

1 **Serum Transthyretin and Aminotransferases are associated with**
2 **Lean Mass in People with Coronary Heart Disease. Further**
3 **Insights from the CARE-CR study**

4
5 **Running title:** Biomarkers of Sarcopenia in CHD

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24 **Word count:** 3,909

25 **Tables:** Three

26 **Figures:** One

27 **Language style:** British English
28

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Abstract

Background

Low muscle mass disproportionately affects people with coronary heart disease compared to healthy controls but is under-researched and insufficiently treated. Inflammation, poor nutrition, and neural decline might contribute to low muscle mass. This study aimed to assess circulatory biomarkers related to these mechanisms (albumin, transthyretin, alanine aminotransferase [ALT], aspartate aminotransferase [AST]) and C-terminal agrin fragment) and their relationship with muscle mass in people with coronary heart disease. Our findings could be beneficial to indicate mechanisms of sarcopenia, detect sarcopenia, and evaluate treatment.

Methods

Serum blood samples from people with coronary heart disease were analysed for biomarker concentrations using enzyme-linked immunosorbent assays. Skeletal muscle mass was estimated using dual X-ray absorptiometry derived appendicular lean mass and reported as skeletal muscle index (SMI; $\text{kg}\cdot\text{m}^{-2}$), and as a proportion of total body mass (appendicular skeletal mass [ASM%]). Low muscle mass was defined as a SMI <7.0 and $<6.0 \text{ kg}\cdot\text{m}^{-2}$, or ASM% $<25.72\%$ and $<19.43\%$ for men and women, respectively. Associations between biomarkers and lean mass were adjusted for age and inflammation.

Results

Sixty-four people were assessed; fourteen (21.9%) had low muscle mass. People with low muscle mass had lower transthyretin (effect size 0.34, $P = 0.007$), ALT (effect size 0.34, $P = 0.008$) and AST (effect size 0.26, $P = 0.037$) concentrations, compared to those with normal muscle mass. SMI was associated with inflammation-corrected ALT ($r = 0.261$, $P = 0.039$) and with inflammation- and age-adjusted AST/ALT ratio ($r = -0.257$, $P = 0.044$). Albumin and C-terminal agrin fragment were not associated with muscle mass indices.

Conclusion

Circulatory transthyretin, ALT and AST were associated with low muscle mass in people with coronary heart disease. Low concentrations of these biomarkers might indicate that low muscle mass is partially explained by poor nutrition and high inflammation in this cohort. Targeted treatments to address these factors could be considered for people with coronary heart disease.

Key words

Agrin, albumin, aminotransferases, biomarkers, coronary heart disease, muscle, sarcopenia, transthyretin

66 1. Introduction

67 Between 1990 and 2019, coronary heart disease (CHD)-related mortality declined at a greater
68 rate (61%) than CHD incidence (37%) (1). In the era of modern medical management, people
69 with a CHD diagnosis live for longer and many will require increased support to manage their
70 long-term health. An important component of healthy ageing is maintaining skeletal muscle
71 mass (SMM) (2, 3). This is particularly relevant in people with CHD where there is a higher
72 incidence of low SMM in people with CHD compared to age- and sex -matched adults (Nichols
73 et al., 2019). Emerging research in people with CHD, shows that low SMM increases the risk
74 of all-cause mortality, fatal or non-fatal major adverse cardiovascular events, lower fitness
75 (peak oxygen uptake; $\dot{V}O_{2peak}$) and poorer quality of life (4-8). However, factors that influence
76 loss of SMM in CHD are poorly defined. The delivery of successful interventions to improve
77 SMM, and subsequently long-term health, in these people requires that we have: (1) the ability
78 to identify those at risk of low SMM early, and (2) a thorough understanding of the factors
79 influencing low SMM. For this purpose, circulatory biomarkers might be useful to complement
80 traditional measures of SMM and strength.

81 Maladaptive processes and behaviours that contribute to loss of SMM and/ or function are
82 complex. There is compelling evidence that these include neural maladaptation (9, 10),
83 inflammation (11, 12), and sub-optimal nutrition (13, 14). Biomarkers which appear to have a
84 central role in these systems need investigating. C-terminal agrin fragment (CAF) is a
85 circulatory by-product of agrin cleavage by synaptic protease neurotrypsin (15), a process
86 which can lead to neuromuscular junction breakdown (16). In healthy older adults (17, 18) and
87 people with heart failure (19), CAF levels are elevated in those with low, compared to with
88 normal, SMM. Thus, declining neural function might contribute to low SMM. However, it is
89 unclear whether these findings exist in older people with CHD. Albumin and transthyretin are
90 acute-phase response proteins which might indicate inflammation-related nutrition risk (20).
91 In hospitalised people with CHD, albumin and transthyretin levels are lower in the presence of
92 sarcopenia (as defined by the Asian Working Group for Sarcopenia) compared to those defined
93 as non-sarcopenic (21). Whether albumin and transthyretin are associated with low SMM using
94 European cut-off points (22), in people with CHD, requires clarification. Finally, alanine
95 (ALT) and aspartate (AST) aminotransferases are liver/skeletal muscle enzymes (23).
96 Circulatory levels of ALT are elevated in people with type 2 diabetes (24) and metabolic
97 syndrome (25), but lower in the presence of age-related syndromes often characterised by
98 under-nutrition, including sarcopenia (26). The AST/ALT ratio is proposed to be higher in
99 those with sarcopenia compared to those without, although few studies have investigated this
100 to date (27, 28).

