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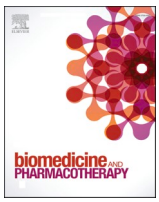

# Effect of statins on mitochondrial function and contractile force in human skeletal and cardiac muscle

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ABSTRACT

*Objectives and Background:* The success of statin therapy in reducing cardiovascular morbidity and mortality is contrasted by the skeletal muscle complaints, which often leads to nonadherence. Previous studies have shown that inhibition of mitochondrial function plays a key role in statin intolerance. Recently, it was found that statins may also influence energy metabolism in cardiomyocytes. This study assessed the effects of statin use on cardiac muscle *ex vivo* from patients using atorvastatin, rosuvastatin, simvastatin or pravastatin and controls.
*Methods:* Cardiac tissue and skeletal muscle tissue were harvested during open heart surgery after patients provided written informed consent. Patients included were undergoing cardiac surgery and either taking statins (atorvastatin, rosuvastatin, simvastatin or pravastatin) or without statin therapy (controls). Contractile behaviour of cardiac auricles was tested in an *ex vivo* set-up and cellular respiration of both cardiac and skeletal muscle tissue samples was measured using an Oxygraph-2k. Finally, statin acid and lactone concentrations were quantified in cardiac and skeletal homogenates by LC-MS/MS.
*Results:* Fatty acid oxidation and mitochondrial complex I and II activity were reduced in cardiac muscle, while contractile function remained unaffected. Inhibition of mitochondrial complex III by statins, as previously described, was confirmed in skeletal muscle when compared to control samples, but not observed in cardiac tissue. Statin concentrations determined in skeletal muscle tissue and cardiac muscle tissue were comparable.
*Conclusions:* Statins reduce skeletal and cardiac muscle cell respiration without significantly affecting cardiac contractility.

## 1. Introduction

Statins have achieved a substantial reduction in mortality and morbidity from cardiovascular disease (CVD) by inhibiting HMG-CoA-reductase, the rate-limiting step of hepatic cholesterol biosynthesis [1–3]. This has resulted in routine prescriptions for CVD patients, with now over 180 million users worldwide. However, 7–29 percent of all statin users experience muscular complaints, varying from common muscle stiffness to rare life-threatening cases of rhabdomyolysis [4–6].

Currently, statin-induced mitochondrial dysfunction is the proposed molecular mechanism behind these muscle complaints [7–10]. The decline in respiration prompts cells to switch to anaerobic glycolysis, increasing lactate production, which may provoke muscle cramps. Especially, complex III (CIII) inhibition seems to play a key role and the decreased mitochondrial oxidative capacity leads to a decline in muscular function [11,12].

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We have postulated five potential molecular mechanisms as to why statin users may suffer from skeletal muscle pains but do not experience cardiac symptoms [13]. Unfortunately, insufficient and largely contradictory studies are available regarding non-human cardiomyocytes. Recently, we demonstrated that statins can reduce viability, cellular respiration and mitochondrial function of human induced pluripotent stem cell-derived (hiPSC) cardiomyocytes [14].

Although hiPSC cardiomyocytes are widely used to evaluate cardiac drug safety, extrapolation of these results to patients remains debatable [15–17]. Here, we studied contractile function of cardiac muscle tissue from patients undergoing cardiac surgery who were using statins compared to matched controls without statin therapy. Oxygen consumption rates and statin concentrations were measured in cardiac and skeletal muscle tissue of these patients.

