Statins Affect Skeletal Muscle Performance: Evidence for Disturbances in Energy Metabolism

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Context: Statin myopathy is linked to disturbances in mitochondrial function and exercise intolerance.

Objectives: To determine whether differences exist in exercise performance, muscle function, and muscle mitochondrial oxidative capacity and content between symptomatic and asymptomatic statin users, and control subjects.

Design: Cross-sectional study.

Setting: Department of Physiology, Radboud University Medical Center.

Participants: Long-term symptomatic and asymptomatic statin users, and control subjects (n = 10 per group).

Interventions: Maximal incremental cycling tests, involuntary electrically stimulated isometric quadriceps-muscle contractions, and biopsy of vastus lateralis muscle.

Main Outcomes Measured: Maximal exercise capacity, substrate use during exercise, muscle function, and mitochondrial energy metabolism.

Results: Peak oxygen uptake, maximal work load, and ventilatory efficiency were comparable between groups, but both statin groups had a depressed anaerobic threshold compared with the control group ($P = 0.01$). Muscle relaxation time was prolonged in both statin groups compared with the control group and rate of maximal force rise was decreased ($P_{time \times group} < 0.001$ for both measures). Mitochondrial activity of complexes II and IV was lower in symptomatic statin users than control subjects and tended to be lower for complex (C) III (CII: $P = 0.03$; CIII: $P = 0.05$; CIV: $P = 0.04$). Mitochondrial content tended to be lower in both statin groups than in control subjects.

Conclusion: Statin use attenuated substrate use during maximal exercise performance, induced muscle fatigue during repeated muscle contractions, and decreased muscle mitochondrial oxidative capacity. This suggests disturbances in mitochondrial oxidative capacity occur with statin use even in patients without statin-induced muscle complaints. (***J Clin Endocrinol Metab*** **103: 75–84, 2018)**

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Abbreviations: ALT, alanine aminotransferase; ANOVA, analysis of variance; ATP, adenosine triphosphate; C, complex; CK, creatine kinase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MFR, maximal rate of force rise; MVC, maximal voluntary contraction; OXPHOS, oxidative phosphorylation; Rt, relaxation time; SERCA, sarcoplasmic reticulum Ca^{2+}-ATPase; SQUASH, Short Questionnaire to Assess Health-Enhancing Physical Activity; SR, sarcoplasmic reticulum; STOMP, Effect of Statins on Skeletal Muscle Function and Performance trial; V_{CO_2}, carbon dioxide production; V_{O_2}, oxygen uptake; $V_{O_{2peak}}$, peak oxygen uptake; VT, ventilatory threshold.

HMG-CoA reductase inhibitors, or statins, effectively reduce blood cholesterol levels and produce a remarkable reduction in cardiovascular events (1). Physical activity also reduces cardiovascular risk, and the combination of statins and high level of physical fitness reduces cardiovascular mortality in patients with hyperlipidemia patients more than either treatment alone (2). However, 7% to 29% of patients are reported to develop muscle complaints while receiving statin treatment (3), and these complaints may be exacerbated by exercise (4–6). Although the mechanisms are poorly understood, statins have been shown to reduce muscle mitochondrial oxidative capacity and content in humans (7–10) and impair exercise-mediated mitochondrial adaptations in skeletal muscle (11).

Few studies have examined the effects of statins on muscle contractile function and exercise performance, and even fewer studies have examined this in statin users with muscle complaints. For example, the Effect of Statins on Skeletal Muscle Function and Performance (STOMP) trial is, to our knowledge, the only randomized, double-blind clinical trial that has examined aerobic exercise performance and muscle strength before and after treatment with placebo or high-dose atorvastatin (12). STOMP researchers found that more patients in the atorvastatin group than in the placebo group developed muscle complaints, but there were no differences in muscle strength and endurance, aerobic performance, or physical activity levels after 6 months of treatment. Only statin-naive individuals were studied in STOMP, however, so the absence of deleterious changes in muscle function and performance may not apply to symptomatic statin users. Also, muscle biopsy specimens were not obtained in the STOMP trial to investigate whether changes in mitochondrial content and/or function occur during statin treatment and whether they relate to muscle complaints and exercise performance.

The aim of the current study was to examine whether differences exist in aerobic exercise performance, muscle contractile function, and muscle mitochondrial oxidative capacity and content between long-term symptomatic and asymptomatic statin users, and control subjects who did not use a statin drug.

Materials and Methods

Participants

Ten statin users with muscle complaints, 10 statin users without muscle or other statin-induced adverse effects, and 10 control subjects not using statins participated in this study; their mean ages ± standard deviation were 61 ± 5 years, 64 ± 3 years, and 61 ± 7 years, respectively. Participants were recruited via advertisements in local newspapers and at pharmacies and general practitioners' offices. Statin users were included if they had been prescribed statins for at least 3 months before the study. Prior temporary statin withdrawal was not exclusionary if it did not occur within the last 3 months before entering the study. Statin users were also included if their regimen had been adjusted (*e.g.*, dose reduction, change of statin type). Muscle symptoms were evaluated for their probability of being statin related using a score of ≥7 on the statin myalgia clinical index score of the Statin Muscle Safety Task Force (13), the short-form McGill pain questionnaire (14) and plasma creatine kinase (CK) concentrations. For the statin myalgia index score, a cutoff of ≥7 was applied. Participants with familial hypercholesterolemia, a cardiovascular event within 1 year of study participation, alanine aminotransferase (ALT), aspartate aminotransferase, or γ-glutamyl transferase levels higher than three times the upper limit of normal, a known hereditary muscle defect, creatine kinase level higher than five times the upper limit of normal, comedication known to have an effect on mitochondrial function (*e.g.*, steroids, metformin), creatinine level <50 μmol/L or >100 μmol/L, or diabetes mellitus were excluded. Written informed consent was obtained from participants before participation, as approved by the Local Committee on Research Involving Human Subjects of the region Arnhem and Nijmegen, Netherlands. This trial is registered in the Netherlands Trial Registry (NTR5505).