101 Associations between SMM and serum CAF (17-19), albumin, transthyretin (21), ALT and
102 AST (26-28) were reported in healthy older adults and people with chronic health conditions.
103 The present study aimed to investigate the association between DXA-estimated skeletal SMM,
104 and serum CAF, albumin, transthyretin, ALT and AST, in people with recently diagnosed
105 stable CHD. We hypothesised that people with CHD and low SMM will have higher CAF
106 levels and AST/ALT ratio and lower albumin and transthyretin levels, compared to people with
107 CHD and preserved SMM.

108

109 2. Materials and methods

110 2.1 Study design and participants

111 Baseline serum blood samples and demographic characteristics used in this cross-sectional
112 study were collected as part of the Cardiovascular and cardiorespiratory Adaptations to Routine
113 Exercise-based Cardiac Rehabilitation (CARE CR) study (29). The CARE CR study protocol
114 was published in detail elsewhere (29). Briefly, clinically stable people with a primary
115 diagnosis of CHD (aged 30-85 years) were referred to the research team by nursing staff, within
116 two weeks of a cardiac event or procedure. Participants provided their written informed consent
117 to participate in the study. The CARE CR study was granted ethical approval by the Humber
118 Bridge NHS Research Ethics Committee- Yorkshire and the Humber (12/YH/0278). Ethical
119 approval for assay analysis of serum samples for biomarkers related to sarcopenia was provided
120 by the Northumbria University Health and Life Sciences Ethics Committee (20933). The main
121 findings from the CARE CR study on patient rehabilitation and cardiorespiratory fitness are
122 published elsewhere (4, 30).

123 **2.2 Body composition**

124 Body mass index (BMI; $\text{kg}\cdot\text{m}^{-2}$) was calculated using mass (kg) and stature (m). Waist and hip
125 circumferences (cm) were measured at one centimetre above the iliac crest and at the widest
126 aspect of the hips, respectively. Appendicular lean mass (ALM), defined as total lean mass in
127 both arms and legs (kg), was measured using dual X-ray absorptiometry (DXA; Lunar iDXA
128 GE Healthcare Buckinghamshire, UK), as a proxy for SMM assessment. ALM is expressed as
129 skeletal muscle index (SMI; $\text{kg}\cdot\text{m}^{-2}$) and as a percentage of total body mass (appendicular
130 skeletal mass; ASM%). Age-adjusted SMI and ASM% were moderately correlated ($r = 0.507$,
131 $P < 0.001$). We defined low SMI as <7.0 and $<6.0 \text{ kg}\cdot\text{m}^{-2}$ (22) and low ASM% as <25.72 and
132 $<19.43\%$ (31) for men and women, respectively.

133 **2.3 Maximal cardiopulmonary exercise test**

134 Cardiopulmonary exercise testing was performed using the modified Bruce treadmill protocol
135 (32), as previously described (4, 29). A 12-lead ECG, ECG-gated automated blood pressure,
136 heart rate, and rate of perceived exertion were monitored throughout. Breath-by-breath
137 metabolic gas exchange data were collected using an Oxycon Pro metabolic cart (Jaeger,
138 Hoechburg, Germany). We report $\dot{V}\text{O}_{2\text{peak}}$ (ml), defined as the mean $\dot{V}\text{O}_2$ over the last 30 s of
139 the test; $\dot{V}\text{O}_{2\text{peak}}$ was adjusted for body mass ($\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) (4).

141 **2.4 Blood sampling and analysis**

142 Participants abstained from strenuous exercise 24-hours prior to attending their baseline study
143 visit. Resting blood samples were drawn by venepuncture and placed in a refrigerated (4°C)
144 centrifuge at 3000 revolutions per minute, for 15 minutes. Albumin, aminotransferases and N-
145 terminal pro-brain natriuretic peptide (NT-proBNP) were analysed at the Hull Royal Infirmary
146 in an accredited biochemistry laboratory, as a single measurement on the day of each blood
147 draw. Calibration and quality controls were conducted in accordance with manufacturer's
148 guidelines. The ABX Pentra 400 biochemistry auto analyser (Horiba, Montpellier, France) was
149 used to analyse high sensitivity C-reactive protein (hs-CRP) in duplicate, in accordance with
150 the manufacturer's quality control guidance (4). Remaining plasma and serum samples were
151 stored at -80°C until analysis.

152 We analysed serum samples in duplicate using commercial enzyme-linked immunosorbent
153 assay (ELISA) for CAF (Abcam #ab216945) and transthyretin (Abcam #ab108895) and
154 followed their standard instructions for serum analysis. Concentrations of transthyretin and
155 CAF were assessed in duplicate and the average of the two measures reported. We re-analysed
156 samples with a coefficient of variation (CV) $>40\%$ and when biomarker concentrations were
157 not within the limits of the standard curve. The CV for the assay analyses of transthyretin and

158 CAF were 7.9 and 5.1%, respectively. Routine health-related serum biomarkers evaluated as
 159 part of the CARE CR study are reported elsewhere, including NT-proBNP, hs-CRP, glucose,
 160 white cell count, total cholesterol, low-density and high-density lipoprotein cholesterol,
 161 estimated glomerular filtration rate and triglycerides (4, 30).