## 2. Materials and methods

### 2.1. Reagents

EGTA (Sigma-Aldrich, #E4378); Potassium hydroxide (Merck, #105032); Calcium carbonate (Sigma-Aldrich, #C4830); Imidazole (Sigma-Aldrich, #I5513); Taurine (Sigma-Aldrich, #T0625); MES hydrate (Sigma-Aldrich, #M8250); Dithiothreitol (Sigma-Aldrich, #D0632); Magnesium chloride hexahydrate (Merck, #105833); ATP disodium salt hydrate (Sigma-Aldrich, #A2383); Phosphocreatine disodium salt hydrate (Sigma-Aldrich, #P7936); Calcium chloride (Merck, #223506); Potassium chloride (Sigma-Aldrich, #P9541); Potassium dihydrogen phosphate (Acros Organics, #7778–77–0); Magnesium sulfate heptahydrate (Merck, #105886); Sodium chloride (Sigma-Aldrich, #S9888); Sodium bicarbonate (Sigma-Aldrich, #S3817); D-(+)-glucose (Sigma-Aldrich, #G7021); Pyruvic acid (Sigma-Aldrich, #P2256); MiR05-Kit (Oroboros Instruments; 60101–01); BSA, fatty acid free (Sigma-Aldrich, #A6003); L-(–)-Malic acid (Sigma-Aldrich, #M1000); Palmitoyl-L-carnitine chloride (Sigma-Aldrich, #P1645); ADP (Sigma-Aldrich, #A4386); L-Glutamic acid (Sigma-Aldrich, #G1251); Rotenone (Sigma-Aldrich, #R8875); Succinate (Sigma-Aldrich, #S2378); Atpenin A5 (MedChemExpress, #HY-126653); glycerophosphate III (Sigma-Aldrich, #G6501); FAD (Sigma-Aldrich, #F6625); Antimycin A (Sigma-Aldrich, #A8674); Sodium L-ascorbate (Sigma-Aldrich, #A4034); TMPD (Sigma-Aldrich, #T3134); Sodium azide (Sigma-Aldrich, #S2002); Cytochrome C (Sigma-Aldrich, #C2506); Saponin (Sigma-Aldrich, #S4521); Dimethyl sulfoxide (DMSO) (Sigma-Aldrich, #276855); Carbogen 95/5 (95 vol% $O_2$ and 5 vol% $CO_2$, Linde Gas Benelux B.V., Schiedam, Netherlands).

### 2.2. Collection of cardiac and skeletal muscle tissue

#### 2.2.1. Subject selection

Adult patients undergoing elective open heart surgery at the Radboud University Medical Center (Nijmegen, The Netherlands) were included in the study. Inclusion criteria were coronary bypass surgery (CABG), aortic valve replacement (AVR), or aortic surgery with extracorporeal circulation. The primary exclusion criteria were emergency surgery, poor cardiac function prior to surgery (left and/or right ventricular failure), permanent atrial arrythmias, and/or a medical history of multisystemic disease. Patients either received statin therapy or were control subjects without statin therapy. In case of statin therapy, the last dose was taken the evening before or the morning before surgery. The study was executed according to the rules regarding the review of research ethics committees (Commissie Mensgebonden Onderzoek Regio Arnhem-Nijmegen). Written informed consent was obtained from all patients prior to inclusion according to Radboud university medical center regulations.

Based on previous observations [11], power calculations indicated that 14 patients were required per group to detect a relative decrease in muscle CIII activity of 20 % mU/U citrate synthasewith a two-sided alpha value of 0.05 and statistical power of 0.9.

#### 2.2.2. Sample harvesting and preparation

Harvesting and preparation of myocardial tissue was performed as described previously [18–20]. Right atrial appendage and pectoral skeletal muscle tissue were harvested from patients during elective cardiac surgery by cardiothoracic surgeons.

The pectoral muscle tissue sample was placed on ice-cold preservation solution BIOPS (2.8 mM $CaK_2EGTA$, 7.2 mM $K_2EGTA$, 20 mM imidazole, 20 mM taurine, 50 mM K-MES, 0.5 mM DTT, 6.6 mM $MgCl_2$, 5.8 mM ATP, 15 mM phosphocreatine, pH 7.1, MiPNet03.02 Oroboros Instruments, Innsbruck, Austria [21–23]) preoxygenated with carbogen 95/5 until one fraction was prepared for respirometry measurements. The remaining tissue was snap-frozen in liquid nitrogen and stored at −80°C to stabilize the statins in either their lactone or acid form [24]. This tissue was used for statin quantification by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

The atrial appendage (auricle) was placed on ice-cold modified Tyrode's solution (1.8 mM CaCl, 4.8 mM KCl, 1.2 mM $KH_2PO_4$, 1.4 mM $MgSO_4$, 118.5 mM NaCl, 24.8 mM $NaHCO_3$, 10.0 mM glucose, 10.0 mM pyruvate) preoxygenated with carbogen 95/5. From the atrial auricle, two trabeculae were dissected for two separate contractile function measurements whilst remaining in Tyrode's solution (see next paragraph). A third muscle fraction was used for the respiration measurements. The remaining tissue was snap-frozen in liquid nitrogen and stored at −80°C until LC-MS/MS analysis.

