. Leg muscle density was reported in HU, which is a measure of radiological density. Formal reproducibility assessment of the CT leg muscle measurements was performed by researchers who were blinded to each other’s measurements (see Supplementary Methods).²²

Follow-Up and Outcomes

Patients were followed up through attendance at outpatient clinics and as an inpatient as part of their normal medical care as previously described.^{23–25} Charts and hospital electronic records of patients were reviewed by a vascular specialist. Data were also linked with hospital admission records obtained from the Queensland Health Admitted Patient Data Collection and the Queensland death register (Data S1).^{19,26} The primary outcomes of the study were the following: (1) major lower limb events, defined as any major lower-limb amputation (ie, above or below the knee), or a lower-limb revascularization procedure, such as bypass or stenting; and (2) major cardiovascular events, defined as myocardial infarction, stroke, or cardiovascular death.

Statistical Analyses

Sample size estimates suggested that ≈200 patients would be sufficient to detect important associations between skeletal muscle characteristics and the primary outcomes (see Supplementary Methods).²⁷ Continuous data were

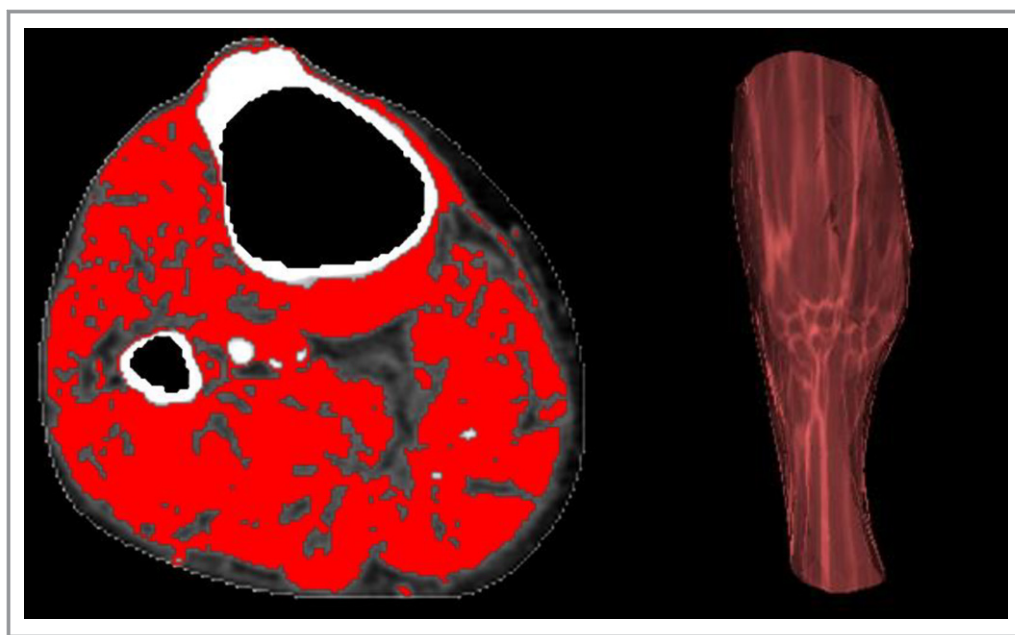


Figure 1. CT assessment of leg muscle characteristics. Example of the assessment of leg muscle characteristics from CT imaging. CT indicates computed tomography.

Table 1. Characteristics of Patients With PAD Included in This Study in Relation to CT-Measured Leg Muscle Density Tertiles

Characteristic	Leg Muscle Density Tertiles			P Value
	Tertile 1 (n=59)	Tertile 2 (n=74)	Tertile 3 (n=90)	
Age, y*	71.6 [66.8–77.7]	67.3 [60.3–74.5]	64.7 [58.0–72.5]	<0.001
Sex (% males)	49 (83)	55 (74)	59 (66)	0.060
Diabetes mellitus	26 (44)	25 (34)	31 (34)	0.398
Current smokers	21 (36)	36 (49)	39 (43)	0.318
Hypertension	28 (47)	24 (32)	40 (44)	0.606
IHD	33 (56)	40 (54)	42 (47)	0.473
Stroke	6 (11)	9 (12)	11 (12)	0.935
Medications				
Aspirin	39 (67)	49 (66)	70 (78)	0.198
Other antiplatelet	10 (17)	8 (11)	13 (14)	0.563
Warfarin	12 (21)	13 (18)	6 (7)	0.031
Statin	36 (62)	54 (73)	58 (64)	0.354
ACEI or ARB	37 (64)	41 (55)	51 (57)	0.586
β-Blocker	24 (41)	21 (28)	26 (29)	0.203
CCB	9 (16)	22 (30)	31 (34)	0.040
Metformin	9 (16)	15 (20)	16 (18)	0.778
Insulin	6 (10)	12 (16)	5 (6)	0.083

Values are frequency and percentage unless otherwise specified. Tertiles of leg muscle density grouped according to equal numbers of major cardiovascular events. Medication history missing for 1 patient; stroke history missing in 2 patients. ACEI indicates angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; CT, computed tomography; IHD, ischemic heart disease; PAD, peripheral artery disease.

*Reported as median and interquartile range.

generally not normally distributed according to histograms and skewness and kurtosis tests. Therefore, they were presented as median and interquartile range, and were compared using Mann–Whitney or Kruskal–Wallis tests as appropriate. Categorical data were presented as absolute numbers and percentages, and were compared using Pearson's χ^2 test.

The associations between leg muscle characteristics and ANGIO Score were assessed using the Kruskal–Wallis test by categorizing ANGIO Score into tertiles. The association between muscle characteristics and ABI was assessed using Spearman's rho for correlation, and Kruskal–Wallis test for association with clinically relevant ABI groups (ABI <0.5, ABI \geq 0.5 and <0.9, ABI \geq 0.9 and <1.4).

Kaplan–Meier curves and multivariate Cox regression were used to assess the associations of muscle characteristics with the primary composite outcomes. Follow-up time was defined as the time between the date of the initial CT scan and occurrence of the first event, or last clinical review. Patients who did not have a primary event were censored at the time of death or at their last recorded follow-up (up until May 5, 2017, after when data were cleaned and analyzed). Kaplan–Meier curves were used to illustrate the risk of lower limb and cardiovascular events across tertiles of leg muscle volume and

density. Multivariate Cox proportional hazard analysis was then used to assess the association of muscle characteristics with primary outcome events, adjusting for relevant confounding variables. Muscle characteristics were grouped into tertiles according to equal numbers of outcome events, and rate ratios (RR) for these categories were plotted against the mean baseline values of each group. This allowed assessment of both strength and pattern of associations. When several groups were being compared with each other, the variance of the log risk in each group (including the reference group) was calculated from the variances and covariances of the log RR values. This yielded group-specific confidence intervals (CIs) for each group (including the reference group) that described the effect of chance on the log risk in that 1 group. It also yielded comparisons across groups that were unaffected by the choice of reference group.^{28,29} Analyses of continuous exposures were conducted and standardized to a 1 SD difference in muscle volume or density. Proportional hazards assumptions were confirmed by the Schoenfeld residuals test, with no violations identified. Additional sensitivity analyses assessed the robustness of the associations with major lower limb amputation outcomes, in a lower-risk subgroup without critical limb ischemia, after excluding the first 6 months of follow-up and limiting cardiovascular events to non-perioperative events

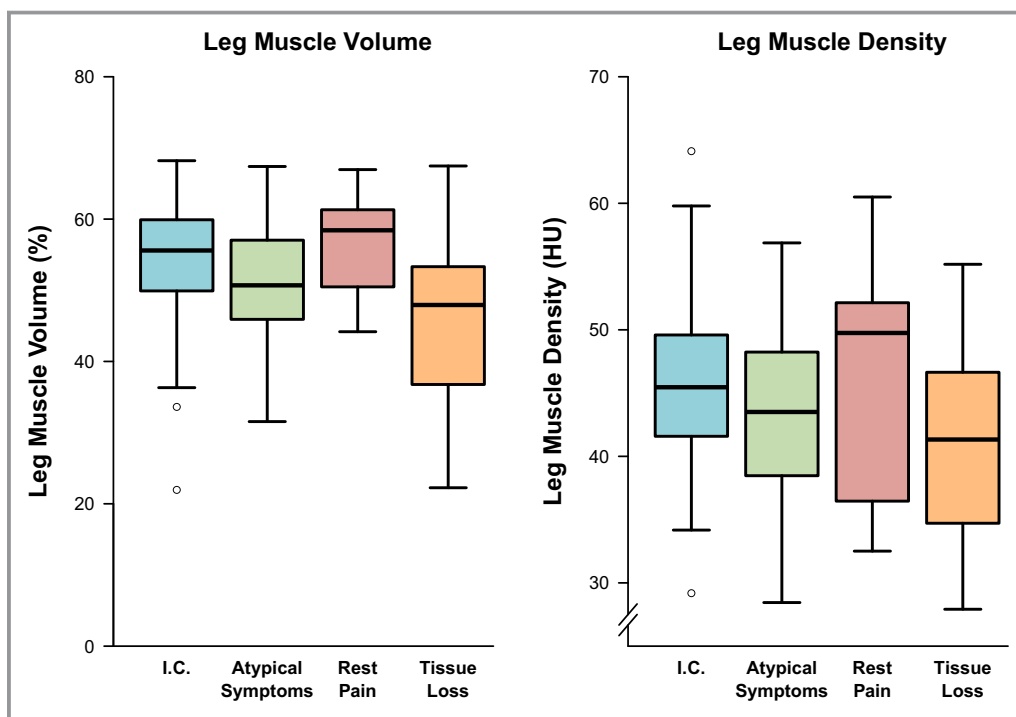


Figure 2. Association between PAD presentation and CT leg muscle characteristics. Box plots are shown illustrating lower limb muscle characteristics according to PAD presentation. The thick band illustrates the median, the box shows the interquartile range, and the whiskers demonstrate the farthest data points that are not outliers. Acute limb ischemia was not included because of insufficient numbers (n=5). CT indicates computed tomography; HU, Hounsfield units; I.C., intermittent claudication; PAD, peripheral artery disease.

(by excluding follow-up after lower-limb revascularization or major amputation). Analyses were conducted using STATA version 15.1 (StataCorp, College Station, TX), and graphics were produced with the R statistical package, version 3.4.4 (www.r-project.org/). All statistical tests were 2-sided and a P value of <0.05 was considered significant.

Results

Population Characteristics

Two hundred sixty patients with PAD were initially included; however, 37 were later excluded because of inadequate views

(n=29), joint prosthesis (n=4), major amputation (n=3), or fracture (n=1), making accurate assessment of the CT images impossible. Ultimately 223 patients were included, of whom 124 (56%) presented with intermittent claudication, 48 (21%) had tissue loss, 35 (16%) had atypical presentations, 11 (5%) had rest pain, and 5 (2%) had acute limb ischemia. The median age of the population was 69.0 [60.8–74.2] years, and 73% of patients were male. Over 40% of patients were current smokers. Regarding medical therapy, 76% of patients were receiving antiplatelet therapy, 70% were taking blood pressure-lowering therapy, and 67% were prescribed a statin. Detailed baseline characteristics of the cohort are shown in Table 1.

Table 2. Association of ANGIO Score Categories With Leg CT Muscle Characteristics

Leg CT Muscle Characteristic	Tertile 1 (n=57)	Tertile 2 (n=70)	Tertile 3 (n=74)	P Value*
Leg muscle volume, %	54.8 [48.7–61.5]	52.4 [45.9–58.8]	51.8 [45.3–58.3]	0.214
Leg muscle density (HU)	45.6 [41.9–50.7]	45.2 [39.8–49.4]	41.6 [35.7–46.3]	0.003

Values are median [interquartile range]. Analysis includes 201 patients who had both leg muscle measurements and ANGIO Score assessment on CT. Tertiles of leg muscle characteristics are grouped according to equal numbers of major cardiovascular events. CT indicates computed tomography; HU, Hounsfield units.

*Kruskal–Wallis test.

Table 3. Lower Limb Skeletal Muscle Characteristics in Relation to Location of Peripheral Artery Occlusions

	Occlusion		No Occlusion		P Value
	N	Median [IQR]	N	Median [IQR]	
Leg muscle volume, %					
Aorto-iliac arteries	56	54.5 [43.5–60.6]	145	52.1 [46.1–58.6]	0.391
Femoro-popliteal arteries	85	54.2 [49.0–59.4]	116	51.7 [44.0–59.2]	0.140
Infragenicular arteries	125	50.3 [44.1–57.5]	76	57.7 [50.6–61.7]	<0.001
Leg muscle density (HU)					
Aorto-iliac arteries	56	44.5 [41.3–50.4]	145	43.5 [37.7–48.7]	0.149
Femoro-popliteal arteries	85	44.4 [38.3–49.8]	116	43.4 [38.7–48.1]	0.279
Infragenicular arteries	125	42.3 [36.1–48.1]	76	46.1 [41.6–50.2]	0.002

Analysis of 201 patients who had both leg muscle measurements and ANGIO Score assessment on CT. Skeletal muscle characteristics were compared with arterial occlusions in the same leg. CT indicates computed tomography; HU, Hounsfield units; IQR, interquartile range.

Reproducibility of the CT Muscle Measurements

Leg muscle volume and density measurements were highly reproducible. The concordance correlation coefficients were >99% for both intraobserver and interobserver measurements, with mean differences of <1% and 0.1 HU for leg muscle volume and density, respectively (Table S1). Intra- and interobserver repeatability were consistent across the

range of muscle measurements assessed (Figures S1 through S3).