162 Normal adult reference values for circulatory markers of interest are:

- 163 • Albumin: 35 to 50 g/L (33).
- 164 • Transthyretin: 30 to 33 mg/dL and 25 to 27 mg/dL in males and females, respectively
 165 (34).
- 166 • ALT: 9.0 to 59.0 U/L and 7.8 to 41.0 U/L in males and females, respectively (35).
- 167 • AST: 11.0 to 34.0 U/L (35)
- 168 • CAF: 0.86 to 4.66 ng/ml (17).

169

170 2.5 Statistical Analysis

171 Statistical analyses were performed by a single researcher using commercially available
 172 software (SPSS version 28, IBM, New York, NY, USA). Distribution of the data was assessed
 173 using visual inspection of histograms, QQ-plots and using the Kolmogorov Smirnov test.
 174 Categorical variables are reported as frequency with percentage. Continuous normally
 175 distributed variables are reported as mean \pm standard deviation. Continuous non-normally
 176 distributed variables are reported as median with interquartile range, or median with range
 177 where the sample size is ≤ 3 people. Demographic characteristics are reported for the whole
 178 cohort and separately for people with normal or low MM (defined as low SMI or low ASM%).
 179 Differences in demographic characteristics between the two groups were assessed using the
 180 Fisher's exact test (categorical variables), a Student's t-test (continuous normally distributed),
 181 or Mann-Whitney U test (continuous non-normally distributed). Two-group comparison of
 182 blood biomarkers between people with normal or low SMM were evaluated using Mann-
 183 Whitney U tests and reported as U statistics, P-values, and effect sizes, calculated using the
 184 following equation (36):

$$185 \quad r = \frac{Z}{\sqrt{n}}$$

186 Absolute r values of 0.2, 0.5 and 0.8 are considered small, moderate and large effect sizes,
 187 respectively (37). The relationship between serum biomarker concentrations, SMI and ASM%
 188 were calculated using Spearman's rank correlations. It is well-established that age and
 189 inflammation influence SMM and some serum biomarkers; people with CHD and low SMM
 190 are significantly older than those with normal SMM (38), whilst albumin and transthyretin
 191 concentrations decrease in the presence of inflammation (39). Accordingly, we also report non-
 192 parametric partial correlations adjusted for age and circulatory hs-CRP concentrations, both
 193 separately and together. An r value of <0.3 , $0.3 - 0.5$, $0.6 - 0.8$, and >0.8 indicated a poor, fair,
 194 moderately strong and very strong associations, respectively (40). Scatterplots of associations
 195 between SMI and circulatory markers were plotted with linear regression lines. Where a marker
 196 was associated with SMI or ASM% or had a significant effect size for low and normal SMM
 197 groups, receiver operating characteristic (ROC) curves were used to investigate the sensitivity
 198 and specificity of predicting low SMM as the dichotomous 'state variable'. We report the area
 199 under the curve (AUC) with 95% confidence interval (CI) and P-values. The AUC value was
 200 interpreted as follows: perfect (1.0), excellent (0.9-0.99), good (0.8-0.89), fair (0.7-0.79), poor
 201 (0.51-0.69), and no value (0.5) (41). The biomarker concentration cut-off points for prediction
 202 of low SMM were selected based on the highest combination of sensitivity and specificity

203 values. We plotted ROC curves and determined biomarker cut-off points for the whole cohort
 204 and then separately for men only. Due to a small sample, ROC curves could not be plotted for
 205 women only.

206 Statistical significance was set at $P < 0.05$.

207 3. Results

208 Sixty-four people were included (63.4 ± 9.8 years; 12.5% female). Participant characteristics,
 209 presenting diagnosis, comorbidities, and medications, are reported in Table 1. Low ASM% and
 210 low SMI were identified in 14.1% ($n = 9$) and 12.5% ($n = 8$) of people, respectively. Three
 211 people had both low ASM% and low SMI (4.7%) and 14 had either low ASM% or low SMI
 212 (21.9%).

213 *[Insert Table 1]*

214 Circulatory biomarker concentrations are reported in Table 2. The distribution of biomarker
 215 concentrations compared to normal reference values (section 2.4) were as follows: albumin,
 216 92.2% ($n = 59$) within, 6.3% ($n = 4$) lower than and 1.6% ($n = 1$) higher than the normal range;
 217 transthyretin, 6.3% ($n = 4$) within, 34.4% ($n = 22$) lower than and 59.4% ($n = 38$) higher than
 218 the normal range; ALT, 90.6% ($n = 58$) within and 9.4% ($n = 6$) higher than the normal range;
 219 AST, 78.1% ($n = 50$) within and 21.9% ($n = 14$) higher than the normal range; and CAF, 78.1%
 220 ($n = 50$) within and 21.9% ($n = 14$) higher than the normal range. There were small to moderate
 221 effect sizes for lower serum transthyretin (effect size 0.34; 29.66 mg/dL versus 37.87 mg/dL,
 222 $P = 0.007$), ALT (effect size 0.34; 20.00 U/L versus 31.00 U/L, $P = 0.008$) and AST (effect
 223 size 0.26; 22.25 U/L versus 27.00 U/L, $P = 0.037$) levels in people with low SMM compared
 224 to those with normal SMM.