### 2.3. High-resolution respirometry measurements of isolated muscle fibers

Respiration rates of the atrial and pectoral muscle fibers were determined using Oxygraph-2k equipped with DatLab5 recording and analysis software (Oroboros Instruments, Innsbruck, Austria) as performed previously [11,12,25,26]. Muscle fibers, both atrial and pectoral, were detached and isolated using forceps in 1 mL ice-cold muscle isolation buffer (BIOPS). Fibers were permeabilised with saponin (50 μg/mL) and mixed gently at 4°C for 20 min. Subsequently, the fibers were thoroughly washed with respiration medium MiR05 (0.5 mM EGTA, 3 mM $MgCl_2$, 60 mM lactobionic acid, 20 mM taurine, 10 mM $KH_2PO_4$, 20 mM HEPES, 110 mM D-sucrose, 1 g/l BSA, fatty acid free, adjusted with 5 M KOH to a pH of 7.1) and vortexed for 5 min at 4°C. Muscle fiber wet weight was measured, after which approximately 7–9 mg of muscle fiber was placed into each Oxygraph-2k chamber containing MiR05 at 37°C. To prevent respiratory inhibition from low oxygen levels, the chambers were set at hyper-atmospheric oxygen concentrations (350–400 nmol/mL) using carbogen (95/5). 20 μM Palmitoyl-L-carnitine and 2 mM malate were added to stimulate fatty acid oxidation-driven oxygen consumption. To stimulate maximal coupled respiration, 2 mM ADP was added. CI respiration was measured by adding the substrate glutamate (10 mM) and the inhibitor rotenone (1 μM). To determine CII respiration, 20 mM succinate as substrate and 50 nM atpenin A5 as inhibitor were added. For the measurement of the individual respiration of CIII and CIV, the substrates glycerophosphate III (10 mM) together with flavin adenine dinucleotide (FAD; 12.5 μM) were injected. Finally, 2 mM ascorbate together with 0.5 mM N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD) were added. Individual inhibition of the complexes, CIII and CIV was achieved using 5 μM antimycin A and ≥100 mM sodium azide, respectively. Quality control was performed by assessing mitochondrial outer membrane intactness through the addition of cytochrome c (10 μM). A cut-off value of 20 percent was used. Complex-driven respiratory rates were measured, and background correction was applied. Negative values were excluded from analyses.

### 2.4. Ex vivo contractile function of cardiac auricles

The experimental setup for the measurement of *ex vivo* contractile

function was based on previous studies [18–20]. In brief, two tissue chambers were perfused with preoxygenated (carbogen 95/5) Tyrode's solution at a final temperature of 37°C. The two dissected trabeculae were vertically suspended in the tissue chambers and linked to the force transducer (Supplementary figure 1). The tissue was able to recover for 20 min in unstretched condition with electrical field stimulation (1 Hz, 60 ms pulse duration, 40 mA pulse current) using platinum ring electrodes positioned at both sides of the trabecula. This allowed the tissue to recover from transportation and preparation. Next, the trabeculae were stretched until maximal contractile force was reached, after which 20 min of equilibration was performed. Subsequently, contractile function was measured for 10 min.

#### 2.4.1. Data recording and data analysis

Contraction waves were recorded using Biopac Student Lab (PRO version, BIOPAC Systems Inc., Goleta, United States) at a sampling rate of 2000 kHz. Before each experiment, a two-point calibration was executed (0 and 10 mN weights). Gain and range were set at x10 and 100 to −100 g respectively.

Data analysis was performed by constructing a smoothened contraction curve using MATLAB (R2018a, The MathWorks, Inc., Natick, Massachusetts, United States; Fig. 1). From this curve, several measures were retrieved as described previously [12,20,27,28], including maximum force (Fmax, peak force), minimum contraction force (cFmin), minimum relaxation force (rFmin), and the difference between Fmax and rFmin ("Δ" F, force differential). Normalised maximal rate of force rise (MFR) was computed as the slope of 25–75 % of the ascending part of the curve and expressed as percentage of peak force [28]. Early relaxation time (earlyRT) and half relaxation time (halfRT) were defined as the time period of 75–50 % and 50–25 % of the descending part of the force curve respectively. The area under the curve (AUC) was determined between Fmin and Fmax on the y-axis and Xmin (of ascending curve) and the Xmax (of the descending curve). Trabeculae that produced a force differential of less than 0.2 g at the end of baseline were excluded.