Medical screening

Eligibility for participation was determined during a medical screening visit. A physician reviewed the medical history and determined the safety of performing the maximal aerobic cycling test and the muscle biopsy. Body mass, height, waist and hip circumferences, and resting heart rate and blood pressure (measured manually; Maxi-Stabil 3; WelchAllyn, Skaneateles Falls, NY) were measured. An electrocardiogram was used to determine heart rhythm. The Short Questionnaire to Assess Health-Enhancing Physical Activity (SQUASH) (15) and the short-form McGill pain questionnaire (14) were used to determine the participants' physical activity levels and the intensity and location of muscle pain and its impact on daily functioning.

Serological markers

Venous blood was collected at the medical screening to measure levels of serum lipids [total, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol, and triglycerides], CK, ALT, aspartate aminotransferase, γ-glutamyl transferase, creatinine, and vitamin D.

Maximal exercise performance

Maximal exercise performance was measured with an incremental cycle ergometer test (Lode Excalibur; Groningen, Netherlands) (16). Peak oxygen uptake ($V_{O_{2peak}}$) was defined as the highest oxygen uptake (30-second average) and was also expressed as a percentage of the predicted maximal oxygen consumption. The first ventilatory threshold (VT_1) was found at the intersection of the carbon dioxide production (V_{CO_2}) *vs* oxygen uptake (V_{O_2}) graph and was determined by using the V-slope method (17). The second ventilatory threshold (VT_2) was found at the inflection of ventilation *vs* V_{CO_2} slope. V_{O_2} at VT_1 and VT_2 was also expressed as a percentage of $V_{O_{2peak}}$ (17). Ventilatory efficiency was defined as the slope of the ventilation to V_{CO_2} calculated over the linear phase of the response up to the VT_1. Maximal oxygen pulse was calculated as oxygen consumption per heart beat. Blood lactate level was measured before and immediately after exercise (Accutrend plus GCT

Cobas; Roche Diagnostics, Sussex, United Kingdom), and the rate of perceived exertion was scored using the Borg scale.

Muscle strength and contractile properties

Electrically stimulated, isometric quadriceps-muscle contractions were obtained through two surface electrodes placed over the distal and proximal parts of the anterior thigh (18). The electrical stimulation was designed to contract the quadriceps muscle at 30% of the maximal voluntary contraction (MVC) (18). A force-frequency relationship was obtained from six bursts of 1-second duration that ranged from 1 to 100 Hz. Contraction and relaxation rates of isometric tetani were calculated as indices of muscle speed. Normalized maximal rate of force rise (MFR) was calculated from the 10- and 100-Hz force differential and expressed as percentage of peak force (19). Early relaxation time (Rt) and half Rt were defined as the time taken for force to decline from 75% to 50% and from 50% to 25% of the peak force, respectively. The early and half Rts were calculated for all stimulation signals (except 10 Hz) and averaged for each participant. In addition, the ratio of the forces generated by the 20- and the 50-Hz stimulations and the stimulus frequency required to generate half-maximal tetanic 100-Hz force were calculated (18). The relative fusion in response to the 10-Hz stimulation was assessed by calculating the force oscillation amplitude (18). The resistance to fatigue was assessed by activating the quadriceps muscle repetitively using 30-Hz bursts of 1-second duration every 2 seconds (on- to off-time ratio, 1s:1s) for 2 minutes. Muscle fatigue resistance was analyzed by calculating the peak force, MFR, and half Rt generated per repetition.

Muscle biopsy specimens

After an overnight fast, a muscle biopsy specimen was collected under local anesthesia from the vastus lateralis muscle (20). Part of the freshly collected specimen (~150 mg) was used to determine mitochondrial adenosine triphosphate (ATP) production, enzyme activity of the individual oxidative phosphorylation (OXPHOS) complexes, and [1-^{14}C]pyruvate oxidation rates. Another portion (~30 mg) was placed in ice-cold muscle isolation buffer (Oroboros Instruments, Innsbruck, Austria) for high-resolution respirometry measurements in muscle fibers using the Oroboros Oxygraph-2k. The remainder of the biopsy specimen was snap-frozen in liquid nitrogen and stored for further analysis. Sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) activity was measured in whole-muscle homogenates as Ca^{2+}-dependent ATPase activity (21).

Mitochondrial ATP production capacity, enzyme activities of individual OXPHOS complexes, and [1-^{14}C]pyruvate oxidation rates

Mitochondria were isolated from freshly collected muscle biopsy specimens and used for measurement of ATP production (22, 23). Enzyme activities of the OXPHOS complexes were determined spectrophotometrically (24, 25). ATP production capacity and enzyme activities of OXPHOS complexes were normalized to the protein content of the muscle biopsy specimen. In addition, citrate synthase was determined spectrophotometrically to assess mitochondrial mass (22). [1-^{14}C]pyruvate plus malate plus ADP rates and [1-^{14}C]pyruvate plus carnitine plus ADP oxidation rates were determined (22).

High-resolution respirometry in isolated muscle fibers

Respiration rates were determined using an Oxygraph-2k equipped with DatLab5 recording and analysis software (Oroboros Instruments) (8). Muscle fibers were permeabilized with saponin (50 μg/mL) at 4°C for 20 minutes (26). After being thoroughly washed with respiration medium MiR05 (Oroboros Instruments), muscle fiber wet weight was determined. Subsequently, ~8 mg of muscle fiber was transferred into each of the chambers of the Oxygraph-2k at hyperatmospheric oxygen concentrations (350 to 400 nmol/mL) to prevent respiratory inhibition from low oxygen. Complex-specific respiratory rates were determined and normalized to the protein content of the muscle biopsy specimen (8).