225 Correlations between circulatory biomarkers, SMI and ASM% are reported in Table 3. Figure
 226 1 shows correlations between SMI and circulatory biomarkers. SMI was associated with hs-
 227 CRP -corrected serum ALT levels ($r = 0.261$, $P = 0.039$) and with hs-CRP and age -corrected
 228 AST/ALT ratio ($r = -0.257$, $P = 0.044$). In men, after correction for hs-CRP levels and age,
 229 SMI was associated with AST ($r = -0.279$, $P = 0.041$) and the AST/ALT ratio ($r = -0.281$, $P =$
 230 0.040). In women, after correction for hs-CRP levels and age, transthyretin was negatively
 231 associated with ASM% ($r = -0.889$, $P = 0.018$).

232 *[Insert Table 2]*

233 *[Insert Table 3]*

234 *[Insert Figure 1]*

235 3.1 ROC curve analysis

236 The prognostic value of transthyretin, ALT, AST, and the AST/ALT ratio for identification of
 237 low SMM was assessed using ROC curve analysis. Including all participants, transthyretin
 238 (AUC 0.739, 95% CI 0.601, 0.876, $P = 0.007$) and ALT (AUC 0.731, 95% CI 0.576, 0.887, P
 239 $= 0.009$) had the greatest predictive capacity to identify low SMM. The AUC for AST level
 240 was 0.684 (95% CI 0.516, 0.851, $P = 0.037$) and non-significant for the AST/ALT ratio (AUC
 241 0.636, 95% CI 0.482, 0.790, $P = 0.123$). The optimal cut-off points to identify low SMM were:
 242 a transthyretin value of ≤ 37.7654 mg/dl (sensitivity 0.857, specificity 0.520), an ALT value of
 243 ≤ 25.00 U/L (sensitivity 0.857, specificity 0.620), and an AST value of ≤ 24.50 U/L (sensitivity
 244 0.714, specificity 0.620).

245 Including men only, ROC curve analyses showed the predictive capacity of transthyretin (AUC
246 0.773, 95% CI 0.603, 0.943, $P = 0.002$), ALT (AUC 0.699, 95% CI 0.509, 0.888, $P = 0.040$)
247 and the AST/ALT ratio (AUC 0.693, 95% CI 0.538, 0.847, $P = 0.014$). The AUC for AST level
248 was non-significant (AUC 0.560, 95% CI 0.357, 0.764, $P = 0.562$). In men, the optimal cut-off
249 points to identify low SMM where: a transthyretin value of ≤ 30.3284 mg/dl (sensitivity 0.778,
250 specificity 0.723), an ALT value of ≤ 25.00 U/L (sensitivity 0.889, specificity 0.660), and an
251 AST/ALT ratio of ≥ 0.9347 (sensitivity 0.778, specificity 0.553).

252

253 **4.0 Discussion**

254 This study aimed to report the association between DXA-estimated SMM and serum albumin,
255 transthyretin, ALT, AST and CAF in people with CHD. People with low SMM had lower
256 serum transthyretin, AST and ALT levels compared to those with normal SMM, with small to
257 moderate effect sizes. SMI was positively associated with ALT level and negatively associated
258 with the AST/ALT ratio. We found no associations between albumin or CAF levels with any
259 SMM index.

260 More than one-fifth of people had low SMM. Similarly, others report a prevalence of 25-30%
261 for low SMM in people with CHD (5, 7, 42). Comparatively fewer (12%) apparently healthy,
262 community-dwelling, older adults have low SMM (43). In the current study, presence of
263 comorbidities associated with SMM loss, such as cancer (44) and COPD (45), likely
264 contributed to the higher prevalence of low SMM. Importantly, in a previous CARE CR
265 publication, ASM% was inversely associated with estimated all-cause mortality risk ($r =$
266 -0.365 , $P = 0.006$) in people with CHD (4). Thus, interventions to prevent or reverse low SMM
267 should be offered to these people. To support the design and implementation of successful
268 interventions, accurate and readily available methods to assess or monitor changes in SMM are
269 needed.

270 **4.1 Albumin**

271 Albumin is a marker of inflammation-related nutritional risk (20). In agreement with previous
272 studies involving people with liver cirrhosis (46), end-stage renal disease (47) and heart failure
273 (48), we found no association between albumin levels and SMM indices in people with CHD.
274 Interestingly, others report both lower (49-51) and or higher (52) albumin concentrations in
275 older adults with low SMM, compared to those with preserved SMM. The use of albumin levels
276 to infer protein energy malnutrition was previously commonplace in clinical practice (53).
277 Given that lean mass reflects the somatic protein store, the assumption followed that albumin
278 might be useful as a marker of lean mass. However, the use of albumin as a biomarker of
279 malnutrition or body composition has not been without criticism (20, 54). The literature lacks
280 consensus on the existence and/ or direction of the association between albumin and SMM-
281 related variables (46-49, 51, 52), likely due to the role of albumin as an acute-phase response
282 protein.