Association of Presenting Symptoms and PAD Severity With CT Muscle Characteristics

Figure 2 shows muscle characteristics for common PAD presentations. Patients with tissue loss had significantly lower

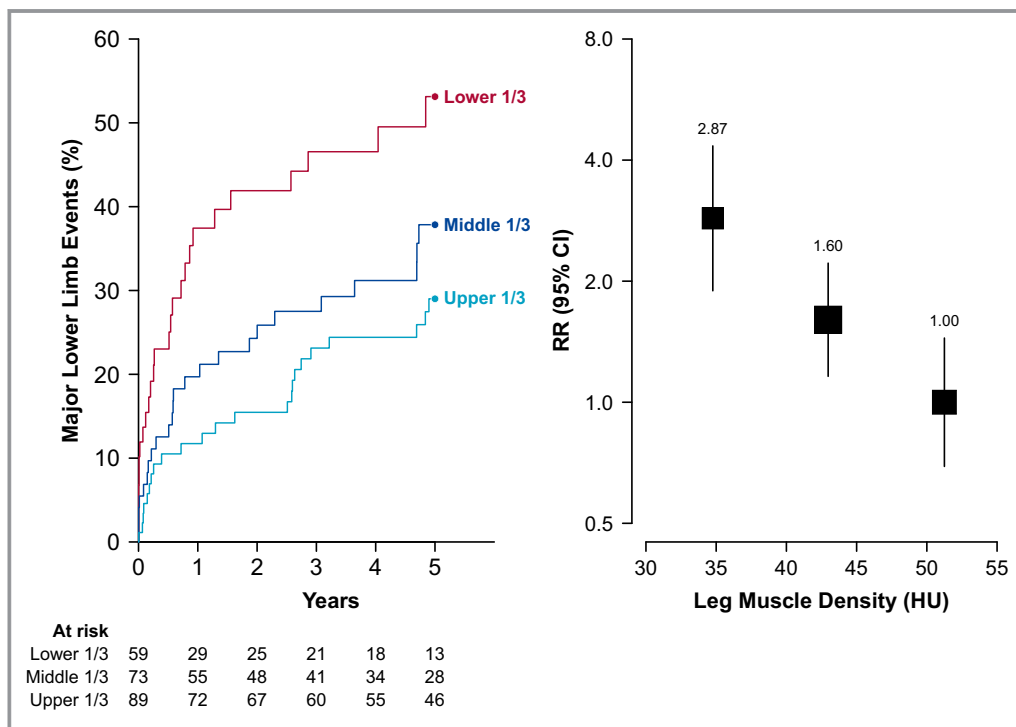


Figure 3. Association between leg muscle density and lower limb events among people with PAD. Left panel shows the risk of lower-limb events over time according to leg muscle density tertile. Right panel shows the relative risk of lower-limb events by density tertile, adjusted for age and sex. The size of the box and vertical lines represent the 95% confidence intervals calculated from the variance of the log risk. CI indicates confidence interval; HU, Hounsfield units; PAD, peripheral artery disease; RR, rate ratio.

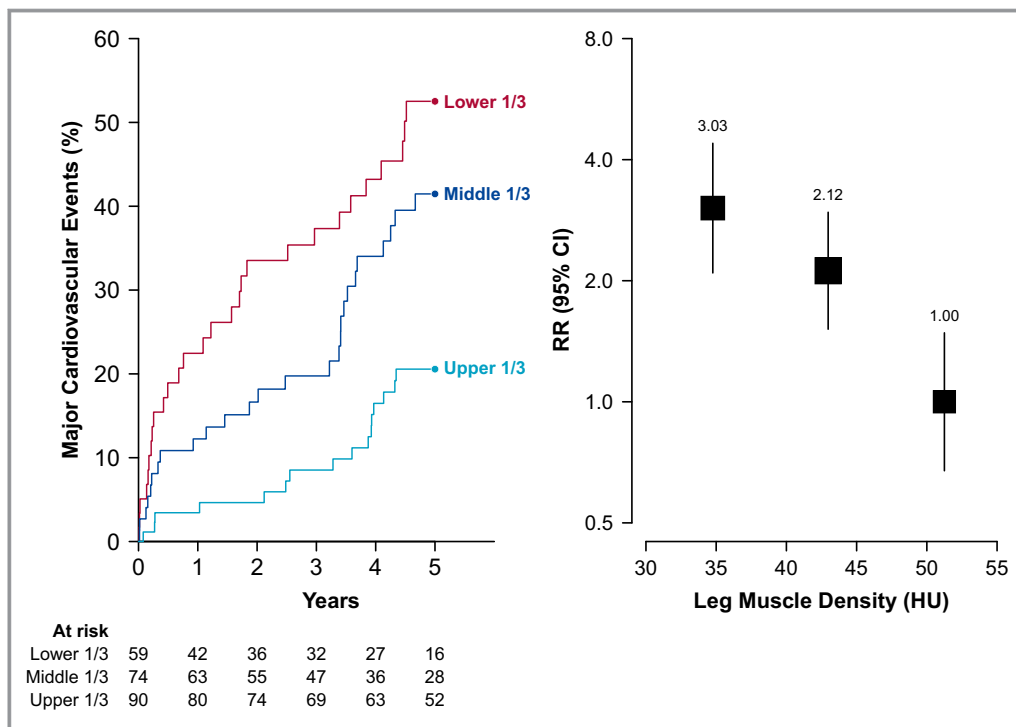


Figure 4. Association between leg muscle density and cardiovascular events among people with PAD. Left panel shows the risk of cardiovascular events over time according to leg muscle density tertile. Right panel shows the relative risk of cardiovascular events by density tertile, adjusted for age and sex. The size of the box and vertical lines represent the 95% confidence intervals calculated from the variance of the log risk. CI indicates confidence interval; HU, Hounsfield units; PAD, peripheral artery disease; RR, rate ratio.

leg muscle volume and density than those with intermittent claudication ($P < 0.001$ for both). People with atypical symptoms had lower calf muscle volume ($P = 0.017$), but similar muscle density ($P = 0.115$) to people with intermittent claudication. Interestingly, those with rest pain had similar muscle characteristics to patients with intermittent claudication. More severe peripheral athero-occlusive disease, measured using the ANGIO Score, was associated with significantly lower leg muscle density, but not muscle volume (Table 2). Many patients (44%) had arterial occlusions at multiple levels. Those with occlusions of the infragenicular arteries had significantly lower leg muscle volume ($P < 0.001$) and density ($P = 0.002$) than those without occluded infragenicular arteries. Interestingly, there was no association between aorto-iliac or femoropopliteal occlusions and leg muscle volume or density (Table 3). ABI measurements were performed in some of the patients ($n = 41$) near the time of CT imaging (median of 1 day [0–25 days]). ABI was not associated with leg muscle characteristics (Tables S2 and S3).

CT Leg Muscle Characteristics and Risk of Lower Limb Events

Patients were followed up for a median of 4.9 [2.6–7.0] years after CT imaging. During this time 92 patients (41%) had at least 1 lower limb arterial revascularization procedure, including 70

endovascular interventions and 46 open operations (some patients had multiple revascularizations). Seventeen patients required a major lower limb amputation, of which 10 were below-knee amputations and 7 were above-knee amputations. Overall 99 (44%) patients had at least 1 lower limb event. There was a strong association between leg muscle density and lower limb events (Figure 3). Patients in the lowest tertile of muscle density had a 3-fold higher risk of revascularization or major amputation (RR 2.87; 95% CI, 1.89–4.34) than those in the highest tertile. Each SD decrease in leg muscle density (7.3 HU) was associated with a 40% higher risk of lower limb events (RR 1.41; CI, 1.11–1.80). The association was lost after adjustment for cardiovascular risk factors and ANGIO Score. In contrast, there was no association between leg muscle volume and lower limb events (Figure S4).

Leg Muscle Characteristics and Risk of Cardiovascular Events

Ninety-seven patients (43%) had at least 1 cardiovascular event during follow-up, comprising 33 myocardial infarctions, 24 strokes, and 40 cardiovascular deaths. Leg muscle density was significantly associated with risk of cardiovascular events (Figure 4). People in the lowest tertile of density had 3 times the risk of cardiovascular events of those in the highest tertile (RR,

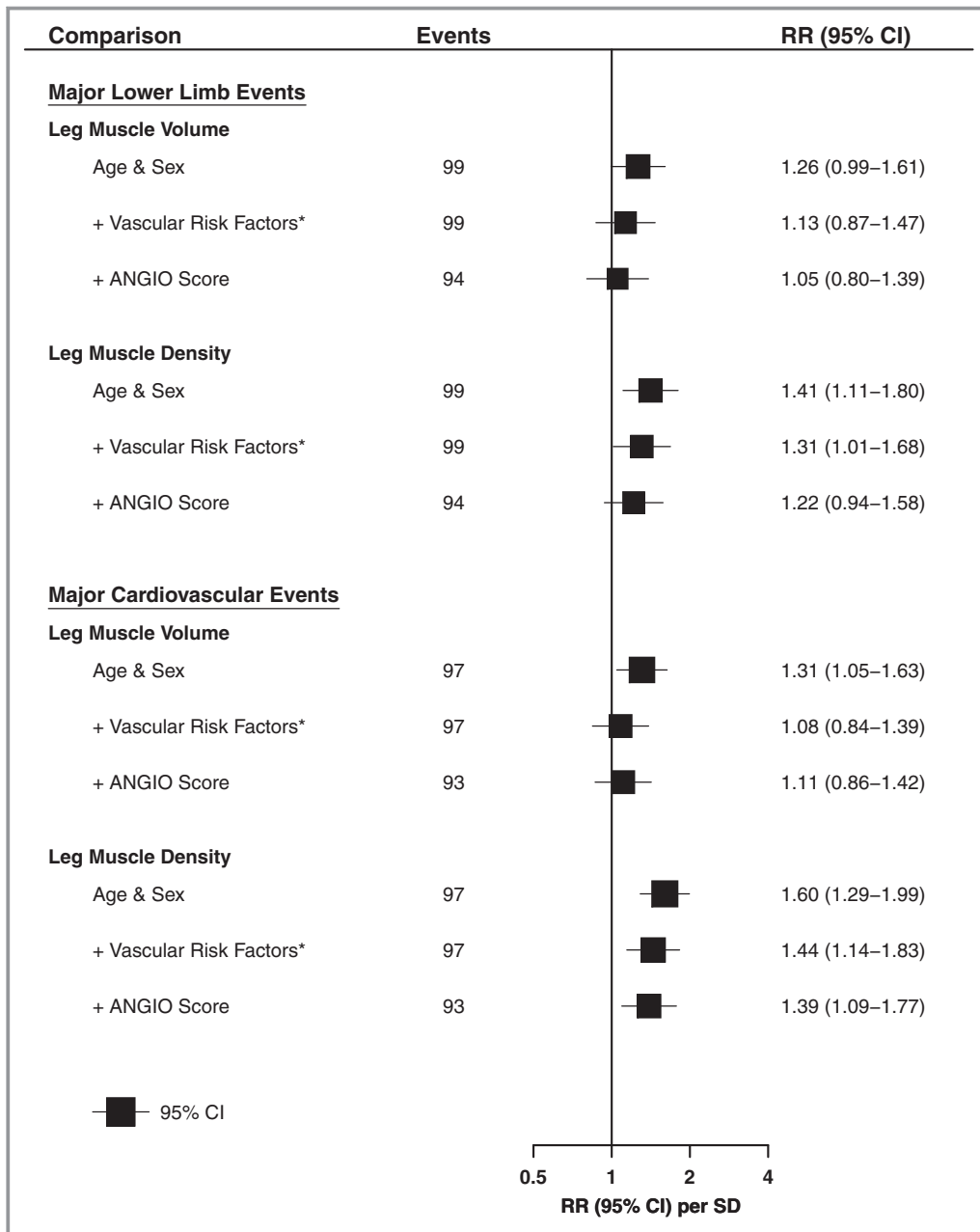


Figure 5. Association of leg muscle characteristics and major adverse events, adjusted for different risk factors. Shown are RRs and 95% CIs per SD difference (lower) in muscle characteristics with different statistical adjustments. Lower-limb event defined as any major lower-limb amputation (above- or below-knee), or peripheral revascularization. Cardiovascular event defined as any non-fatal myocardial infarction, non-fatal stroke, or cardiovascular death. *Additional adjustment for clinical presentation, smoking history, diabetes mellitus, ischemic heart disease, prior stroke, and statin use. CI indicates confidence interval; SD, standard deviation; RR, rate ratio.

3.03; CI, 2.09–4.39). Each SD reduction in leg muscle density (7.3 HU) was associated with a 60% higher risk of cardiovascular events (RR, 1.60; CI, 1.29–1.99), and remained a strong predictor of cardiovascular events after adjusting for cardiovascular risk factors and angiographic PAD severity (Figure 5). In contrast, the association of leg muscle volume with cardiovascular events was only half as strong (RR 1.31, CI,

1.05–1.63; per 1 SD lower muscle volume), and disappeared after adjusting for additional confounders (Figure 5, Figure S5).

Sensitivity Analyses

Sensitivity analyses demonstrated that leg muscle density was also significantly associated with major lower limb

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amputations after adjusting for age and sex (density: RR 1.76, CI, 1.02–3.06, per lower SD; Figure S6). The association between leg muscle density and cardiovascular events remained after restricting the comparison to patients without critical limb ischemia (intermittent claudication or atypical leg symptoms only; Figure S7). In contrast, leg muscle density was no longer associated with major lower limb events after excluding people with critical limb ischemia (Figure S8). The associations between low leg muscle density and outcome remained after excluding the first 6 months of follow-up (Figures S9 and S10), and when considering only non-perioperative cardiovascular events (Figure S11).

Discussion

This study showed strong associations between low leg muscle density and important clinical events in a high-risk PAD population. Patients in the lowest tertile of muscle density had 3 times the risk of lower-limb events and cardiovascular events of those in the highest tertile of muscle density. The association of muscle density with cardiovascular (but not lower limb) events remained after adjusting for cardiovascular risk factors and angiographic PAD severity, suggesting that low leg muscle density is an independent predictor of worse cardiovascular outcome in this population.