Statistical analysis

Differences among the three groups were assessed by descriptive statistics. Continuous variables were reported as mean ± standard deviation or median with 25th and 75th quartiles. Differences were tested by one-way analysis of variance (ANOVA) for normally distributed variables, and by Kruskal–Wallis one-way ANOVA for skewed variables. Where significant main effects were found, Bonferroni *post hoc* tests were performed to identify specific pairwise comparisons. Categorical data were reported in proportions and tested by a χ^2 test. Group comparisons of parameters measured at multiple time points were assessed by two-way repeated measures ANOVA. Linear regression analyses were conducted to identify correlations between variables. $P < 0.05$ was considered statistically significant. Analyses were performed using SPSS version 22.0 (IBM, Armonk, NY).

Results

Participants' characteristics

Characteristics of the participants are presented in Table 1. Seven of the 10 symptomatic statin users had switched statins or lowered their dose because of muscle adverse effects, but all 10 were still experiencing muscular discomfort during the study, as evidenced by the higher pain-rating index. Duration of statin use was not different between symptomatic and asymptomatic statin users (symptomatic users: 5 ± 3 years; asymptomatic: 9 ± 6 years; $P = 0.11$). CK concentrations were similar among groups, but symptomatic statin users had a wider range and higher upper levels of CK. Sex, age, height, weight, body mass index, and waist and hip circumferences were similar among the participants, as was resting blood pressure and heart rate. Total physical activity level, assessed by the SQUASH questionnaire, was also similar among statin users and control subjects. Both groups of statin users had higher levels of leisure time physical activity than control subjects (symptomatic statin users *vs* control subjects, $P = 0.02$; asymptomatic statin users *vs* control subjects, $P = 0.01$), but there were no differences in activity between symptomatic and asymptomatic statin users. This suggests increased activity was not alone in

Table 1. Participant Characteristics

Characteristics	Symptomatic Statin Users (n = 10)	Asymptomatic Statin Users (n = 10)	Control Subjects (n = 10)	*P* Value[a]
Participants, male/ female, no.	6/4	8/2	7/3	0.71
Age, median (Q_{25}–Q_{75}), y	61 (56–65)	65 (63–66)	61 (54–67)	0.29
Height, mean ± SD, m	1.71 ± 0.08	1.73 ± 1.07	1.77 ± 0.11	0.35
Weight, mean ± SD, kg	74.7 ± 7.1	80.3 ± 10.9	84.4 ± 13.1	0.15
BMI, mean ± SD, kg/m^2	25.6 ± 2.4	26.8 ± 2.6	26.7 ± 2.4	0.50
Waist circumference, mean ± SD, cm	91.2 ± 9.2	95.3 ± 9.0	94.7 ± 9.7	0.58
Hip circumference, mean ± SD, cm	99.8 ± 5.2	101.9 ± 4.0	104.0 ± 7.2	0.26
Systolic blood pressure, mean ± SD, mm Hg	134 ± 11	138 ± 13	134 ± 15	0.70
Diastolic blood pressure, mean ± SD, mm Hg	79 ± 5	82 ± 7	85 ± 7	0.12
Heart rate at rest per min, mean ± SD	65 ± 5	63 ± 10	64 ± 14	0.97
PRI, median (Q_{25}–Q_{75}), AU	33.3 (23.2–60.0)	0.0 (0.0–4.1)	3.7 (0.0–29.2)	0.03[b,c]
CK, median (Q_{25}–Q_{75}), U/L	146.5 (81.8–244.0)	97.5 (55.5–158.8)	102.5 (75.3–160.0)	0.34
Total physical activity level, median (Q_{25}–Q_{75}), METh/wk	113 (74–166)	104 (75–145)	74 (50–116)	0.35
Commuting	0 (0–0.0)	0 (0–0.5)	0 (0–20)	0.43
Activities at home or work	71 (4–122)	21 (8–76)	33 (20–73)	0.46
Leisure time activities	33 (20–56)	31 (21–83)	17 (10–22)	0.02[b,d]
Sport activities	2 (0–7)	4 (0–37)	9 (0–28)	0.55
Cardiovascular medication, no.				
Antiplatelet drugs	7	4	1	0.03
β-blockers	5	3	0	0.05
Angiotensin II receptor antagonists	1	1	0	1.00
Angiotensin-converting enzyme inhibitors	2	2	1	1.00
Diuretics	4	2	1	0.43
Statins	10	10	0	<0.001
Type, no.				0.61
Simvastatin	4	3	—	
Atorvastatin	5	3	—	
Pravastatin	1	3	—	
Fluvastatin	0	1	—	
Dose, mg/d, no.				0.31
10	5	1	—	
20	3	4	—	
40	2	5	—	

Abbreviations: —, not applicable; AU, arbitrary unit; BMI, body mass index; CK, creatine kinase; METh/wk, metabolic equivalent hours per week; PRI, pain rating index; Q, quartile; SD, standard deviation.

[a] *P* values are from ANOVA, Kruskal–Wallis one-way ANOVA, or χ^2 tests, and significance is set at $P < 0.05$. When significant differences between groups are found, pairwise comparisons were performed by Bonferroni *post hoc* testing.

[b] *Post hoc* result significantly different between asymptomatic statin users and control subjects.

[c] *Post hoc* test result significantly different between symptomatic statin users and control subjects.

[d] *Post hoc* test result significantly different between symptomatic and asymptomatic statin users.

contributing to the muscle complaints in statin users. Statin-treated participants used more antiplatelet drugs and tended to use more β-blockers than control subjects. There were no differences in statin type or dose between symptomatic and asymptomatic statin users.