283 The inflammation-induced reduction in albumin concentration is underpinned by: decreased
284 albumin synthesis during stress response to prioritise synthesis of essential proteins, increased
285 capillary permeability prompting a shift of albumin from the intravascular to the interstitial
286 space, and a shortened albumin half-life resulting from tissue catabolism (20). In older adults,
287 serum albumin is inversely associated with common inflammatory cytokine, CRP (55). We
288 found no difference in hs-CRP between people with normal or low SMM (table 2). This could
289 explain the similar albumin levels between groups. Additionally, Chen et al. (2022) speculated
290 that sex-specific hormones levels might also impact the association between SMM and albumin

291 levels, after finding these variables to be positively associated in men and negatively associated
292 in women. However, our study included a small sample of women, and we were unable to
293 investigate this hypothesis.

294 **4.2 Transthyretin**

295 Transthyretin levels were significantly lower in people with low versus normal SMM. Similar
296 to albumin, transthyretin is a marker of inflammation-related nutritional risk (20), a key
297 component of malnutrition related to acute or chronic disease (56). Amino acid availability,
298 from dietary protein intake, was proposed to mediate the relationship between transthyretin and
299 lean mass (34). This is because amino acid ingestion promotes lean tissue accretion (57) and
300 also modulates transthyretin synthesis in the liver (58). A strong, positive association ($r = 0.58$)
301 between transthyretin levels and SMI was previously reported in people at a geriatric outpatient
302 hospital (51). Around 40% of people in the study by Sergi and colleagues (51) were
303 underweight ($BMI < 20 \text{ kg.m}^{-2}$). Poorer nutritional status likely contributed to a more
304 pronounced inflammatory environment and lean mass loss in this study (51), potentially
305 explaining the strong association between transthyretin and SMI, compared to a non-significant
306 association in the present study ($r = 0.246$, $P = 0.05$). Nevertheless, our detection of
307 significantly lower transthyretin levels in people with low compared to normal SMM is a
308 promising finding, as it become increasingly apparent that transthyretin assessment might have
309 clinical utility as part of a comprehensive medical evaluation (59).

310 **4.3 Aminotransferases**

311 Assessment of liver enzymes ALT and AST is routine in clinical practice (60). As a catalyst in
312 the alanine -glucose cycle, ALT converts pyruvate to amino acid alanine in skeletal muscle and
313 converts alanine back to pyruvate (for glucose production) in the liver (61, 62). A similar cycle
314 is catalysed by AST, where the amino acid and product are aspartate and oxaloacetate,
315 respectively (62). Circulatory levels of ALT and AST are elevated in Type 2 diabetes (24) and
316 metabolic syndrome (25), conditions characterised by insulin resistance and hepatic steatosis.
317 We, and others, demonstrate that ALT levels appear to be lower in the presence of low SMM
318 (26, 50). Contrastingly, in a cross-section of $>12,000$ adults without liver-related disorders,
319 ALT levels were elevated in those with low SMM compared to normal SMM (63). The
320 direction of the relationship between AST and SMM is similarly contested. We found lower
321 AST concentrations in people with low SMM compared to normal SMM. Others report that
322 low SMM coincided with higher AST concentrations in people with (64, 65) and without (63)
323 liver disease.

324 Multiple factors likely influence the inconsistency in these findings. First, damaged liver cells
325 release ALT and AST into circulation, explaining their higher serum concentrations in people
326 with liver disorders (66). Secondly, participants with low SMM in the study by Yoo and
327 colleagues (63) were more often obese with higher fasting blood glucose and insulin levels
328 compared to the normal SMM group, consistent with the theory that aminotransferase levels
329 are elevated in the presence of higher metabolic risk. In the present study, people with reduced
330 SMM had higher average body fat and comparable BMI to people with normal SMM. It could
331 be speculated that differences in intra-abdominal and intra-hepatic steatosis, together with diet
332 quality/alcohol consumption might have influenced aminotransferase concentrations.

333 Additionally, both ALT and AST require vitamin B₆ as a cofactor, meaning that vitamin B₆
334 deficiency might contribute to low circulatory ALT and AST (67). Furthermore, vitamin B₆ is
335 mostly stored in striated muscle (68); thus, where lean mass is reduced a smaller pool of vitamin
336 B₆ is available to act as a cofactor for AST and ALT. An estimated 31 and 24% of community-
337 dwelling men and women (≥ 65 years) are at risk of inadequate vitamin B₆ dietary intake (69).

338 Although not assessed in this study, addressing any dietary deficiencies in people with CHD
339 and low SMM should be prioritised.

340 **4.4 C-terminal agrin fragment**

341 Studies involving older adults (17, 70, 71), people with lung disease (71, 72) and with heart
342 failure (19, 72) have reported an association between high circulatory CAF levels and low
343 SMM. This association is proposed to originate from degeneration of the neuromuscular
344 junction with ageing. Agrin is cleaved by neurotrypsin during normal neural development (15).
345 Excessive agrin cleavage from over-expression of neurotrypsin causes agrin to become
346 deactivated and the neuromuscular junction to break down (16). The product of this breakdown,
347 CAF, is released into the circulation (73). However, the effect of degeneration and remodelling
348 of the neuromuscular junction on SMM loss is debated, with polarising studies arguing that
349 this process contributes to (74) or is protective against (75) muscle atrophy.