This is the first study to report a highly reproducible method of assessing 3-dimensional CT leg muscle characteristics in patients with PAD. Lower extremity muscle characteristics have previously been assessed on a single slice on cross-sectional imaging,¹⁴ which might not be representative of the entire leg musculature. In the prospective WALCS II (Walking and Leg Circulation Study), lower-leg muscle cross-sectional density, but not area was associated with a significantly higher risk of cardiovascular mortality.¹⁴ People in the lowest tertile of cross-sectional muscle density had triple the cardiovascular death risk of those in the highest tertile, consistent with the present study. Interestingly, leg muscle size was not associated with cardiovascular death in WALCS II, but was moderately associated with cardiovascular events in the present study after adjusting for age and sex. This could be because of the larger number of cardiovascular outcome events in the current study. Leg CT muscle characteristics do not appear to be associated with cardiovascular events in the general population. The InChianti study of 934 community-dwelling people found no association between cross-sectional calf muscle density or size with mortality after adjusting for age and sex,³⁰ suggesting that the prognostic value of leg muscle density may be unique to patients with PAD.

Interestingly, low leg muscle density, but not volume, was independently associated with risk of major cardiovascular

events. Previous studies demonstrate that decline in muscle strength in older adults is much more rapid than concomitant loss of muscle mass.³¹ This could explain why low muscle density, but not volume, was independently associated with risk of major cardiovascular events. Another possible explanation is that low muscle density may result from myocyte fat infiltration, which could influence cardiometabolic risk through pro-inflammatory adipokines.³² Factors contributing to muscle density on CT imaging are not well established. Biopsy studies suggest that patients with PAD have a lower proportion of type I muscle fibers, reduced capillary density, increased muscle apoptosis, and mitochondrial dysfunction.^{33–36} Moreover, the average type I muscle fiber size and mitochondrial activity correlate strongly with 6-minute walking distance.³⁷ The lower muscle density in patients with more severe PAD, as assessed from CT angiography, likely reflects these muscle phenotype characteristics of patients with PAD. Currently no research has been performed to assess the effect of established PAD treatments, such as exercise, or novel approaches, such as mitochondrial-modifying drugs, on leg muscle phenotype in patients with PAD, making this an important area for future PAD research.^{16,38}

A number of possible explanations exist for the association between low leg muscle density and cardiovascular events. It is possible that this association simply reflects a negative correlation between leg muscle density and severity of coronary and cerebrovascular atherosclerosis, as was the case for peripheral atherosclerosis. The association of low muscle density with cardiovascular events remained after adjusting for established cardiovascular risk factors and the severity of PAD, as estimated by the ANGIO Score. This suggests that either a factor we did not adjust for, such as physical activity, or an intrinsic effect of the leg muscle phenotype that occurs in patients with PAD, is responsible for this association. There is some preliminary evidence suggesting that skeletal muscle itself has functional roles that may act to reduce cardiovascular risk.³⁹ For example, preclinical studies demonstrate that healthy muscle secretes an array of anti-inflammatory and cardioprotective cytokines.^{40,41} It is possible, although unproven, that the observed association may be explained in part by a reduction in cardiovascular protective muscle cytokines.³⁹ Further research is needed to investigate this concept.

The rates of major clinical events in this study were high, emphasizing the need for better management of patients with PAD. There was imperfect uptake of effective cardiovascular medical therapies in the population. Combined use of effective triple medical therapy, including an antiplatelet agent, blood pressure–lowering, and a statin, can reduce the risk of cardiovascular events by as much as 50%.^{42–44} However, even with effective cardiovascular medications, the

residual risk of major adverse events in patients with PAD is still high. Novel interventions in addition to medications are needed. This study tentatively suggests that interventions that improve lower limb muscle density may reduce the risk of cardiovascular events in PAD patients. Further research is needed to better understand the reasons responsible for the association between low muscle density and cardiovascular events, and to identify and test interventions that uniquely improve leg muscle density in PAD patients.

This study has a number of strengths and limitations. The strengths include long-term follow-up and a highly reproducible method of assessing 3-dimensional leg muscle tissue from CT scans. The limitations include retrospective data collection, the select PAD population recruited, and the small sample sizes, particularly for data on ABI. Because data were collected retrospectively, there was incomplete data on biochemical measurements and body-mass index and routinely collected clinical data may not have been assessed uniformly. The effect of reverse causation and unmeasured confounders should be considered, given the retrospective cohort design. The study included patients with PAD who were referred to a tertiary vascular surgery clinic and who underwent CT imaging as part of their clinical care. The findings may not be generalizable to patients with PAD in the community with milder symptoms. ABI measurements were only available in a small subset of patients, so more moderate associations between muscle characteristics and ABI may not have been detected. Physical activity was not assessed as part of this study, so we were not able to adjust for this in the prognostic models. Greater physical activity has previously been shown to be associated with increased calf muscle density.⁴⁵ Finally, there were too few major lower-limb amputations to robustly assess the associations between CT leg muscle characteristics and major amputation alone.

In conclusion, this study showed a strong association between low leg muscle density and clinical events in patients with PAD. Further research is needed to better understand the reasons for this association.

Sources of Funding

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

SUPPLEMENTARY MATERIAL

The association of computed tomographic leg muscle characteristics with lower limb and cardiovascular events in patients with peripheral artery disease

Supplemental Methods

Definitions of clinical characteristics	3
Ankle-brachial pressure index assessment	3
ANGIO Score assessment	4
Reproducibility of leg muscle measurements from CT images	4
Electronic data-linkage with outcome events	4
Sample size estimation	5

Supplemental Tables

Reproducibility of lower limb muscle characteristics assessed on computed tomography.	6
Correlation of ankle-brachial pressure index with lower extremity CT muscle characteristics.	7
Association of ankle-brachial pressure index categories with lower extremity CT muscle characteristics.	8

Supplemental Figures

Intra-observer differences (observer 1) for CT leg muscle volume (A) and density (B).	9
Intra-observer differences (observer 2) for CT leg muscle volume (A) and density (B).	10
Inter-observer differences for CT leg muscle volume (A) and density (B).	11
Association between leg muscle volume and major lower limb events among people with PAD.	12
Association between leg muscle volume and major cardiovascular events among people with PAD.	13
Association between leg muscle density and major lower limb amputations among people with PAD.	14
Association between leg muscle density and cardiovascular events among people with IC or atypical symptoms.	15
Association between leg muscle density and lower limb events among people with IC or atypical symptoms.	16
Association between leg muscle density and lower limb events after 6 months.	17
Association between leg muscle density and cardiovascular events after 6 months.	18
Association between leg muscle density and non-perioperative cardiovascular events.	19

Supplemental Methods

Definitions of clinical characteristics

Clinical characteristics collected included: presenting complaint, age, sex, diabetes, hypertension, smoking history, ischaemic heart disease (IHD), history of stroke and current medications. Hypertension and diabetes were defined by history or treatment for these conditions. Cigarette smoking classification was based on smoking history, and grouped as current smoker, previous smoker or never smoked. IHD was defined by a history of myocardial infarction, angina or coronary revascularization. Current prescriptions for aspirin, other antiplatelet agents, warfarin, beta-blockers, calcium channel blockers, angiotensin pathway inhibitors, metformin, insulin and statins were recorded.

Ankle-brachial pressure index assessment

A 5-MHz Doppler probe (MD 6; Hokanson, Bellevue, Washington, USA) angled at 45° to the direction of blood flow was used to assess arterial signals in the right and left brachial artery, dorsalis pedis artery and posterior tibial artery. The sphygmomanometer cuff was inflated until the Doppler signal disappeared, and deflated slowly until the signal returned. The highest Doppler pressure measurement in each ankle (dorsalis pedis or posterior tibial) was divided by the highest brachial pressure to obtain an ankle-brachial pressure index (ABI) value for each foot. ABI comparisons were restricted to patients with compressible pedal arteries (ABI <1.4; toe-brachial index measurements were not performed for those with incompressible arteries).

ANGIO Score assessment

A previously validated angiographic scoring system, named the ANGIO score, was applied to CT angiograms. Scoring comprised assessment of the ten major arteries supplying the lower limbs. Arteries were scored 0, 1 or 2 according to the degree of stenosis or occlusion: 0, no stenosis or stenosis below 50 per cent; 1, non-occlusive stenosis of at least 50 per cent; and 2, complete occlusion of the artery. Values for all arteries from both lower limbs were added to produce an overall score for the patient. Higher scores indicate more severe lower extremity PAD.

Reproducibility of leg muscle measurements from CT images

All CT imaging readings were performed by two researchers (DRM and TAS). Prior to commencing measurements these researchers received training in CT analysis and performed over 10 hours of trial measurements. The reproducibility of their measurements was assessed by both researchers examining CT images of 30 legs from 15 randomly selected patients. Reproducibility was assessed on two occasions separated by at least one week, with blinding to previous measurements. Intra- and inter-observer reproducibility were assessed using concordance correlation coefficient, average coefficient of variation, mean difference and 95% limits of agreement, and Bland-Altman plots.

Electronic data-linkage with outcome events

Myocardial infarction and stroke were defined as events requiring hospital admission diagnosis, including International Statistical Classification of Diseases and Related Health Problems-10 (ICD-10) codes in the range I21.0-I22.9 and I60.0-I64 (inclusive), respectively. Mortality status, date and cause of death were obtained from the Queensland death register. Cardiovascular death was defined by cause of death in

the ICD-10 range I00–I99. Patients were censored at the first event, or at the date of last review (or linked data retrieval) if they did not experience an event.

Sample size estimation

A sample size calculation was performed according to the planned Cox regression analysis, examining the association of leg muscle characteristics with cardiovascular events. Monte-Carlo simulations suggest that a multivariate regression model is powered sufficiently when 10 outcome events per degree of freedom of the predictor variable are observed.¹ We estimated the incidence of both primary outcome events to be approximately 40% over 5-years, and planned to adjust for 8 variables.² Based on these estimates we considered that a sample size of approximately 200 patients was adequate to detect this effect size.

Supplemental Tables

Table S1: Reproducibility of lower limb muscle characteristics assessed on computed tomography.

CT Leg Muscle Characteristic	CCC (95% LoA)	Average CoV	Mean Difference (95% LoA)
Intra-Observer Reproducibility 1			
Percent Leg Muscle Volume	0.998 (0.997-0.999)	0.29%	0.00 (-0.01 to 0.01)
Leg Muscle Density (HU)	0.999 (0.999-1.000)	0.14%	-0.04 (-0.46 to 0.37)
Intra-Observer Reproducibility 2			
Percent Leg Muscle Volume	0.999 (0.998-1.000)	0.28%	0.00 (-0.01 to 0.00)
Leg Muscle Density (HU)	1.000 (1.000-1.000)	0.09%	0.04 (-0.07 to 0.15)
Inter-Observer Reproducibility*			
Percent Leg Muscle Volume	0.997 (0.995-0.999)	0.48%	0.00 (-0.01 to 0.00)
Leg Muscle Density (HU)	1.000 (1.000-1.000)	0.12%	-0.05 (-0.27 to 0.16)

CT, computed tomography; CCC, concordance correlation coefficient; CoV, coefficient of variation; LoA, limits of agreement; HU, Hounsfield units.

*Average measurements of first observer compared to average measurements of second observer.

Table S2: Correlation of ankle-brachial pressure index with lower extremity CT muscle characteristics.

CT Leg Muscle Characteristic	Correlation Coefficient	Standard Error	p-value
Leg Muscle Volume (%)	-0.29	0.14	0.067
Leg Muscle Density (HU)	0.02	0.16	0.887

Analyses conducted in 41 patients who underwent routine ankle-brachial pressure index assessment at the time of CT imaging, and restricted to those with compressible arteries (ankle-brachial pressure index <1.4). Correlation assessed using Spearman's method. The standard error of the correlation coefficient is estimated as $(1-r^2)/\sqrt{N}$. CT, computed tomographic; HU, Hounsfield units.

Table S3: Association of ankle-brachial pressure index categories with lower extremity CT muscle characteristics.

CT Leg Muscle Characteristic	ABI \geq0.9	ABI \geq0.5, $<$0.9	ABI $<$0.5	<i>p</i>-value*
Leg Muscle Volume (%)	56.1 [49.5-60.2]	56.2 [49.9-58.8]	59.4 [53.9-61.2]	0.245
Leg Muscle Density (HU)	43.8 [43.7-49.4]	45.5 [38.0-49.8]	43.1 [41.0-46.8]	0.757

Comparison restricted to people with compressible arteries (ABI $<$ 1.4). Values are median [interquartile range]. CT, computed tomographic; HU, Hounsfield units; ABI, ankle-brachial pressure index.

*Kruskal-Wallis test.