Serological markers

Total serum cholesterol levels were higher in control subjects than in symptomatic statin users ($P = 0.03$) and tended to be higher than in asymptomatic statin users ($P = 0.08$). LDL cholesterol levels tended to be higher in control subjects than in symptomatic statin users (symptomatic statin users *vs* control subjects, $P = 0.05$; asymptomatic statin users *vs* control subjects, $P = 0.36$). HDL cholesterol levels and the LDL/HDL ratio were similar among groups. Liver and kidney function measurements were not different among groups, although ALT levels were higher in symptomatic statin users ($P = 0.03$). Vitamin D levels were similar among groups (Table 2).

Maximal exercise performance

$V_{O_{2peak}}$ was similar in the three groups but tended to be lower in statin users when expressed as percentage of predicted $V_{O_{2peak}}$ ($P = 0.10$). There were no differences in maximal work load, peak heart rate, or perceived exhaustion among the groups. Also, maximal oxygen pulse and ventilatory efficiency were similar among groups. VT_1, reflecting the ventilatory drive necessary to buffer

Table 2. Serological Markers

	Symptomatic Statin Users (n = 10)	Asymptomatic Statin Users (n = 10)	Control Subjects (n = 10)	*P* Value[a]
Cholesterol, mmol/L	4.8 ± 0.7	4.9 ± 0.6	5.7 ± 0.8	0.02[b]
HDL cholesterol, mmol/L	1.6 ± 0.5	1.3 ± 0.3	1.8 ± 0.4	0.08
LDL cholesterol, mmol/L	2.7 ± 0.9	3.0 ± 0.5	3.5 ± 0.7	0.05[b]
LDL/HDL risk index, ratio	1.9 ± 1.0	2.4 ± 0.7	2.1 ± 0.7	0.47
AST, U/L	25.9 ± 3.4	24.0 ± 5.0	23.6 ± 4.5	0.46
ALT, U/L	28.8 ± 7.0	25.8 ± 7.3	20.9 ± 4.7	0.03[b]
γ-GT, median (Q_{25}–Q_{75}),U/L	20.5 (18.0–29.0)	25.5 (19.5–42.5)	26.5 (21.5–29.5)	0.47
Creatinine, μmol/L	77.8 ± 15.7	82.3 ± 14.5	83.5 ± 15.7	0.68
Vitamin D, nmol/L	67.60 ± 32.76	58.80 ± 21.13	47.80 ± 18.47	0.22

Data given as mean ± standard deviation unless otherwise indicated.

Abbreviations: AST, aspartate aminotransferase; Q, quartile; γ-GT, γ-glutamyl transferase.

[a] *P* values are from ANOVA, Kruskal–Wallis one-way ANOVA, or χ^2 tests, and significance is set at $P < 0.05$. When significant differences between groups were found, pairwise comparisons were performed by Bonferroni *post hoc* testing.

[b] *Post hoc* significantly different between symptomatic statin users and control subjects.

lactic acid production, was significantly lower in symptomatic statin users compared with control subjects (P = 0.03), but did not differ from VT_1 of the asymptomatic statin group (P = 0.08). VT_2, the second curvilinear rise in ventilation, occurred at a lower percentage of Vo_{2peak} in both groups of statin users compared with control subjects (symptomatic statin users *vs* control subjects, P = 0.01; asymptomatic statin users *vs* control subjects, P = 0.01). These data indicate statin users switch to anaerobic metabolism sooner and that this effect may be more pronounced in symptomatic statin users. This is supported by the higher end-exercise lactate values in symptomatic statin users compared with both asymptomatic statin users (P = 0.02) and control subjects (P = 0.05). End-exercise respiratory exchange ratio values were similar among groups (Table 3).

Muscle strength and contractile properties

The MVC force in symptomatic statin users tended to be lower than in control subjects (P = 0.05; Table 4), but not after correction for body weight. The median current needed to produce 30% of MVC was ~16% lower in symptomatic statin users than control subjects (P = 0.03). Typical force responses of the quadriceps muscle to 100- and 10-Hz stimulations for the three groups are shown in Fig. 1(a) and 1(b) and quantified in Table 4. Findings for the other stimulation frequencies (1, 20, 30, and 50 Hz) were similar (data not shown). The MFR and the contractile speed were similar between statin users and control subjects for both 10- and 100-Hz stimulations (Table 4). Low-frequency stimulation (10 Hz) of the quadriceps muscle produced unfused tetani in all three groups [Fig. 1(b)], and the force oscillations were similar among groups (Table 4). However, muscle half Rt after a single contraction tended to be lower in statin users compared with control subjects (symptomatic statin users *vs*. control subjects, P = 0.08; asymptomatic statin users *vs* control subjects, P = 0.09), whereas early Rt was similar. The force responses to different stimulation frequencies demonstrate a lower response in symptomatic statin users compared with control subjects [P = 0.03; Fig. 1(c) and 1(d)]. When these forces were normalized for peak force resulting from 100-Hz stimulation, the force-frequency relationship for all three groups was similar [Fig. 1(d)]. Consequently, the stimulation frequency necessary to elicit half the maximal 100-Hz force (freq 50 N) did not differ among groups, nor did the ratio of the forces generated by the 20- and the 50-Hz stimulations (Table 4). Resistance to fatigue was not different among the groups [Fig. 1(e)]. In contrast, early Rt was significantly prolonged in both groups of statin users compared with control subjects [$P_{time \times group} < 0.001$; Fig. 1(f)]. Similarly, the MFR of each contraction decreased more rapidly in both groups of statin users compared with control subjects [$P_{time \times group} < 0.001$; Fig. 1(g)]. *Ex vivo* analysis of SERCA activity in muscle biopsy specimens revealed a 30% higher activity in asymptomatic statin users compared with control subjects (asymptomatic statin users, 89.3 ± 18.0 mU/mg protein; control subjects, 68.5 ± 9.2 mU/mg protein; P = 0.04), whereas no differences were found between symptomatic statin users and control subjects (symptomatic statin users, 72.2 ± 12.8 mU/mg protein; P = 1.0).