### 2.5. Tissue statin concentrations

Homogenates were made of heart tissue in PBS (20 % w/v) and skeletal muscle tissue (10 % w/v) with the use of a turrax (IKA Germany). The difference in homogenate preparation is due to the skeletal tissue being more fibrous and tougher to homogenize compared to cardiac tissue. 50 μL of the homogenates were deproteinated with 50 μL of methanol with the internal standards (acid and lactone form of deuterated components of rosuvastatin (Ros-d6 [4 ng/mL]), atorvastatin (Ator-d5 [2 ng/mL]) and simvastatin (Sim-d6 [40 ng/mL])) and 100 μL of acetonitrile. After centrifugation, the clear supernatant was transferred into a vial or a 96-well plate suitable for LC-MS/MS injection. All sample handlings were performed on ice as much as possible. For the calibration, control tissue homogenates were used, and the acids or lactone forms of each statin were added in a separate calibration curve in the range from zero to 20 ng/mL in tissue. All compound concentrations were quantified using an Acquity UPLC (Waters, Milford, MA, USA) coupled to a Xevo TQ-S (Waters) triple quadropole mass spectrometer. The compounds were separated using an Acquity UPLC BEH C18 column (Waters, 2.1 x 50 mm, 1.7 μm). The mobile phase consisted of solvent A (0.2 mM ammonium fluoride in water) and solvent B (0.2 mM ammonium fluoride in Methanol/Acetonitril 25:75). Positive and negative ionization mode with selective reaction monitoring were used. To exclude control patients who may have been taking statins without a prescription or through herbal medicines (*e.g.* red yeast rice with lovastatin) all samples were screened for the presence of statins. For every run, a calibration curve was constructed for each compound in matrix and the intrarun variation ranged between 1 % and 7 %.

Instrument control and data acquisition were performed by Masslynx software packages (version 4.1 SCN 945). Data processing and analysis were performed using TargetLynx software.

Detection of all compounds was based on isolation of the protonated molecular ion, [M + H]+ or the deprotonated molecular ion, [M – H]- and subsequent MS/MS fragmentations and a multi reaction monitoring (MRM) were carried out. The MRM transitions used are listed in supplementary table 1.

### 2.6. Statistical analysis

All analyses were performed using SPSS statistics (IBM SPSS statistics, version 25, Armonk, New York, United States) and GraphPad Prism (GraphPad software Inc., version 9). Data are presented as mean ± standard error of the mean (SEM). Differences between the two groups were analyzed with an independent samples *t*-test and $\chi 2$ test. Differences between respiration rates in statin users and non-statin users, who were not matched, were therefore tested for significance using an unpaired *t*-test with Welch's correction when variances were significantly different according to F-test. All substrates and inhibitors were added sequentially and independent values for all complexes were determined without an overall rate. An α-level of 0.05 was used to determine statistical significance. To exclude contraction waves containing background interference, ten contraction waves were manually selected for analysis. Force differential values were averaged and regarded as