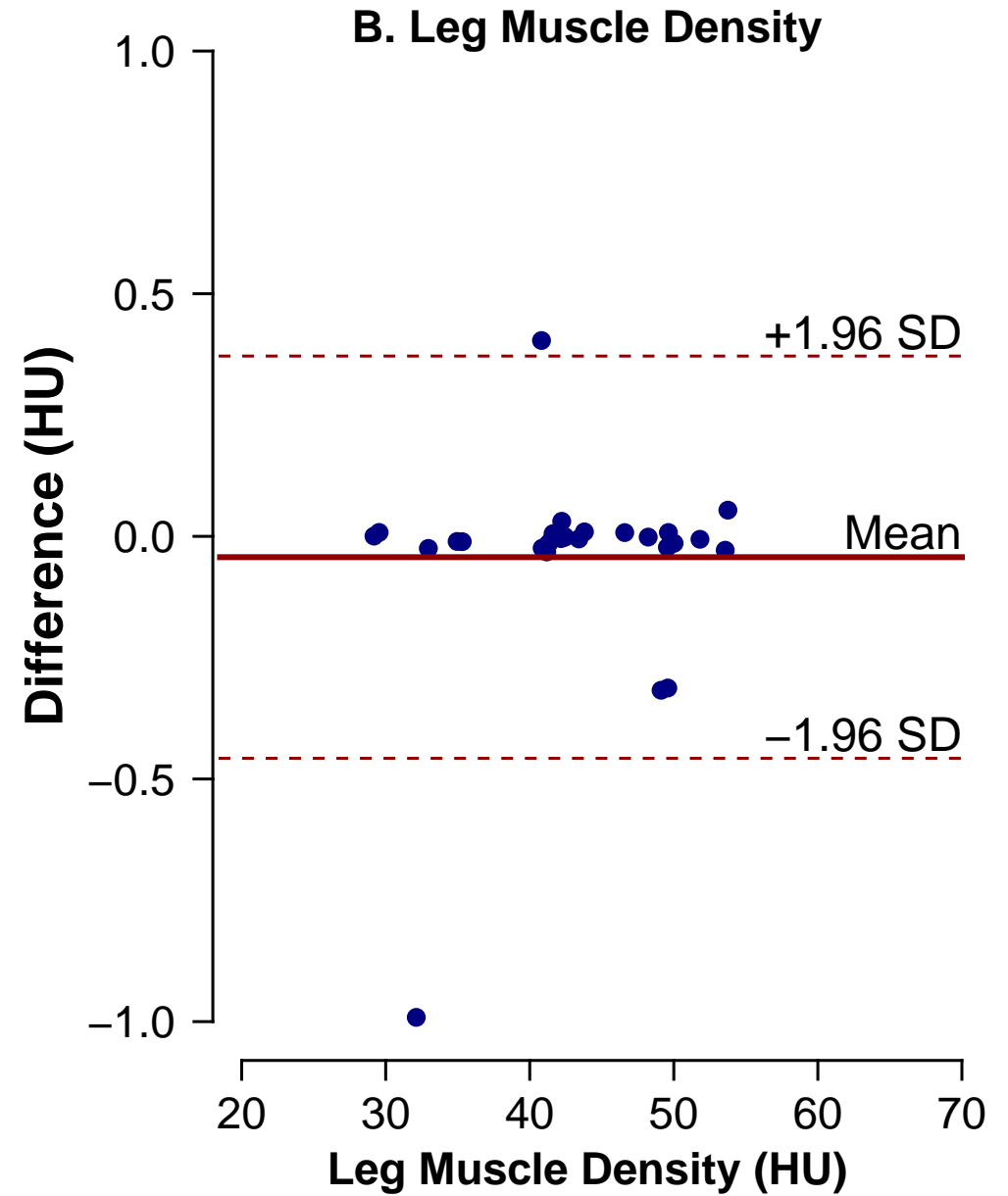
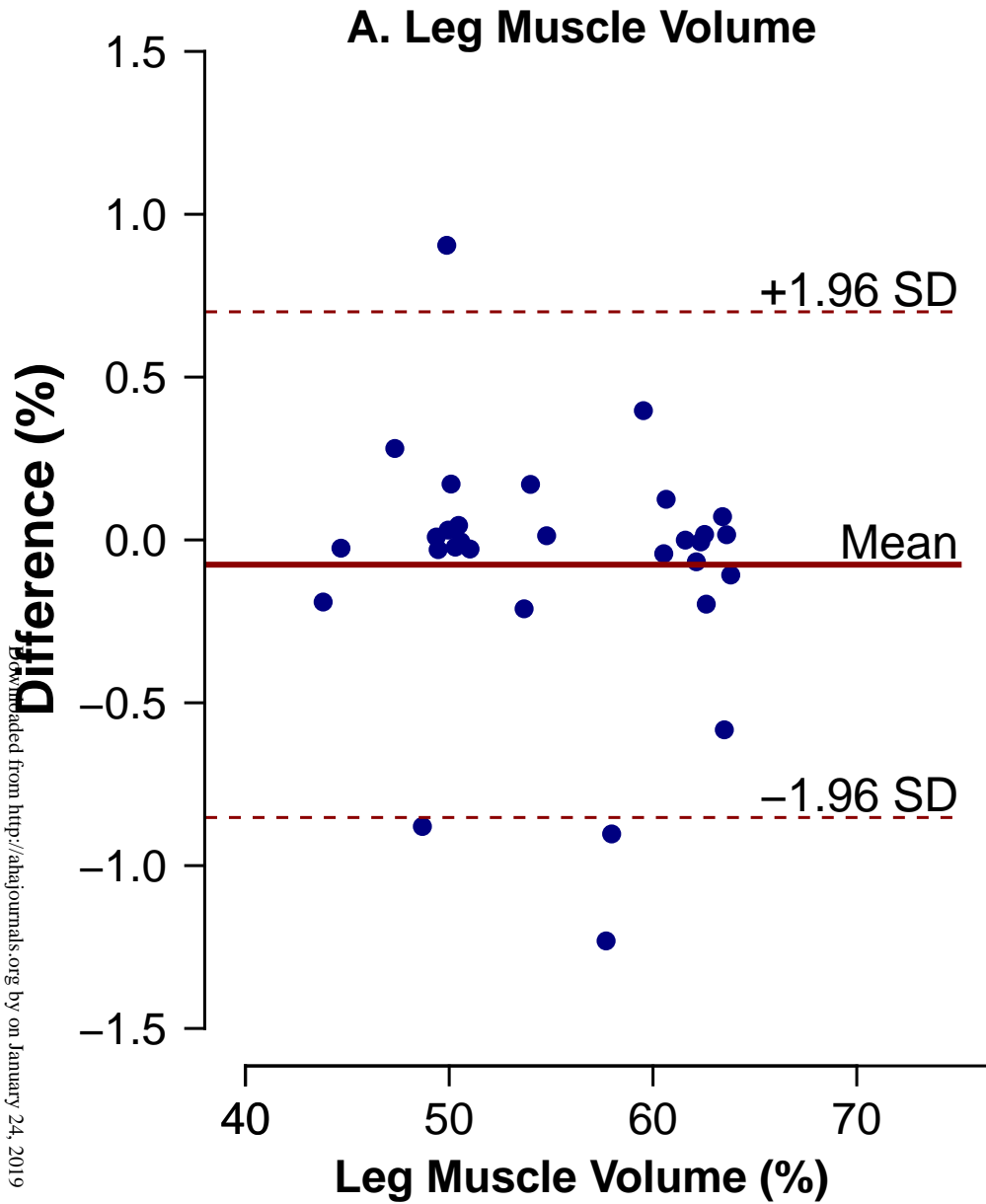


Figure S1: Intra-observer differences (observer 1) for CT leg muscle volume (A) and density (B).

The thick horizontal line represents the mean difference, and the dotted horizontal lines represent the 95% limits of agreement. SD, standard deviation; HU, Hounsfield units.

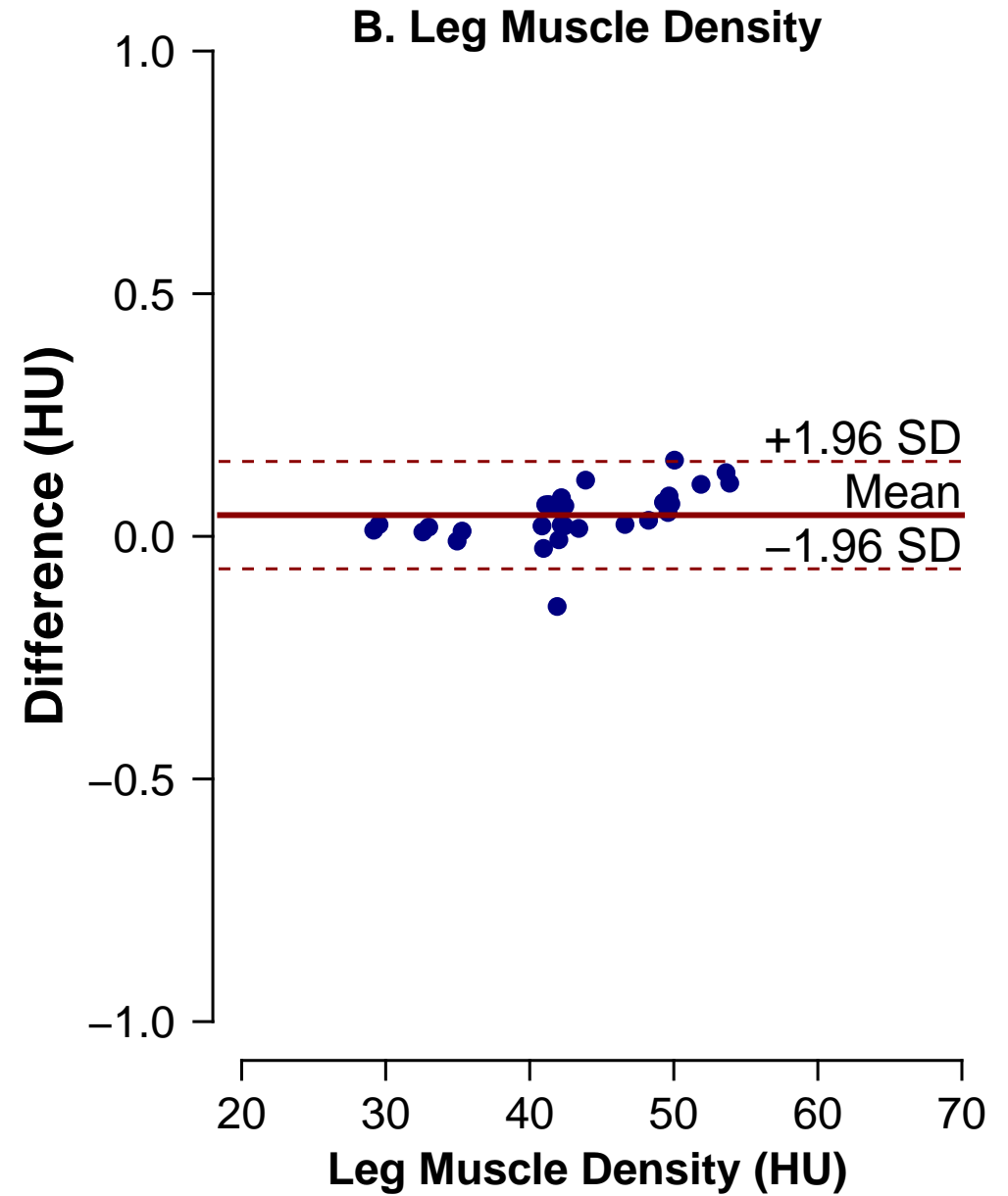
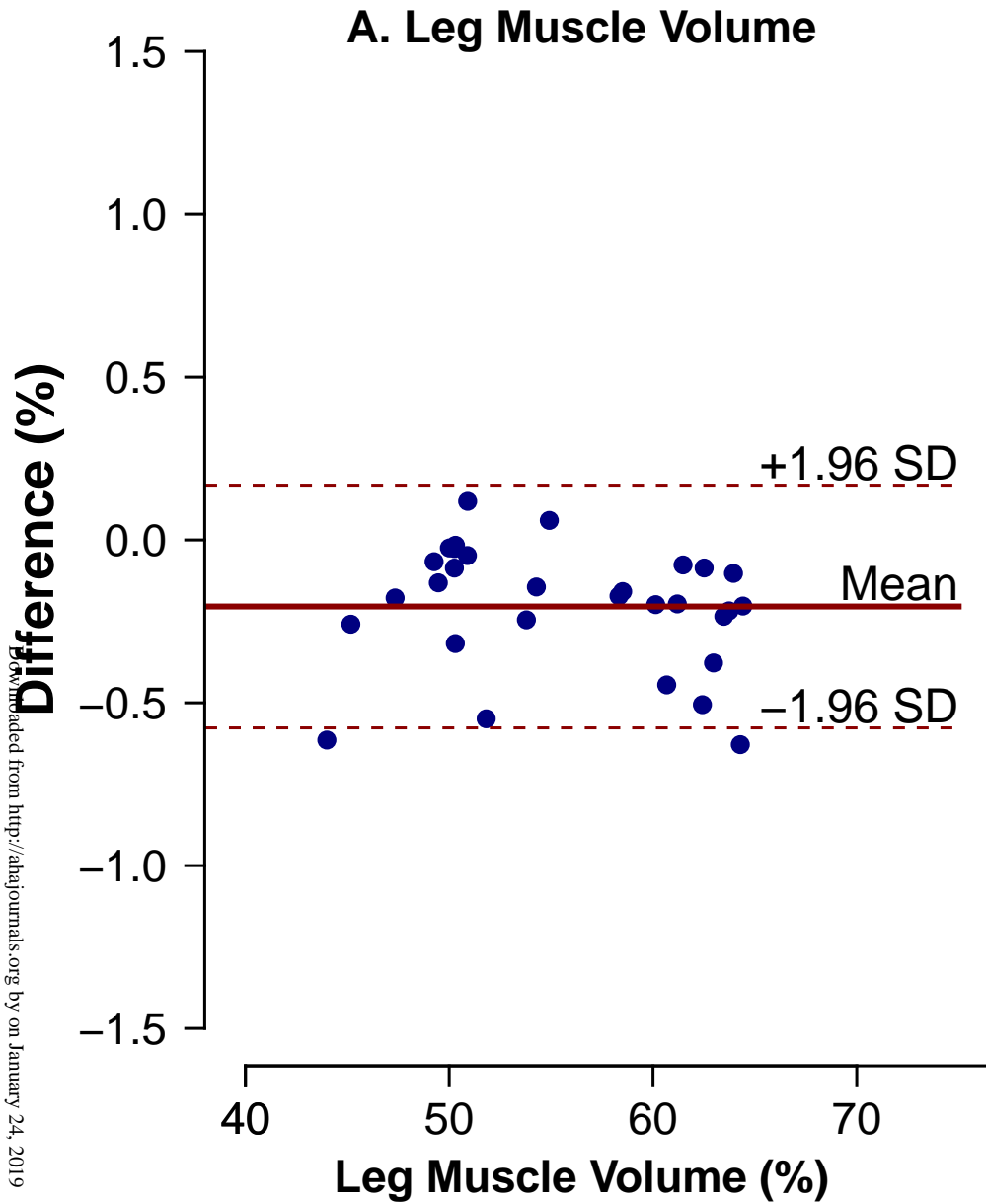


Figure S2: Intra-observer differences (observer 2) for CT leg muscle volume (A) and density (B).