Mitochondrial energy metabolism

Muscle from symptomatic statin users demonstrated lower mitochondrial activity for complexes II and IV than control subjects and a tendency for lower complex (C) III

Table 3. Maximal Exercise Performance

	Symptomatic Statin Users (n = 10)	Asymptomatic Statin Users (n = 10)	Control Subjects (n = 10)	*P* Value[a]
Vo_{2peak}, mL/min	2293 ± 527	2489 ± 688	2966 ± 898	0.14
Vo_{2peak}, median (Q_{25}–Q_{75}), mL/min/kg	29.6 (25.9–35.8)	28.1 (25.8–38.6)	34.2 (26.2–40.1)	0.87
Vo_{2peak}, % predicted Vo_{2max}	90.8 ± 7	90.5 ± 8	98.4 ± 8	0.10
Maximum work load, median (Q_{25}–Q_{75}), W	200 (142–237)	201 (167–262)	237 (168–325)	0.39
Maximum heart rate per min	164 ± 14	154 ± 18	169 ± 10	0.11
Maximum O_2/heart rate, mL	15.1 ± 3.2	19.1 ± 5.2	20.0 ± 7.0	0.12
VE/V_{CO_2} slope	28.4 ± 2.6	29.8 ± 3.2	31.4 ± 3.8	0.15
Vo_2 at VT_1, median (Q_{25}–Q_{75}), mL/min	1249 (994–1501)	1529 (1378–2093)	1896 (1666–3199)	0.01*b*
Vo_2 at VT_1, %Vo_{2peak}	54.9 ± 12.2	65.3 ± 11.0	71.4 ± 10.5	0.02[b]
Vo_2 at VT_2, median (Q_{25}–Q_{75}), mL/min	1693 (1553–2282)	2050 (1657–2424)	2878 (1894–4214)	0.10
Vo_2 at VT_2, %Vo_{2peak}	81.8 ± 8.2	80.9 ± 10.8	94.4 ± 6.3	<0.01[b,c]
Vo_2 at RER = 1, median (Q_{25}–Q_{75}), mL/min	1744 (1385–2004)	2270 (1679–2873)	2645 (1585–3637)	0.14
RER end-exercise	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	0.32
Lactate preexercise, mmol/L	2.1 ± 0.9	1.8 ± 0.5	1.7 ± 0.5	0.59
Lactate end-exercise, mmol/L	10.8 ± 1.8	8.1 ± 1.1	8.5 ± 2.2	0.02[b,d]
Preexercise Borg scale score, median (Q_{25}–Q_{75})	6 (6–7.3)	7 (6–11)	6 (6–7)	0.30
End-exercise Borg scale score, median (Q_{25}–Q_{75})	19 (16.5–19)	19 (17.5–19)	17 (16.5–18)	0.08

Data given as mean ±SD unless otherwise indicated.

Abbreviations: Q, quartile; RER, respiratory exchange ratio; VE, ventilation.

[a] *P* values are from ANOVA, Kruskal–Wallis one-way ANOVA, or χ^2 tests, and significance is set at $P < 0.05$. When significant differences between groups are found, pairwise comparisons were performed by Bonferroni *post hoc* testing.

[b] *Post hoc* significantly different between symptomatic statin users and control subjects.

[c] *Post hoc* significantly different between asymptomatic statin users and control subjects.

[d] *Post hoc* significantly different between symptomatic and asymptomatic statin users.

activity [CII, $P = 0.03$; CIII, $P = 0.05$; CIV, $P = 0.04$; Fig. 2(a)]. CIII activity was inversely related to the pain-rating index in symptomatic statin users; patients with the highest level of pain demonstrated the lowest CIII activity [$r = -0.65$; $P = 0.04$; Fig. 2(b)]. Symptomatic statin users had ~28% lower muscle ATP production capacity than control subjects [symptomatic statin users, 44.9 ± 22.2 nmol/h/mg protein; asymptomatic statin users, 53.8 ± 18.2 nmol/h/mg; and control subjects 65.1 ± 21.7 nmol/h/mg protein; Fig. 2(c)]. [1-^{14}C]pyruvate oxidation rates in the presence of malate [$P = 0.03$; Fig. 2(d)] or carnitine [$P = 0.045$; Fig. 2(e)] were also significantly lower in symptomatic statin users than in control subjects. Mitochondrial respiration measurements in isolated muscle fibers showed β-oxidation– and glycerol-3-phosphate dehydrogenase–driven respiration were lower in symptomatic statin users than in control subjects (β-oxidation–driven respiration, $P = 0.048$; glycerol-3-phosphate dehydrogenase–driven respiration, $P = 0.02$), with non-significant decreases in CI-, CII-, and CIV-driven oxygen consumption [Fig. 2(f)]. Citrate synthase activity, a marker of mitochondrial density, revealed a tendency toward lower mitochondrial density in statin users [$P = 0.08$; Fig. 2(g)]. Mitochondrial density, in turn, was directly related to Vo_{2peak} in the three study groups [$r = 0.46$; $P = 0.02$; Fig. 2(h)].

Discussion

In this study, we show statin-induced changes in substrate use during maximal exercise performance, muscle fatigue during repeated muscle contractions, and disturbances in mitochondrial oxidative capacity of the muscle. We are unaware of other studies that used electrostimulation to examine muscle contractile function and fatigability in statin users. This approach is independent of the participants' effort and motivation; therefore, it is ideal for objectively measuring muscle function in patients with myopathy.