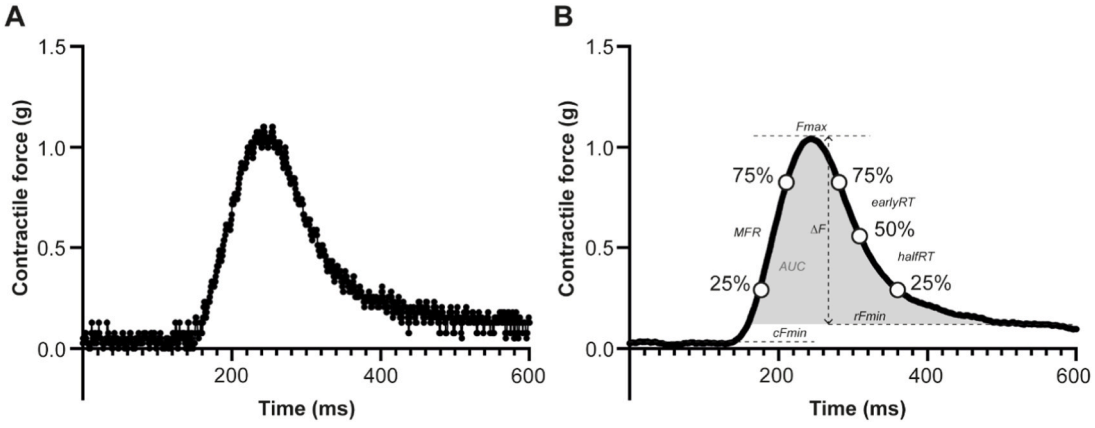


**Fig. 1.** Cardiac auricle contraction wave. Example of an electrically induced (40 mA) contraction wave in a trabecula. From the raw data (A) a smoothened curve was constructed (B), which allowed the retrieval of several kinetic parameters. This included the minimal contractile force before and after contraction (cFmin and rFmin, respectively), maximal (peak) force (Fmax), the force difference between rFmin and Fmax (ΔF), time period from 75–50% (earlyRT) and 50–25 % (halfRT) of the descending part of the curve, the slope from 25–75% of the ascending part of the curve expressed as peak force (MFR) and the area under the curve (AUC).

contractile force. Similarly, mean MFR, earlyRT, and halfRT values and the AUC were calculated for each auricle and patient. The difference between intracellular statin concentration as measured by LC-MS/MS and calculated unbound and total therapeutic plasma concentrations based on pharmacokinetic characteristics obtained from literature were compared using the Mann-Whitney test [29–33]. The non-parametric paired Wilcoxon test was used to compare intracellular statin concentrations between cardiac and skeletal muscle tissue.

## 3. Results

### 3.1. Patient characteristics

A total of 33 patients provided written informed consent. Three patients were excluded due to the unregistered use of a PCSK9 inhibitor, missing skeletal muscle tissue or infeasibility to collect cardiac tissue due to perioperative complications. Statin users were treated with significantly more comedication ($p<0.001$), especially ACE-inhibitors, antiplatelet therapy, and proton pump inhibitors. Moreover, significantly more statin users underwent CABG (10 *vs.* 1, respectively; $p=0.002$), whereas control subjects underwent more aortic valve replacements (2 *vs.* 11, respectively; $p<0.001$). Statins used were atorvastatin (n=11), rosuvastatin (n=2), simvastatin (n=1) and pravastatin (n=1). Age, sex, BMI, and comorbidities were similar between statin users and controls, as were left ventricular function and atrial dilatation as determined on preoperative transthoracic echocardiogram. All baseline characteristics of the remaining 30 patients are shown in Table 1.

**Table 1**
Baseline characteristics. Baseline characteristics of patients undergoing elective surgery, either on statins or without statin therapy. Data are presented as absolute numbers or mean ± SD. *AF: atrial fibrillation; BMI: body mass index; PCI: percutaneous coronary intervention; PAV: peripheral arterial disease; TIA: transient ischemic attack.*

| | Statin users | Non-statin users (controls) | *P-value* |
|---|---|---|---|
| **Number (N)** | 15 | 15 | 1.000 |
| **Sex** | | | |
| **Female** | 2 | 5 | 1.000 |
| **Male** | 13 | 10 | 0.390 |
| **Age (years)** | 69 ± 8 | 67 ± 6 | 0.574 |
| **BMI (kg/m$^2$)** | 27 ± 3 | 28 ± 6 | 0.576 |
| **Hypertension** | 10 | 5 | 0.143 |
| **Diabetes** | 2 | 0 | 0.483 |
| **Previous PCI** | 4 | 0 | 0.100 |
| **PAV** | 0 | 0 | 1.000 |
| **Stroke/TIA** | 3 | 1 | 0.598 |
| **Hypercholesterolemia** | 3 | 1 | 0.598 |
| **COPD** | 0 | 0 | 1.000 |
| **Paroxysmal AF** | 3 | 1 | 0.598 |
| **Drugs** | | | |
| ***Statins*** | 15 | 0 | <0.001 |
| ***Atorvastatin*** | 11 | 0 | |
| ***Rosuvastatin*** | 2 | 0 | |
| ***Simvastatin*** | 1 | 0 | |
| ***Pravastatin*** | 1 | 0 | |
| ***Other cholesterol lowering drugs*** | 0 | 0 | 1.000 |
| ***Betablocker*** | 10 | 4 | 0.066 |
| ***Calcium antagonist*** | 7 | 2 | 0.109 |
| ***ACE inhibitor*** | 10 | 0 | <0.001 |
| ***ARB*** | 3 | 5 | 0.682 |
| ***Diuretics*** | 5 | 1 | 0.169 |
| ***Antiplatelet*** | 12 | 2 | <0.001 |
| ***(N)OAC*** | 3 | 1 | 0.598 |
| ***Nitrates*** | 4 | 0 | 0.100 |
| ***Insulins*** | 1 | 0 | 1.000 |
| ***PPI*** | 11 | 3 | 0.009 |
| **Total number of drugs** | 6.9 ± 2.1 | 1.7 ± 1.4 | <0.001 |
| **Left ventricular function (%)** | 55.2 ± 0.9 | 55.0 ± 0.0 | 0.340 |
| **Dilated atria** | 3 | 2 | 1.000 |