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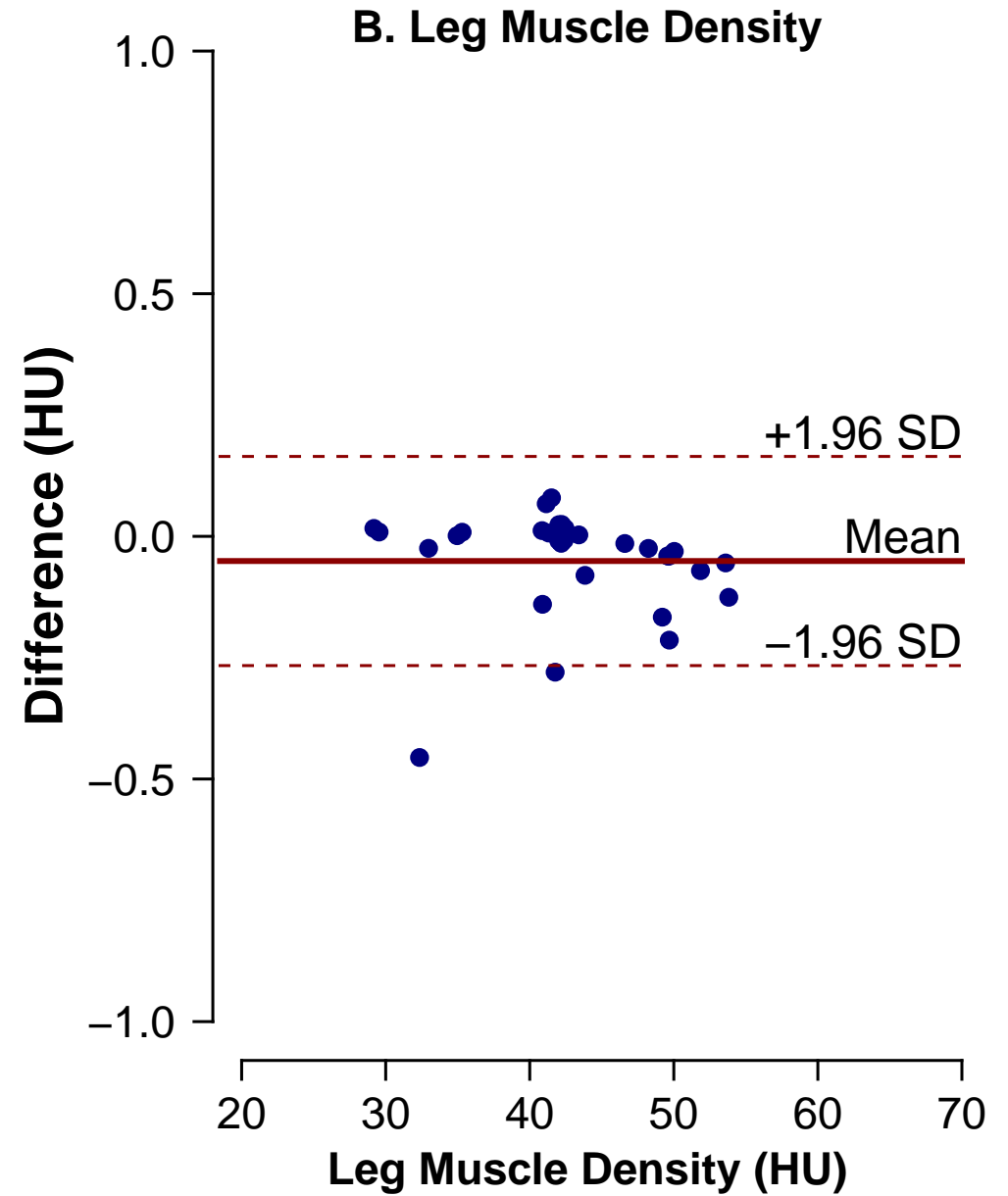
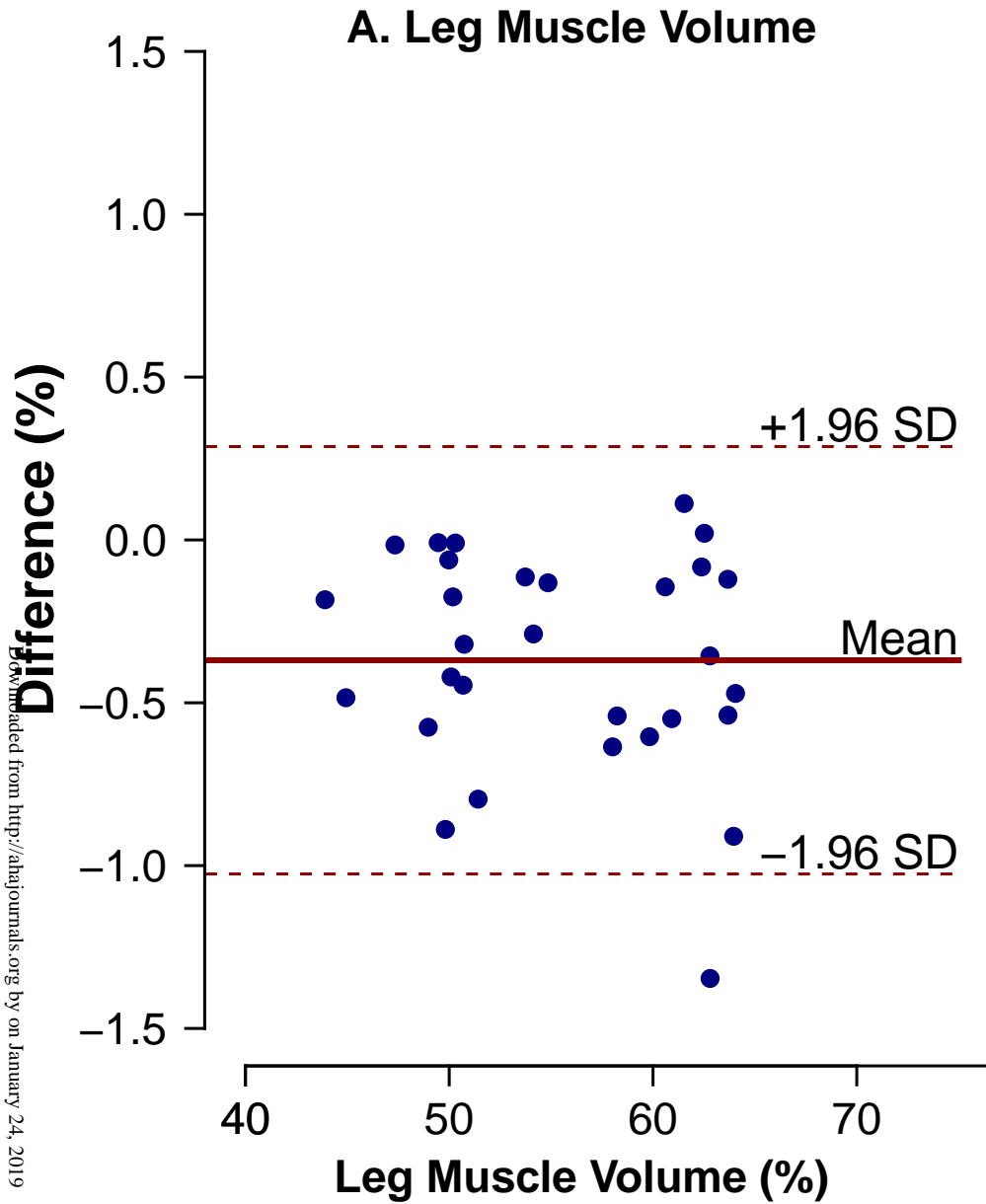


Figure S3: Inter-observer differences for CT leg muscle volume (A) and density (B).

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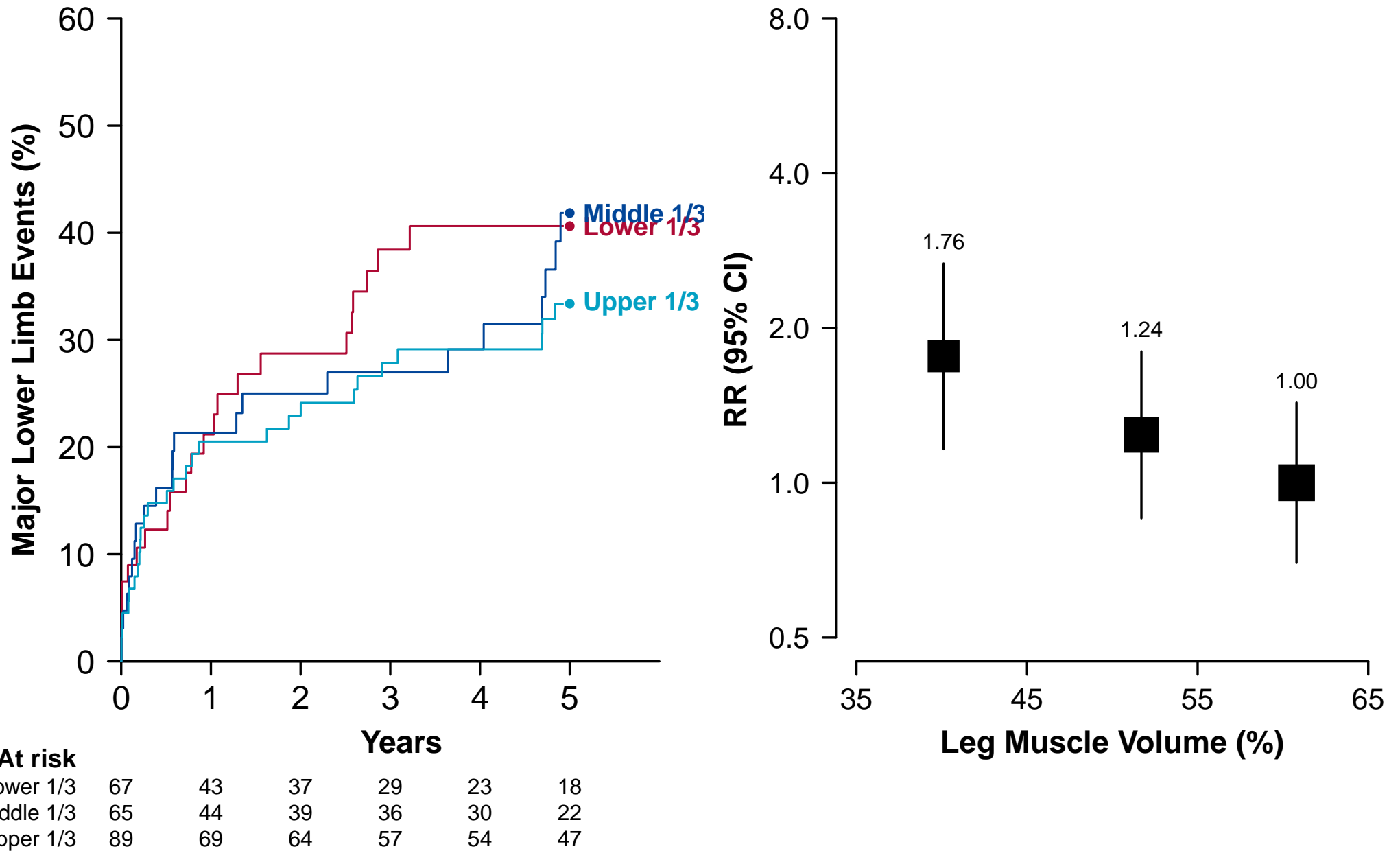
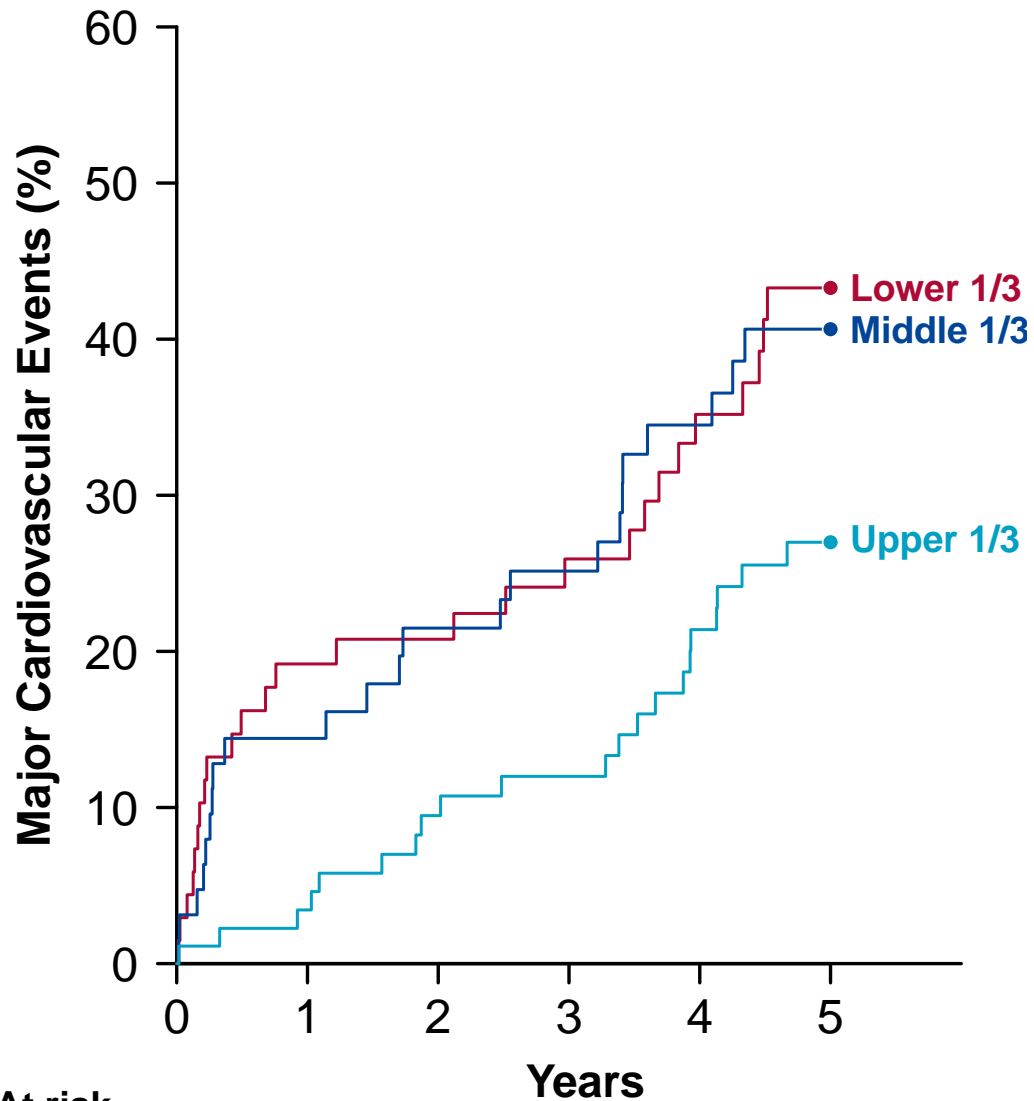


Figure S4: Association between leg muscle volume and major lower limb events among people with PAD.