350 We found no association between CAF levels and SMM indices in people with CHD. Others
351 have reported similar non-significant findings when assessing possible associations between
352 CAF and presence of frailty in people with CHD, although an assessment of SMM was not
353 included in their definition of frailty (76). Sánchez-Castellano, Martín-Aragón (77) found no
354 difference in CAF levels between low and normal SMM groups with hip fracture and suggested
355 that elevated CAF levels in both groups indicated neuromuscular degeneration was present in
356 both. In contrast, median CAF values in the low and normal SMM groups were within the
357 normal limits in the present study (0.86 to 4.66 ng/ml; 17), suggesting that circulatory CAF has
358 limited utility as biomarker for low SMM in this cohort.

359 **4.5 Strengths and limitations**

360 We assessed, in a secondary analysis, multiple proposed biomarkers for low SMM in people
361 with CHD, contributing to our understanding of the factors influencing this complex and under-
362 researched pathology. We included assessment of four biomarkers which are already
363 commonly assessed in clinical practice (albumin, transthyretin, ALT and AST), aiding the
364 potential transition of our findings into practice.

365 This study is potentially limited by our use of DXA-derived lean mass to estimate SMM. DXA
366 assessment is the current reference standard, but is limited by the production of variability
367 related to different devices and software versions (78) and the absence of a universally agreed
368 cut-off point for low SMM (79). Furthermore, DXA derived lean mass can be interpreted in
369 several ways (i.e., corrected for stature, body mass or body fat percentage), which often
370 produce conflicting findings when analysed in relation to circulatory biomarkers. This might
371 limit the comparability of our findings with other, similar research. Finally, we included a small
372 sample of women and there was no assessment of muscle strength or function.

373 **4.6 Future research**

374 Future research should evaluate the association between albumin, transthyretin,
375 aminotransferases, CAF and measures of muscular strength alongside SMM. Whether these
376 markers change with targeted lifestyle interventions also requires investigation. Additionally,
377 there appears to be sex differences in median biomarker concentrations and their correlations
378 with SMM indices, although our small sample of women limits the certainty of this finding.
379 Future research might further investigate sex differences in SMM biomarkers in people with
380 CHD.

381 **4.7 Conclusion**

382 This study aimed to identify associations between SMM indices and circulatory biomarkers in
383 people with CHD. Lower levels of serum transthyretin, AST and ALT were present in people
384 with CHD and low SMM, compared to those with normal SMM. To assist with practical
385 application, we also identified the cut-off points below which transthyretin, ALT and AST
386 indicate high likelihood of low SMM. We found no association between albumin, CAF and
387 SMM indices, suggesting that these markers have limited utility as markers for low SMM in
388 this cohort.

389

390 **Abbreviations**

391 ALT = alanine aminotransferase

392 ASM% = appendicular skeletal muscle

393 AST = aspartate aminotransferase

394 CAF = C-terminal agrin fragment

395 CHD = coronary heart disease

396 DXA = dual X-ray absorptiometry

397 Hs-CRP = high sensitivity C-reactive protein

398 SMI = skeletal muscle index

399 SMM = skeletal muscle mass

400

401 **Figure captions**

402 Figure 1. Correlations between skeletal muscle index and circulatory (A) albumin, (B)
403 transthyretin, (C) AST/ALT ratio, (D) ALT, (E) AST, and (F) C-terminal agrin fragment, in
404 people with coronary heart disease (n = 64). ALT = alanine aminotransferase, AST =
405 aspartate aminotransferase

406

407 **Acknowledgements**

408 We would like to thank Hull and East Riding Cardiac Trust Fund and Northumbria University
409 for providing financial support enabling blood sample analysis. We extend our gratitude to
410 Wendy Summer, Lesley Richardson, and Emma Smith for their support recruiting people to
411 this study. We would also like to thank Julie Davis, Joan Weston and Stella Rimmer for the
412 support they provided during data collection.

413 **Funding**

414 Financial support for blood sample analysis was provided by Hull and East Riding Cardiac
415 Trust Fund (Hull, East Yorkshire, UK) and Northumbria University (Newcastle upon Tyne,
416 UK).

417 **Conflicts of interest**

418 The authors declare that the research was conducted in the absence of any commercial or
419 financial relationships that could be construed as a potential conflict of interest.

420

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629 Table 1 Patient baseline characteristics