### 3.2. Effect of statin use on skeletal muscle respiration

The individual respiration rates of skeletal muscle fibers were measured using the Oxygraph-2k. Statins reduced mitochondrial CIII activity by 62 % as compared to controls under the condition of CI and CII inhibition (1.62 *vs.* 4.28 pmol•s$^{-1}$•mg wet weight$^{-1}$ for respectively statin users and controls; $p=0.015$) and showed a trend towards inhibition of CI-linked respiration (reduction by 59 %, 1.04 *vs.* 2.51 pmol•s$^{-1}$•mg wet weight$^{-1}$ for respectively statin users and controls; $p=0.080$)(see Fig. 2B and D). Fatty acid oxidation (FAO), CII- and CIV-driven respiration remained unchanged (see Fig. 2A, C and E).

### 3.3. Effect of statin use on cardiac muscle respiration

In cardiac muscle fibers statins significantly reduced complex I-driven respiration by 58 % (2.28 *vs.* 5.45 pmol•s$^{-1}$•mg wet weight$^{-1}$ for respectively statin users and controls; $p=0.022$)(see Fig. 3B). Also, FAO (55 % reduction; 2.79 *vs.* 6.14 pmol•s$^{-1}$•mg wet weight$^{-1}$ for respectively statin users and controls; $p=0.014$) and complex II (44 %; 6.46 *vs.* 11.55 pmol•s$^{-1}$•mg wet weight$^{-1}$ for respectively statin users and controls; $p=0.043$) were significantly inhibited (see Fig. 3A and C). In contrast to the skeletal muscle fibers, statins did not alter CIII activity as much as in cardiac muscle tissue (3.72 *vs.* 5.49 pmol•s$^{-1}$•mg wet weight$^{-1}$ for respectively statin users and controls; $p=0.090$). Moreover, as in skeletal muscle, CIV-driven respiration was not affected (see Fig. 3D and E).

### 3.4. Effect of statin use on cardiac muscle contractile force

Contractile function of cardiac auricle tissue samples was measured by an *ex vivo* set-up as shown in supplementary figure 1. Statin use resulted in a decrease in both the difference between minimal and maximal contractile force (ΔF; 0.855 *vs.* 1.233 N; $p=0.062$) and maximal force rise (ΔF/Δt; 0.009 *vs.* 0.014 N/ms; $p=0.061$)(see Fig. 4A and D). Contraction parameters related to contraction and relaxation time (MFR, early RT and half RT) and the AUC did not show any differences between statin users and controls (see Fig. 4B, C, E and F).

### 3.5. Statin concentrations in cardiac and skeletal muscle of statin users

Atorvastatin showed higher measured intracellular concentrations as compared to unbound plasma concentrations from literature (heart 14 ± 7 pmol/mg wet weight vs unbound 0.12 ± 0.01 pmol/mL; skeletal 9 ± 2 pmol/mg wet weight vs unbound 0.12 ± 0.01 pmol/mL), however, total plasma concentrations from literature and measured intracellular concentration were not different (heart 14 ± 7 pmol/mg wet weight vs total plasma 6.2 ± 0.5 pmol/mL; skeletal 9 ± 2 pmol/mg wet weight vs total plasma 6.2 ± 0.5 pmol/mL), suggesting equilibrium between intracellular and plasma protein binding (see supplementary table 2). There were no significant differences in atorvastatin acid and lactone concentrations between heart and skeletal muscle tissue (acid 8 ± 6 pmol/mg wet weight vs. 4.6 ± 1.7 pmol/mg wet weight, $p = 0.278$ and lactone 5.3 ± 1.4 pmol/mg wet weight vs. 4.1 ± 1.0 pmol/mg wet weight, $p = 0.206$, respectively; supplementary table 3). For rosuvastatin (n=2), simvastatin (n=1) and pravastatin (n=1) similar results were found (see supplementary table 3). No significant correlations were observed between atorvastatin tissue concentrations and respiration and contractility measurements except for atorvastatin acid and contractility (delta F; R=0.722 and p=0.018 and maxdF/dt; R=0.645 and p=0.044).