When single stimulation frequencies were applied, our study did not find differences in force response or contractile properties among symptomatic and asymptomatic statin users or control subjects, whereas half Rt tended to be shorter in both groups of statin users than in control subjects. In contrast, when participants were exposed to multiple contractions to assess fatigability of the muscle, a clear difference between statin users and control subjects was demonstrated. Although the relative force decline over time was not statistically different among groups, early Rt was prolonged, and the rate of MFR of each consecutive contraction decreased more rapidly in both statin groups compared with control subjects. These results contrast with other studies that did

Table 4. Muscle Strength and Contractile Properties

	Symptomatic Statin Users (n = 10)	Asymptomatic Statin Users (n = 10)	Control Subjects (n = 10)	*P* Value[a]
MVC, mean ± SD, N	522 ± 163	632 ± 112	697 ± 176	0.05[b]
MVC, mean ± SD, N/kg	6.9 ± 1.1	7.9 ± 1.1	8.2 ± 1.5	0.17
Current to approximate 30% MVC, mA	101.0 (82.8–117.8)	121.5 (101.3–125.5)	121.0 (108.8–158.8)	0.03[b]
Freq 50 N, mean ± SD, Hz	15.4 ± 1.4	15.7 ± 1.6	15.0 ± 1.5	0.65
Ratio 20/50, mean ± SD	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.88
Force oscillation amplitude, %	16.5 (12.0–24.5)	20.2 (17.2–29.5)	13.1 (10.3–21.4)	0.26
Early Rt, ms	25.1 (22.6–30.0)	22.38 (20.5–24.1)	25.4 (20.8–29.9)	0.34
Half Rt, ms	35.1 (30.7–44.3)	35.3 (32.6–40.9)	46.9 (39.1–63.0)	0.04
MFR 10 Hz, %/ms	0.8 (0.8–1.0)	0.9 (0.8–1.2)	0.9 (0.7–1.1)	0.60
MFR 100 Hz, %/ms	1.3 (1.0–1.4)	1.1 (1.0–1.3)	1.1 (1.0–1.4)	0.46
Contractile speed 10 Hz ms	74.5 (73.0–79.0)	75.5 (74.8–78.5)	73.0 (55.3–79.5)	0.70
Contractile speed 100 Hz ms	25.5 (19.8–28.3)	29.5 (24.0–34.5)	35.0 (25.3–41.5)	0.06

Data presented median (Q_{25}–Q_{75}) unless otherwise indicated.

Abbreviations: SD, standard deviation; Q, quartile.

[a] *P* values are from ANOVA, Kruskal–Wallis one-way ANOVA, or χ^2 tests, and significance is set at $P < 0.05$. When significant differences between groups are found, pairwise comparisons were performed by Bonferroni *post hoc* testing.

[b] *Post hoc* significantly different between symptomatic statin users and control subjects.

not find a relationship between statin use or statin myopathy and alterations in muscle strength or performance (12, 27–29). All these studies, however, used voluntary maximal isometric-strength measurements to study muscle function, which could account for the difference in results. However, one study showed that statin users required more contractions to achieve their maximal isometric force, suggesting pain may affect voluntary muscle performance (27). This is in line with the results of the STOMP trial, in which participants with myopathies had decreased muscle strength irrespective of statin or placebo treatment (12). These data collectively raise the question of whether single, maximal voluntary contractions are the best tool to detect changes in muscle function with statin use. This idea is strengthened by the fact that others, in agreement with our results, found statin myopathy was associated with a delay in time to peak power output during repeated maximal contractions