Left panel shows the risk of major lower limb events over time according to leg muscle volume tertile. Right panel shows the relative risk of major lower limb events by leg muscle volume tertile, adjusted for age and sex. The size of the box and vertical lines represent the 95% confidence intervals calculated from the variance of the log risk. RR, rate ratio; CI, confidence interval.



At risk

Lower 1/3	68	53	48	41	35	23
Middle 1/3	65	50	44	40	33	25
Upper 1/3	90	82	73	67	58	48

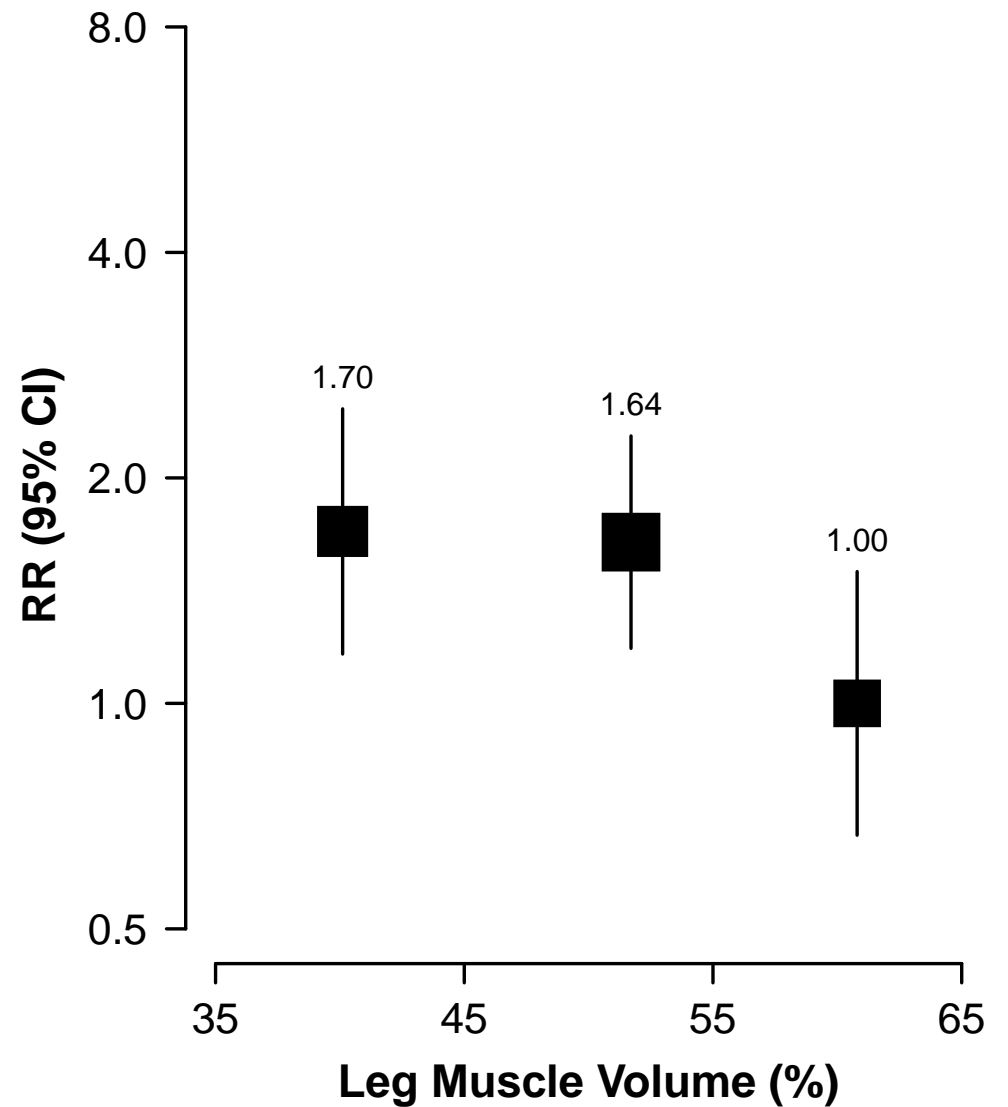
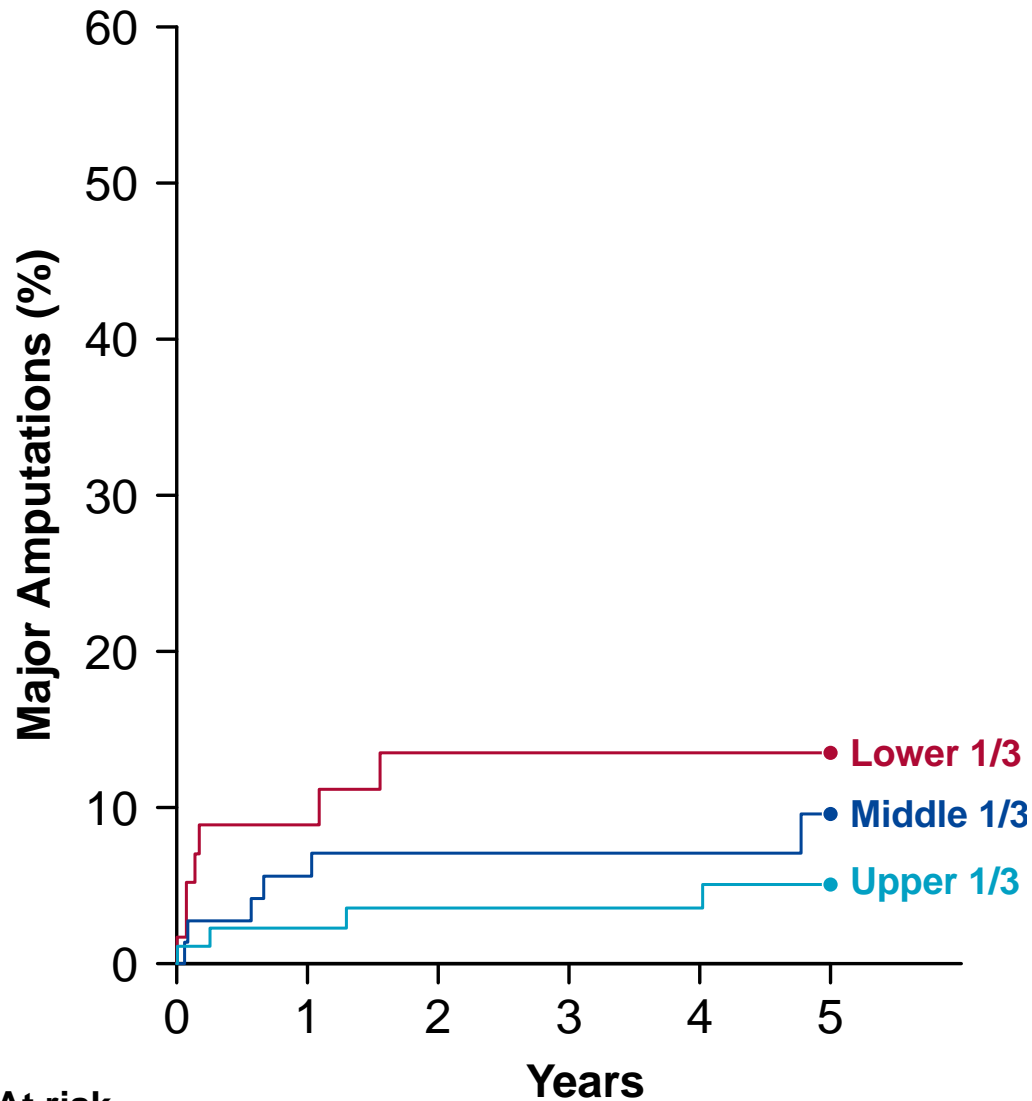


Figure S5: Association between leg muscle volume and major cardiovascular events among people with PAD.

Left panel shows the risk of major cardiovascular events over time according to leg muscle volume tertile. Right panel shows the relative risk of major cardiovascular events by leg muscle volume tertile, adjusted for age and sex. The size of the box and vertical lines represent the 95% confidence intervals calculated from the variance of the log risk. RR, rate ratio; CI, confidence interval.



At risk

Lower 1/3	59	40	36	33	27	18
Middle 1/3	74	65	57	51	42	35
Upper 1/3	90	78	72	68	64	53

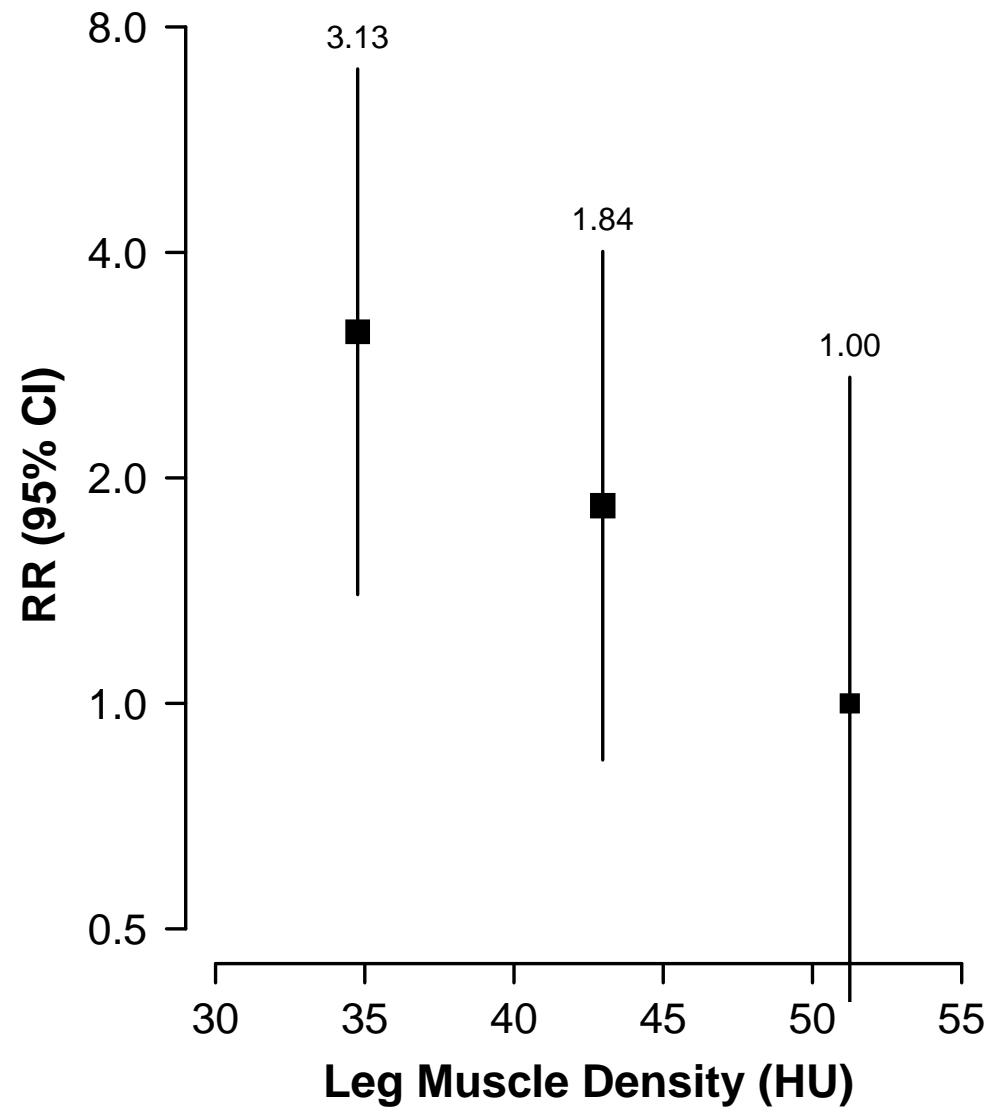
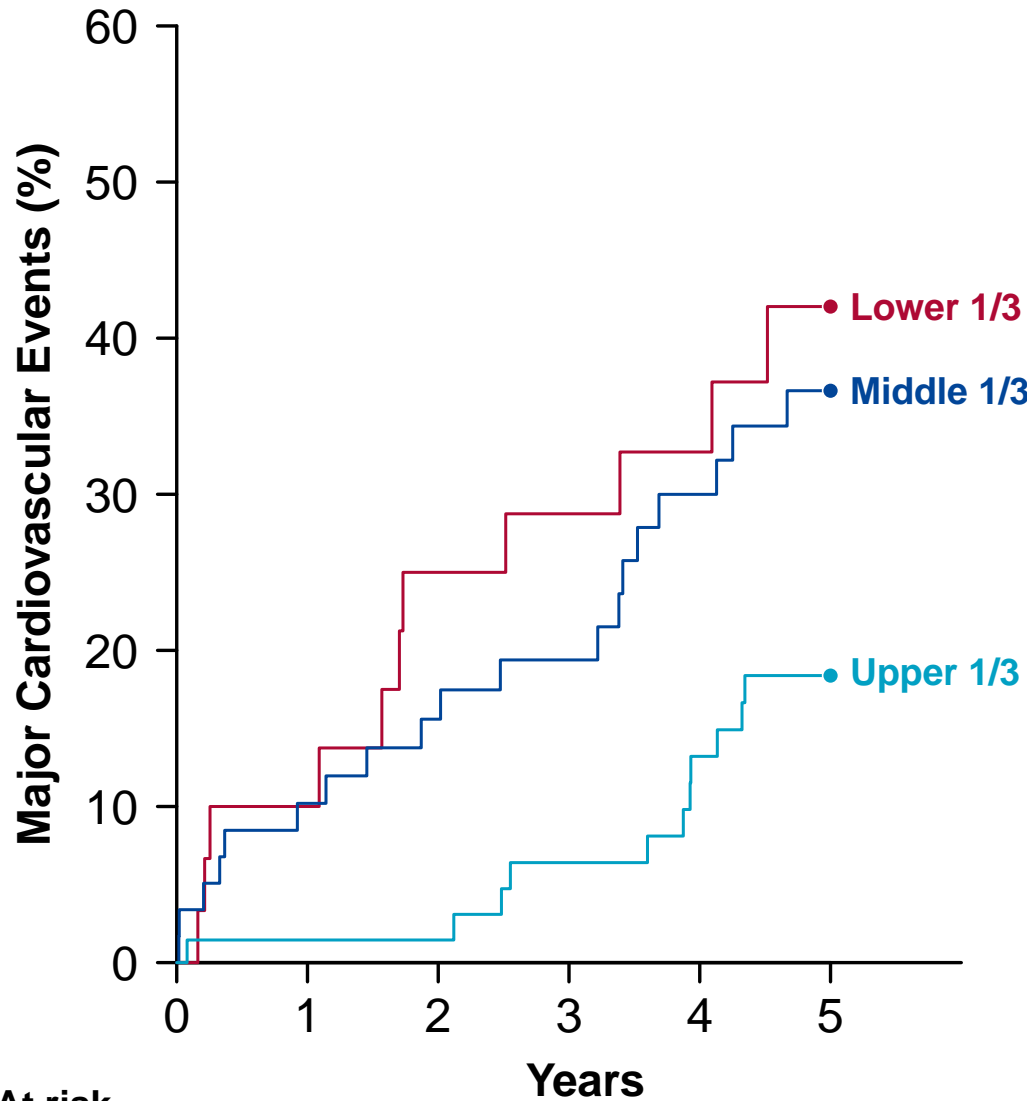