Variable	Mean \pm standard deviation or Frequency (%)		
	All people (n=64)	Low SMM (n=14)	Normal SMM (n=50)
Age (years)	63.4 \pm 9.8	67.6 \pm 10.6	62.2 \pm 9.4
Female	8 (12.5)	5 (35.7)*	3 (6.0)
Body mass index (kg.m ⁻²)	28.9 \pm 3.9	28.9 \pm 5.5	28.9 \pm 3.3
Body fat content (%)	36.1 \pm 6.9	41.9 \pm 8.6**	34.5 \pm 5.4
Waist/ Hip circumferences ratio ^{a, b}	0.97 (0.93, 1.02)	0.96 (0.86, 1.0)	0.97 (0.93, 1.0)
Appendicular lean mass (kg)	23.8 \pm 4.6	18.9 \pm 3.2**	25.2 \pm 3.9
Skeletal muscle index (kg.m ⁻²)	8.7 \pm 1.7	6.9 \pm 1.2**	9.2 \pm 1.4
Appendicular skeletal mass (%) ^a	28.7 (26.2, 30.8)	24.4 (22.0, 25.3)**	29.1 (27.9, 31.0)
$\dot{V}O_{2peak}$ (ml.kg ⁻¹ .min ⁻¹)	23.9 \pm 6.0	20.1 \pm 5.6*	24.6 \pm 5.8
Left ventricular ejection fraction (%)	55.1 \pm 7.0	53.9 \pm 8.7	55.5 \pm 6.5
N-terminal pro-brain natriuretic peptide (NT-proBNP; pg.L ⁻¹) ^{a, b}	172.0 (64.7, 344.0)	357.0 (112.8, 998.0)*	138.0 (55.8, 273.5)
Presenting diagnosis			
ST-elevation MI (STEMI)	14 (21.9)	3 (21.4)	11 (22.0)
Non-ST-elevation MI (non-STEMI)	21 (32.8)	4 (28.6)	17 (34.0)
Elective percutaneous coronary intervention (PCI)	17 (26.6)	3 (21.4)	14 (28.0)
Coronary artery bypass graft (CABG)	6 (9.4)	2 (14.3)	4 (8.0)
Angina	6 (9.4)	2 (14.3)	4 (8.0)
Comorbidities			
Hypertension	30 (46.9)	8 (57.1)	22 (44.0)
Type 2 diabetes	12 (18.8)	3 (21.4)	9 (18.0)
Chronic obstructive pulmonary disease (COPD)	3 (4.7)	2 (14.3)	1 (2.0)
Hyperlipidaemia	43 (67.2)	9 (64.3)	34 (68.0)
Previous PCI	13 (20.3)	4 (28.5)	9 (18.0)
Previous MI	13 (20.3)	2 (14.2)	11 (22.0)
Previous CABG	5 (7.8)	2 (14.3)	3 (6.0)
Previous cardiac valve surgery	1 (1.6)	0	1 (2.0)
Previous transient ischemic attack	6 (9.4)	3 (21.4)	3 (6.0)
Cancer	10 (15.6)	5 (35.7)*	5 (10.0)
Medications			
Aspirin	62 (96.9)	13 (92.9)	49 (98.0)
Clopidogrel	19 (29.7)	6 (42.9)	13 (26.0)
Ticagrelor	32 (50.0)	4 (28.6)	28 (56.0)
Beta-blockers	57 (89.1)	12 (85.7)	45 (90.0)
Angiotensin converting enzyme (ACE)-inhibitors	38 (59.4)	10 (71.4)	28 (56.0)
Statins	61 (95.3)	14 (100.0)	47 (94.0)

Diuretics	7 (10.9)	3 (21.4)	4 (8.0)
Nitrates (non-GTN)	15 (23.4)	2 (14.3)	13 (26.0)
GTN spray	58 (90.6)	12 (85.7)	46 (92.0)

630 ^a values are median (interquartile range), ^b n=63, GTN = glyceryl trinitrate, MI = myocardial
631 infarction. *P <0.05 or **P <0.01 compared to normal SMM group.

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636 Table 2 Circulatory biomarker concentrations in people with coronary heart disease with low or normal skeletal muscle mass (SMM). Values are
637 median (range) where n = 3. All other values are median (interquartile range).

Biomarker	All					Men					Women				
	Low SMM (n = 14)	Normal SMM (n = 50)	U	ES	P-value	Low SMM (n = 9)	Normal SMM (n = 47)	U	ES	P-value	Low SMM (n = 5)	Normal SMM (n = 3)	U	ES	P-value
Albumin (g/L)	37.50 (36.00, 39.25)	38.50 (37.00, 41.00)	283.00	.14	.274	38.00 (36.00, 39.50)	39.00 (37.00, 41.00)	170.00	.12	.352	37.00 (36.00, 40.00)	37.00 (36.00, 38.00)	7.00	.05	.877
Transthyretin (mg/dl)	29.66 (18.36, 34.08)	37.87 (28.83, 53.63)	183.00	.34	.007**	28.64 (17.51, 34.96)	37.88 (28.87, 54.55)	96.00	.34	.010*	32.07 (22.23, 35.28)	24.34 (24.08, 50.34)	7.00	.05	.881
Alanine aminotransferase (U/L)	20.00 (17.00, 24.00)	31.00 (21.75, 41.25)	188.00	.34	.008**	20.00 (19.50, 24.00)	32.00 (22.00, 42.00)	127.50	.25	.061	17.00 (14.50, 28.00)	21.00 (19.00, 24.00)	3.00	.47	.180
Aspartate aminotransferase (U/L)	22.25 (18.00, 29.13)	27.00 (23.00, 34.75)	221.50	.26	.037*	24.00 (22.25, 31.50)	27.50 (23.00, 35.50)	186.00	.08	.569	18.00 (17.25, 20.25)	25.50 (22.50, 27.00)	.00	.79	.025*
AST/ALT	1.17 (0.93, 1.25)	0.91 (0.69, 1.23)	255.00	.19	.123	1.20 (0.92, 1.31)	0.86 (0.68, 1.23)	130.00	.25	.069	1.13 (0.76, 1.24)	1.07 (1.06, 1.42)	6.00	.16	.655
C-terminal agrin fragment (ng/ml)	3.89 (3.10, 4.24)	3.67 (3.11, 4.48)	335.00	.03	.808	4.15 (2.55, 4.53)	3.63 (3.05, 4.36)	206.00	.02	.902	3.74 (3.37, 4.02)	4.83 (4.02, 5.25)	1.00	.69	.053
Hs C-reactive protein (mg/L) ^a	2.51 (0.42, 4.33)	1.19 (0.50, 3.41)	303.00	.10	.445	2.62 (0.58, 4.63)	1.18 (0.48, 3.41)	164.00	.14	.289	1.87 (0.32, 4.25)	2.96 (2.17, 8.86)	4.00	.37	.297