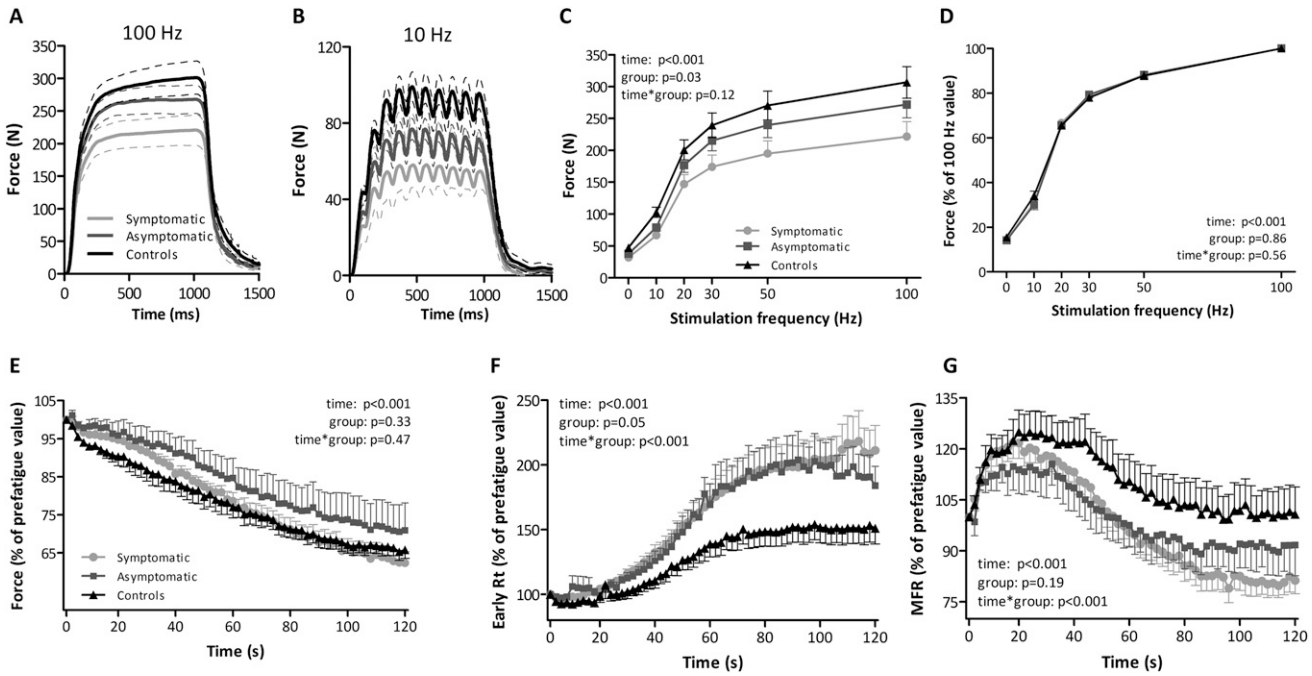

Figure 1. Muscle strength, fatigability, and contractile properties. (a, b) In these examples of typical force responses resulting from (a) 100-Hz and (b) 10-Hz stimulation in quadriceps muscle, forces are normalized for mean force in the 10-Hz signal and for peak force in the 100-Hz signal. (c, d) Force–frequency relationship in quadriceps muscle. Force responses to different stimulation frequencies are given in (c) absolute forces and (d) normalized for peak isometric 100-Hz force. (e, f) Force responses during the fatigue protocol (120 seconds). (e) Force responses are plotted every second during the complete fatigue protocol. (f) Force decline is expressed as a percentage of prefatigue value. Early Rt expressed as a percentage of prefatigue value. (g) MFR is expressed as a percentage of prefatigue value. Data are presented as mean ± standard deviation.