Figure S6: Association between leg muscle density and major lower limb amputations among people with PAD.

Left panel shows the risk of major amputations over time according to leg muscle density tertile. Right panel shows the relative risk of major amputations by leg muscle density tertile, adjusted for age and sex. The size of the box and vertical lines represent the 95% confidence intervals calculated from the variance of the log risk. RR, rate ratio; CI, confidence interval; HU, Hounsfield units.



At risk	0	1	2	3	4	5
Lower 1/3	30	24	20	18	16	11
Middle 1/3	59	52	46	40	32	26
Upper 1/3	70	64	60	55	51	43

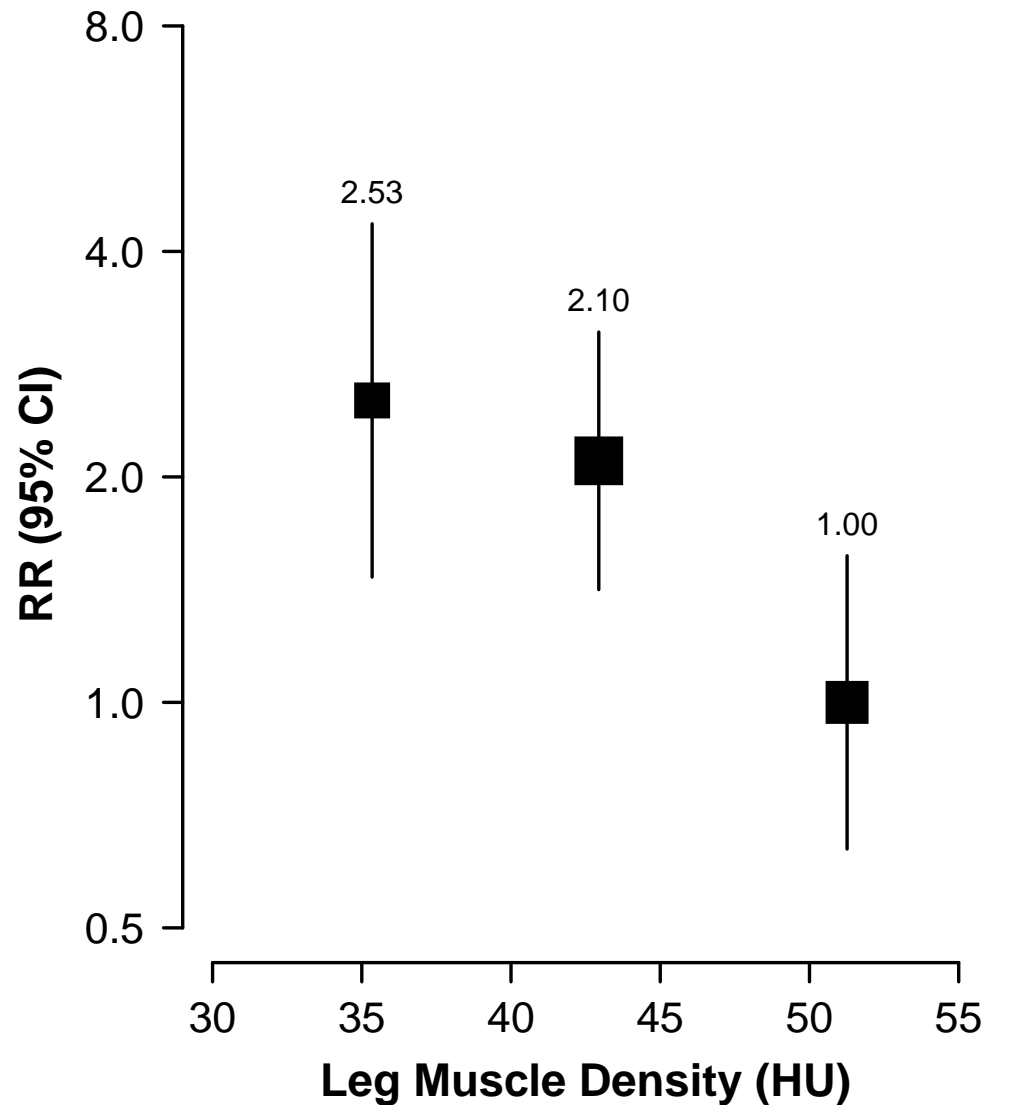


Figure S7: Association between leg muscle density and cardiovascular events among people with IC or atypical symptoms. Left panel shows the risk of major cardiovascular events over time according to leg muscle density tertile. Right panel shows the relative risk of major cardiovascular events by leg muscle density tertile, adjusted for age and sex. The size of the box and vertical lines represent the 95% confidence intervals calculated from the variance of the log risk. RR, rate ratio; CI, confidence interval; HU, Hounsfield units; IC, intermittent claudication.

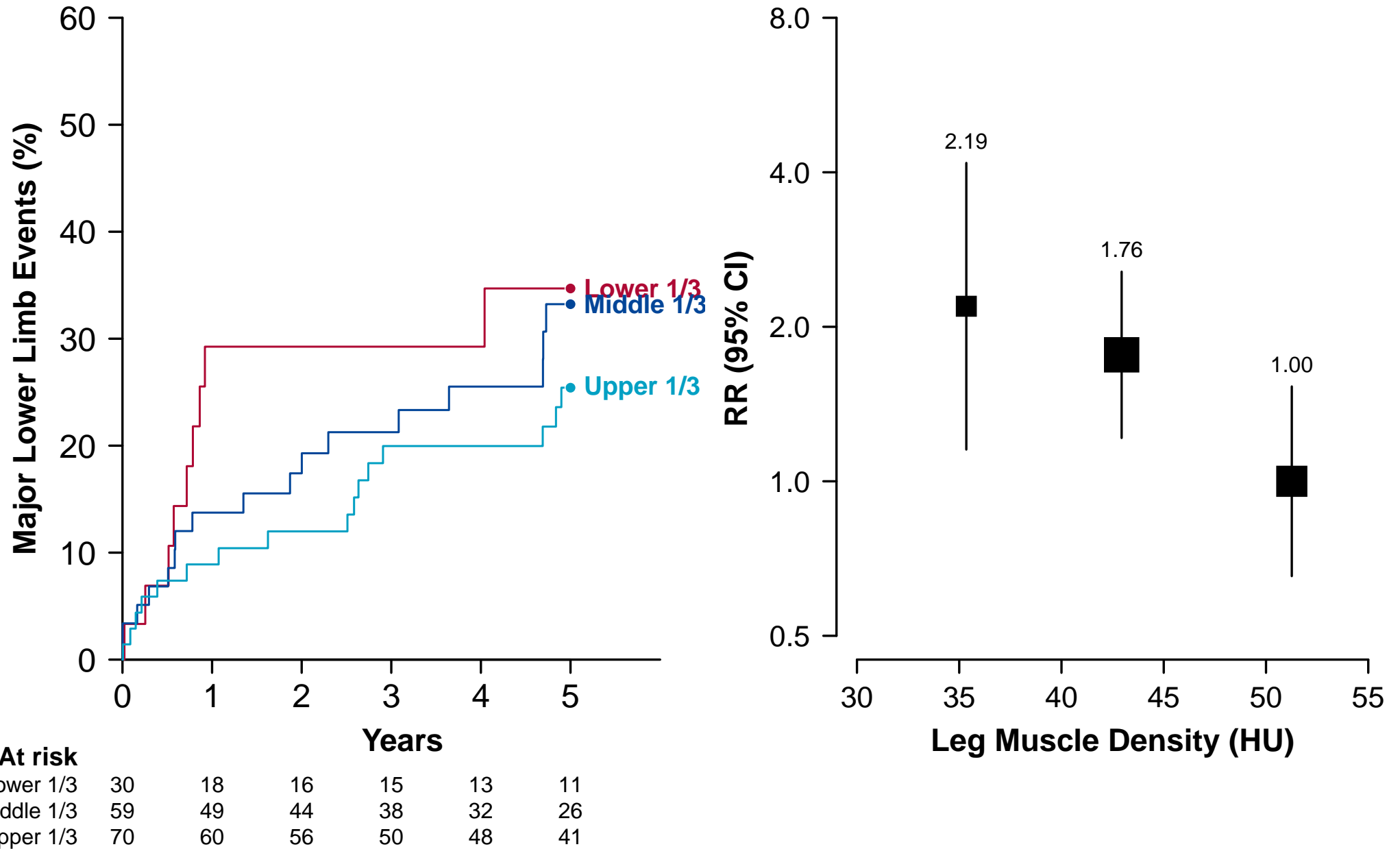


Figure S8: Association between leg muscle density and lower limb events among people with IC or atypical symptoms.

Left panel shows the risk of major lower limb events over time according to leg muscle density tertile. Right panel shows the relative risk of major lower limb events by leg muscle density tertile, adjusted for age and sex. The size of the box and vertical lines represent the 95% confidence intervals calculated from the variance of the log risk. RR, rate ratio; CI, confidence interval; HU, Hounsfield units; IC, intermittent claudication.

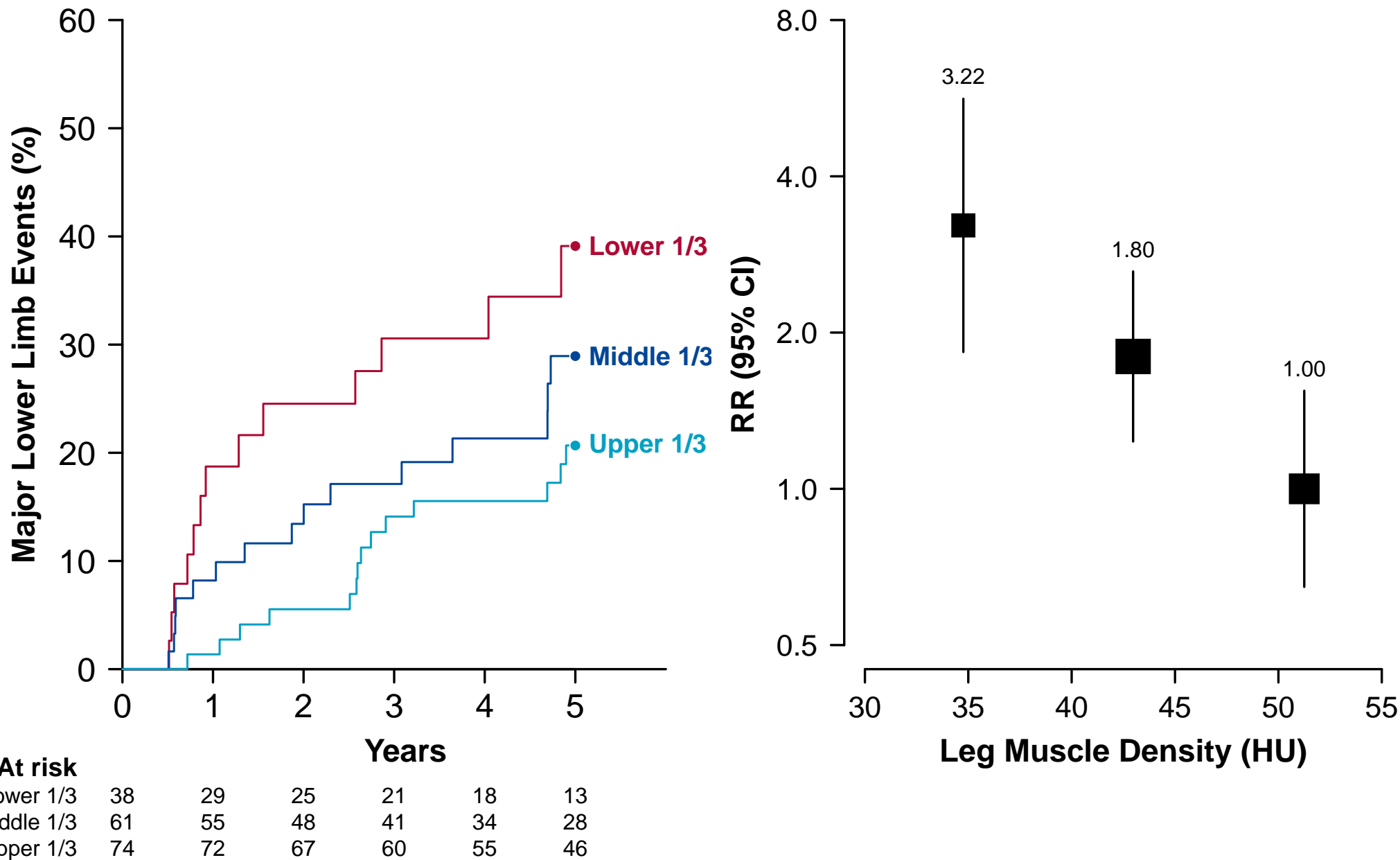


Figure S9: Association between leg muscle density and lower limb events after 6 months.