638 *P <0.05 or **P <0.01 compared to normal SMM group. AST/ALT = alanine aminotransferase/aspartate aminotransferase ratio, ES = effect
639 size, U = U statistic from Mann-Whitney U test. Low SMM was skeletal muscle index <7.0 and <6.0 kg.m⁻², or appendicular skeletal muscle

640 <25.72% and <19.43%, for men and women, respectively. ^a values from a subset of people included in the present study were been reported
641 elsewhere (4)

642 Table 3 Correlations between SMI, ASM% and serum biomarkers.

	All (n=64)		Men (n=56)		Women (n=8)	
	SMI	ASM%	SMI	ASM%	SMI	ASM%
Albumin						
Spearman's corr. (r)	.229	.179	.147	.104	.593	.272
P-value	.069	.157	.279	.447	.121	.515
Partial corr. (r) ^a	.217	.141	.143	.071	.738	.114
P-value	.087	.271	.297	.607	.058	.807
Partial corr. (r) ^b	.082	.151	-.023	.081	.765*	.198
P-value	.524	.236	.868	.557	.045	.671
Partial corr. (r) ^c	.072	.116	-.034	.036	.763	.248
P-value	.579	.369	.809	.795	.078	.636
Transthyretin						
Spearman's corr. (r)	.246	.132	.208	.048	.048	-.857**
P-value	.050	.297	.124	.725	.911	.007
Partial corr. (r) ^a	.237	.101	.204	.002	.030	-.839*
P-value	.061	.433	.135	.987	.949	.018
Partial corr. (r) ^b	.213	.120	.142	.033	.075	-.874
P-value	.094	.349	.302	.808	.872	.010*
Partial corr. (r) ^c	.206	.090	.134	-.017	.054	-.889*
P-value	.109	.484	.335	.901	.919	.018
Alanine aminotransferase (ALT)						
Spearman's corr. (r)	.271*	.209	.144	.048	.190	.095
P-value	.030	.098	.289	.726	.651	.823
Partial corr. (r) ^a	.261*	.175	.141	.023	.200	.051
P-value	.039	.170	.304	.868	.668	.913
Partial corr. (r) ^b	.158	.186	-.032	.016	.193	.106
P-value	.216	.144	.819	.917	.678	.820
Partial corr. (r) ^c	.150	.156	-.040	-.020	.277	-.016
P-value	.245	.226	.776	.886	.596	.977
Aspartate aminotransferase (AST)						
Spearman's corr. (r)	.038	.181	-.169	-.001	.238	.190
P-value	.766	.152	.213	.993	.570	.651
Partial corr. (r) ^a	.034	.176	-.171	-.013	.236	.339
P-value	.791	.168	.212	.927	.610	.457
Partial corr. (r) ^b	-.017	.168	-.275*	-.018	.277	.290
P-value	.897	.188	.042	.895	.547	.528
Partial corr. (r) ^c	-.019	.165	-.279*	-.033	.279	.313
P-value	.825	.199	.041	.812	.592	.546
AST/ALT ratio						
Spearman's corr. (r)	-.360**	-.089	-.386**	-.031	-.048	.000
P-value	.003	.484	.003	.823	.911	1.00
Partial corr. (r) ^a	-.351**	-.043	-.384**	-.001	-.117	.361
P-value	.005	.736	.004	.997	.803	.427

Partial corr. (r) ^b	-.264*	-.054	-.285*	-.002	-.025	.135
P-value	.036	.675	.035	.986	.958	.773
Partial corr. (r) ^c	-.257*	-.011	-.281*	.037	-.139	.393
P-value	.044	.932	.040	.792	.793	.441
C-terminal agrin fragment						
Spearman's corr. (r)	-.042	-.180	-.008	-.161	.429	.286
P-value	.741	.154	.956	.235	.289	.493
Partial corr. (r) ^a	-.029	-.146	-.002	-.133	.429	.303
P-value	.823	.253	.988	.334	.337	.509
Partial corr. (r) ^b	-.048	-.182	-.015	-.163	.436	.311
P-value	.706	.152	.912	.234	.329	.497
Partial corr. (r) ^c	-.038	-.148	-.008	-.134	.476	.277
P-value	.770	.250	.955	.333	.340	.595

643 corr., correlation. *P < 0.05, **P < 0.01. ASM% = appendicular skeletal muscle; SMI =
644 skeletal muscle index (kg.m⁻²). Non-parametric partial correlations are corrected for (a) high-
645 sensitivity C-reactive protein, (b) age, and (c) high-sensitivity C-reactive protein and age.

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