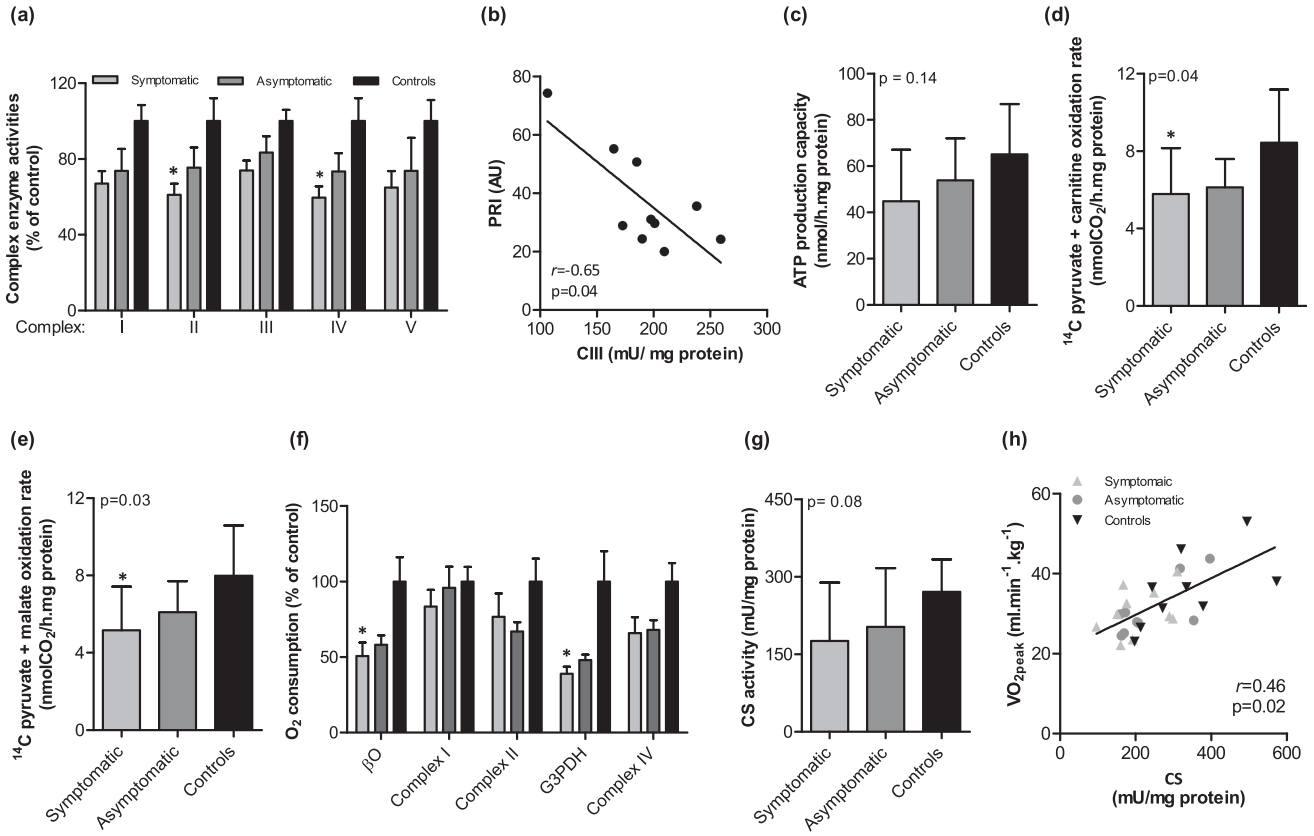

Figure 2. Mitochondrial energy metabolism. Mitochondrial fractions were prepared from fresh muscle biopsy specimens. (a) Enzyme activities of CI to CV corrected for muscle protein content and normalized to control subjects (CI, 18.8 ± 4.2 mU/mg protein; CII, 65.2 ± 20.8 mU/mg protein; CIII, 271.6 ± 42.9 mU/mg protein; CIV, 299.0 ± 70.0 mU/mg protein; and CV, 93.6 ± 27.7 mU/mg protein). (b) Enzyme activity of CIII correlates with PRI in symptomatic statin users. (c) ATP production rate normalized to muscle protein content. Oxidation rates for ^{14}C-labeled substrates were calculated as nanomoles of $^{14}CO_2$ per hour per milligram of protein. (d) ^{14}C-pyruvate plus malate and (e) ^{14}C-pyruvate plus carnitine oxidation rates normalized to muscle protein content. (f) Mitochondrial respiratory rates based on single or convergent electron flows in freshly permeabilized muscle fibers. The following substrates were used in combination with 4 mM adenosine diphosphate to determine complex-specific respiratory rates: palmitoyl-L-carnitine (20 μM) was used to estimate fatty acid βO-driven respiration. Glutamate (10 mM) plus malate (4 mM) were used as substrates for CI, succinate (10 mM) for CII, and ascorbate (2 mM) plus *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (0.5 mM) for CIV. G3PDH-driven respiration was measured in the presence of glycerol-3-phosphate (20 mM) and flavine adenine dinucleotide (10 μM). Rotenone (0.5 μM) and aptenin A5 (50 nM) were added to inhibit CI and CII, respectively. Finally, cytochrome c (10 mM) was added to check the intactness of the mitochondrial outer membrane. Respiration data were corrected for muscle protein content and normalized to control subjects (all data given as pmol/s/ mg protein: βO, 8.1 ± 3.7; CI, 6.6 ± 1.9; CII, 20.1 ± 9.1; G3PDH, 6.5 ± 3.7; and CIV, 35.6 ± 11.0). (g) CS activity normalized to muscle protein content. (h) CS activity correlated with Vo_{2peak} during the maximal incremental cycling test. Data are presented as mean ± standard deviation, if normally distributed; and as median with 25th and 75th quartile when skewed. $*P < 0.05$ is significantly different between symptomatic statin users and control subjects. βO, β-oxidation; CS, citrate synthase; G3PHD, glycerol 3-phosphate dehydrogenase; PRI, pain-rating index.

but not during single, maximal isometric-strength measurements (27).

Skeletal muscle fatigue is largely dependent on Ca^{2+} regulation by the sarcoplasmic reticulum (SR) (30, 31). Muscle contraction is initiated by the release of Ca^{2+} by the SR. Relaxation is accomplished by Ca^{2+} reuptake into the SR by the SERCA. Disturbances in Ca^{2+} homeostasis are linked to cramps, myalgia, and other adverse effects of statins on muscle (9, 32). More specifically, some suggest that by inducing mitochondrial alterations and mitochondrial membrane depolarization, statin use can lead to an elevated cytosolic Ca^{2+} concentration, which influences SERCA activity (9, 32). In the current study, SERCA activity was 30% higher in asymptomatic statin users compared with control subjects, whereas there were no differences between symptomatic statin users and control subjects. Because the mitochondrial energy-generating capacity of the asymptomatic statin users was already reduced, these findings suggest an initial disturbance in mitochondrial energy production renders statin users more susceptible to disturbances in muscle Ca^{2+} homeostasis and hence muscle fatigability. A possible explanation for the lack of an increase in SERCA activity in symptomatic statin users is that large amounts of ATP are needed to drive SERCA pumping (33).

Mitochondrial dysfunction is frequently cited as a mechanism for statin-induced myopathy. Disturbances in mitochondrial quantity (7, 34, 35) and quality (7–10, 36)

are postulated to cause statin-associated adverse effects in muscle. We found a decrease in mitochondrial CII and CIV activity in symptomatic statin users compared with control subjects in our study, and a tendency for lower CIII activity. Asymptomatic statin users showed intermediate activity levels for the mitochondrial complexes. Mitochondrial content, assessed by citrate synthase activity, showed a similar pattern, which suggested a reduction in mitochondrial number in statin users. Overall, this suggests ATP production capacity was lowest in the symptomatic group of statin users and highest in control subjects, although the differences between groups did not reach statistical significance. These results agree with the observation that mitochondrial respiration is inhibited because of reduced complex activities in both symptomatic and asymptomatic statin users (9). The results of our study and of Sirvent *et al.* (9) suggest mitochondrial dysfunction may be an early event in the pathophysiology of statin-induced myopathy, because there is evidence of mitochondrial dysfunction even in asymptomatic statin users. However, this would imply statins have a direct mitochondrial target. In that respect, we have shown the coenzyme Q binding site on CIII is a target of statins in C2C12 myoblasts and confirmed this in muscle tissue from patients with statin-related myopathies (8). The current study observed a strong inverse correlation between CIII activity and pain intensity in the symptomatic statin users, which further confirms the importance of this complex in these adverse effects.

The statin-induced changes in muscle energy metabolism are also apparent on the whole-body level during maximal exercise. VT1 and VT2 occurred at a lower percentage of $V_{O_{2peak}}$ in symptomatic statin users and VT2 occurred at a lower percentage of $V_{O_{2peak}}$ in asymptomatic statin users. This indicates statin users buffered lactic acid production at a lower level of their maximal capacity. Symptomatic statin users also had higher end-exercise lactate levels. Others also found that resting respiratory exchange ratio increased with statin therapy in healthy participants and was also elevated in patients with myopathy after cessation of statin therapy (37).

Even though our study findings clearly show aerobic exercise performance may be limited by substrate metabolism, we found no evidence that statins reduce aerobic fitness. $V_{O_{2peak}}$, maximal work load, maximal heart rate, ventilatory efficiency, and overall physical activity levels measured by the SQUASH were comparable between statin users and control subjects. Similarly, both a 12-week trial (29) and a 6-month (12) trial found no effect of statins on exercise performance, but both trials had a short duration, and statin-induced changes in exercise performance may require a longer time to develop.

The cross-sectional nature of this study does not allow us to draw definite conclusions about the causality of statin-induced muscle complaints and changes in muscle performance. However, we feel this study stresses the possibility that changes in muscle performance occur inherent to statin use.

Conclusion

Statin users switch to anaerobic metabolism sooner during maximal exercise performance, are more prone to muscle fatigability during repeated muscle contractions, and have a reduced mitochondrial oxidative capacity of the muscle than nonstatin users. This suggests disturbances in mitochondrial oxidative capacity occur with statin use even in patients without statin-induced muscle complaints. Additional longitudinal studies of patients treated with statins or placebo are required to determine if mitochondrial changes are an early step in the pathological process of statin myopathy and, hence, could serve as a diagnostic tool in the future, and whether enhancement of mitochondrial function could reduce adverse effects of statins on muscle.

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