Left panel shows the risk of major lower limb events over time according to leg muscle density tertile. Right panel shows the relative risk of major lower limb events by leg muscle density tertile, adjusted for age and sex. The size of the box and vertical lines represent the 95% confidence intervals calculated from the variance of the log risk. RR, rate ratio; CI, confidence interval; HU, Hounsfield units.

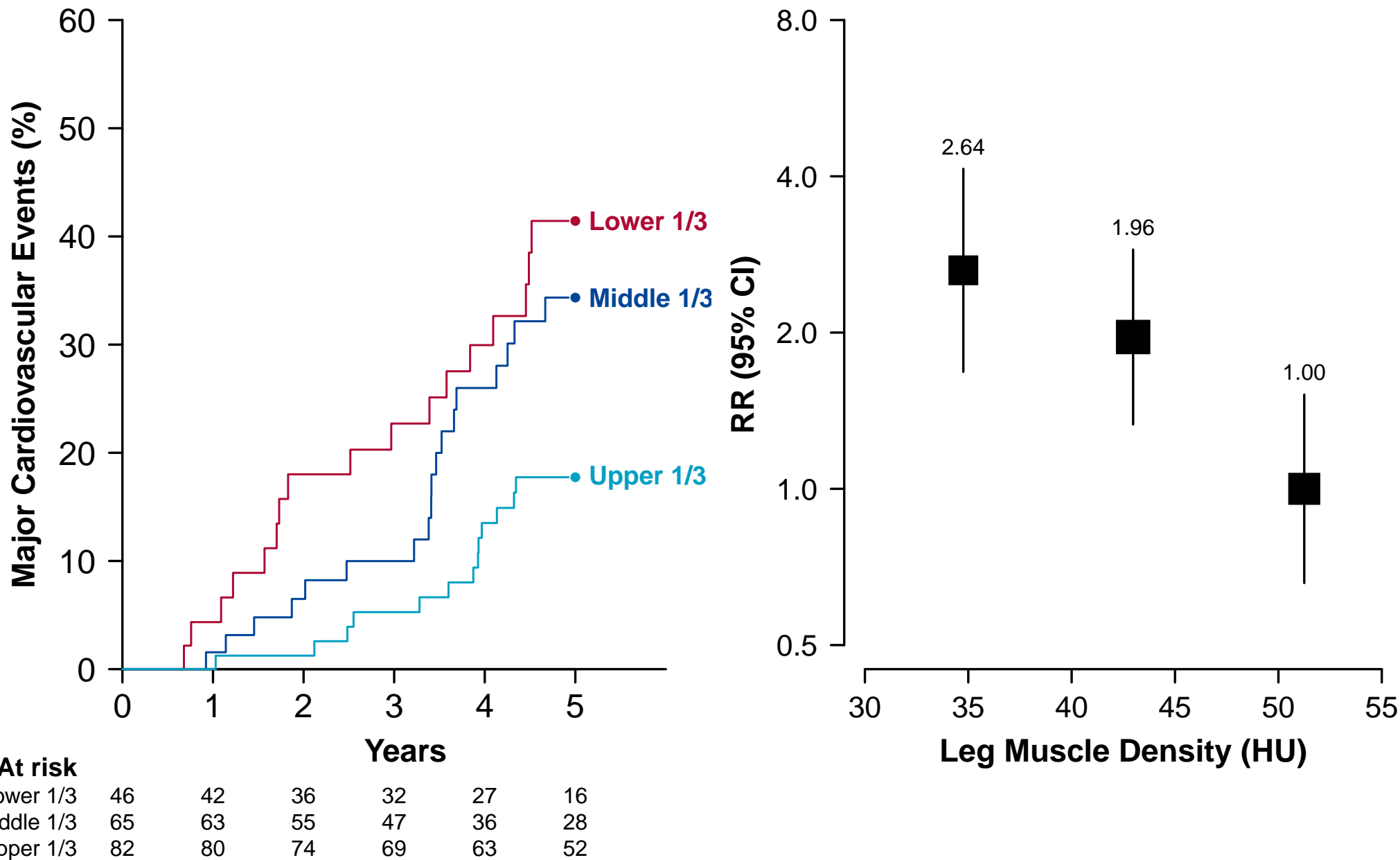
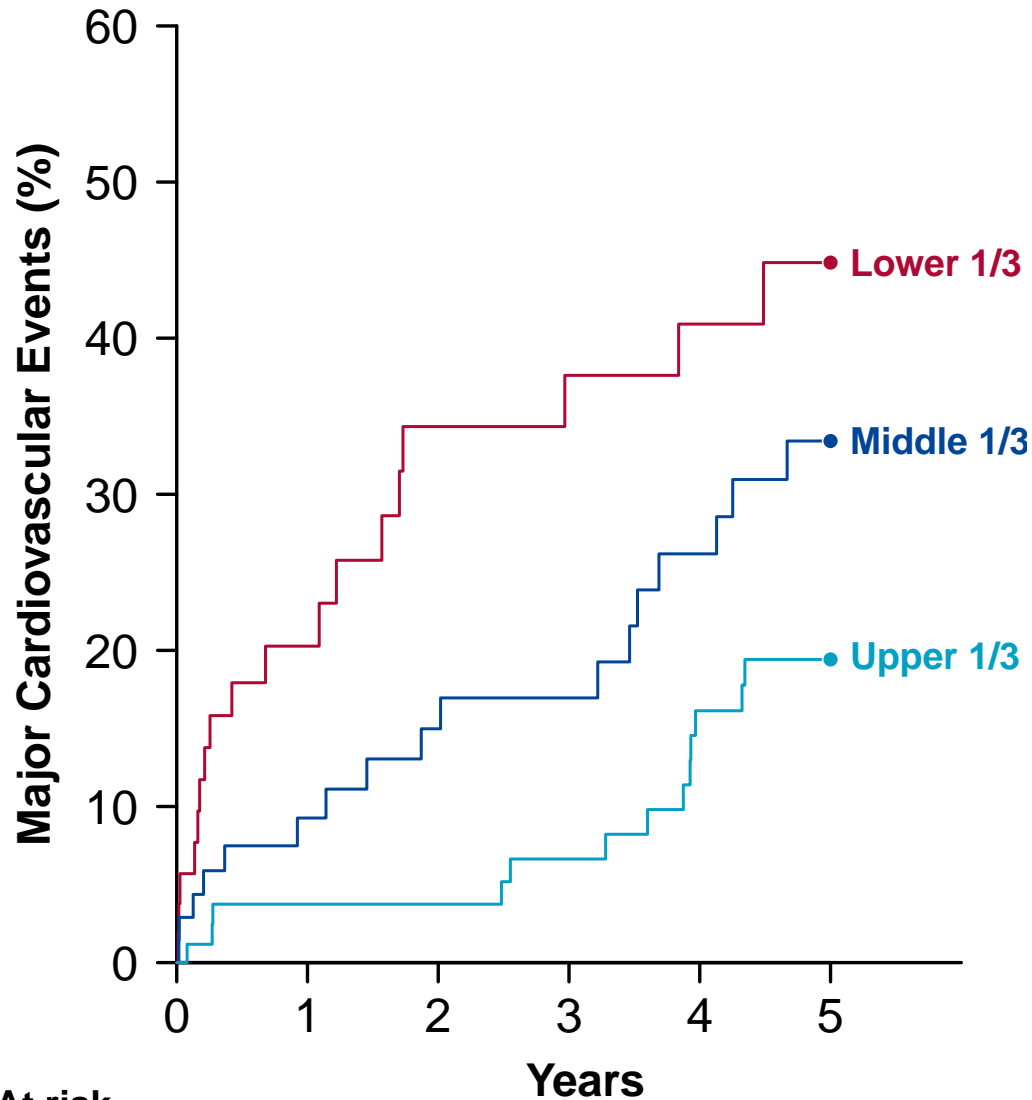


Figure S10: Association between leg muscle density and cardiovascular events after 6 months.

Left panel shows the risk of major cardiovascular events over time according to leg muscle density tertile. Right panel shows the relative risk of major cardiovascular events by leg muscle density tertile, adjusted for age and sex. The size of the box and vertical lines represent the 95% confidence intervals calculated from the variance of the log risk. RR, rate ratio; CI, confidence interval; HU, Hounsfield units.

**At risk**

Lower 1/3	59	29	23	19	17	12
Middle 1/3	73	51	44	38	31	23
Upper 1/3	89	72	67	60	53	43

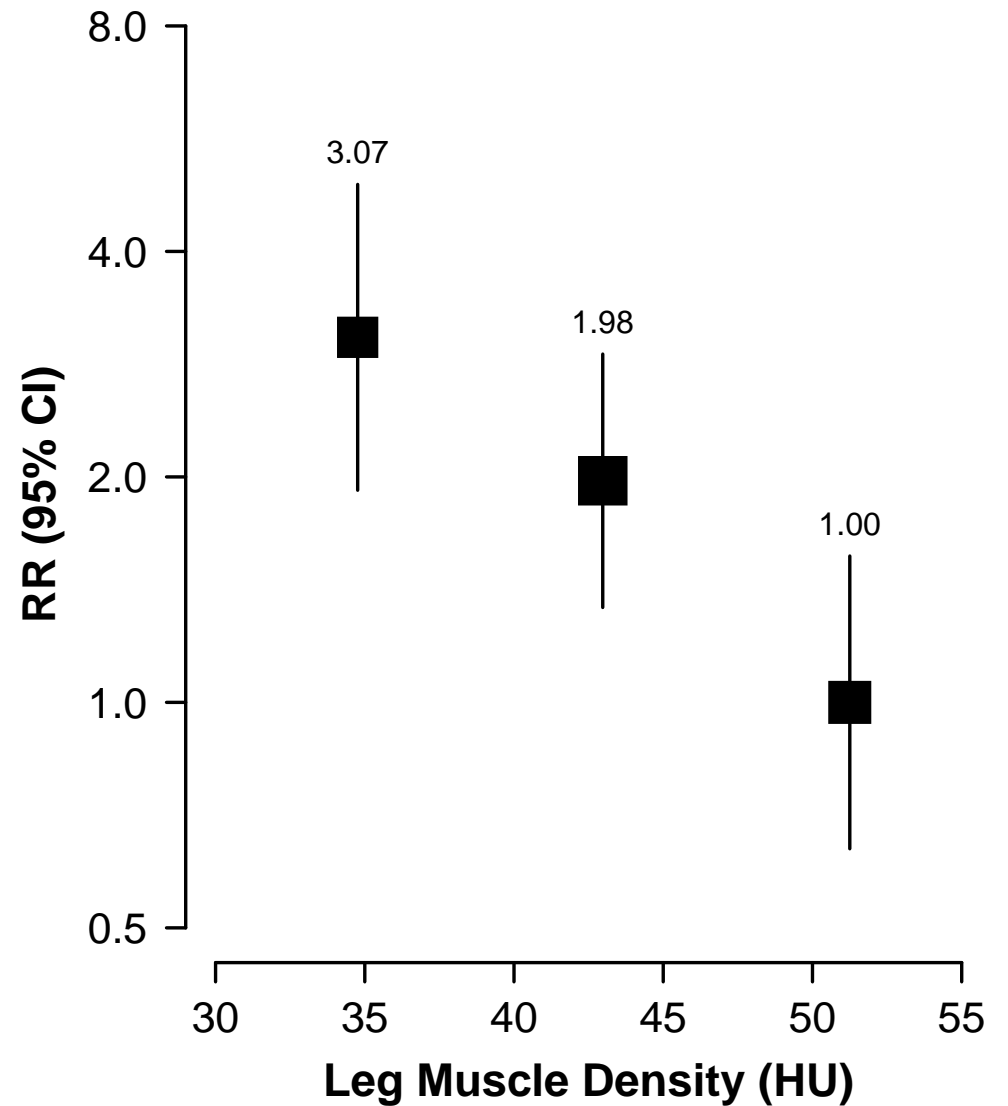


Figure S11: Association between leg muscle density and non-perioperative cardiovascular events.

Left panel shows the risk of major cardiovascular events over time according to leg muscle density tertile. Right panel shows the relative risk of major cardiovascular events by leg muscle density tertile, adjusted for age and sex. Follow-up excludes time after any lower limb revascularisation or major amputation. The size of the box and vertical lines represent the 95% confidence intervals calculated from the variance of the log risk. RR, rate ratio; CI, confidence interval; HU, Hounsfield units.

Supplemental References

1. Vittinghoff E and McCulloch CE. Relaxing the rule of ten events per variable in logistic and Cox regression. *Am J Epidemiol.* 2007;165:710-718.
2. Morris DR, Singh TP, Moxon JV, Smith A, Stewart F, Jones RE and Golledge J. Assessment and validation of a novel angiographic scoring system for peripheral artery disease. *Br J Surg.* 2017;104:544-554.