## 4. Discussion

Statins are undoubtedly effective in reducing the morbidity and mortality from CVD patients [34], although the most commonly reported side effect, statin-associated muscle symptoms (SAMS), remains

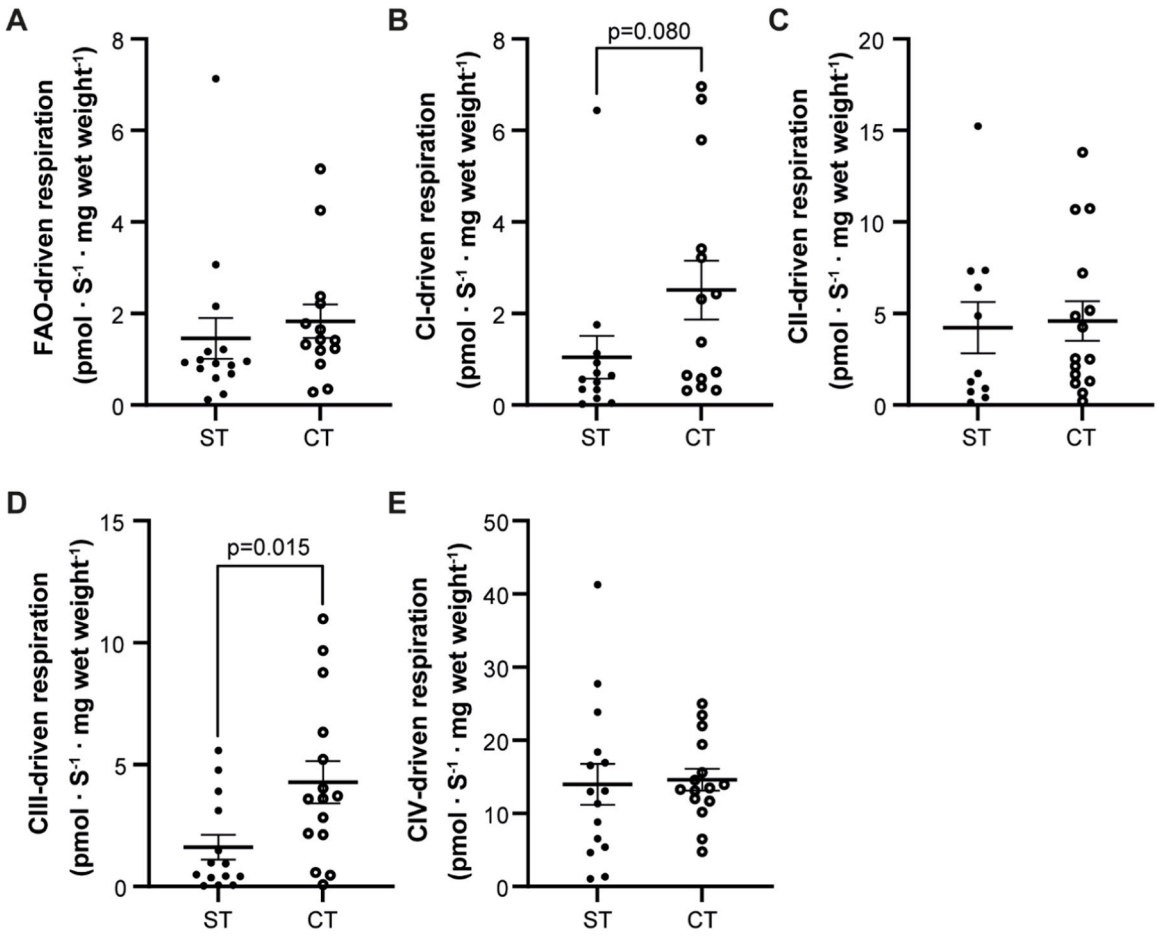


**Fig. 2.** Effect of statins on skeletal muscle respiration. Enzyme activities as measured by the Oxygraph 2k in skeletal muscle tissue from patients receiving statin treatment (ST) and control patients (CT). Fatty acid oxidation (A) and respiration driven by complex I-IV (B-E) was calculated after stimulating and inhibiting one complex after the other. Every dot represents the mean of two replicates from one patient. Unpaired *t*-test with Welch's correction when variances were significantly different according to F-test, significant if $p < 0.05$, mean ± SEM, n=11–15 for statin patients, n=14–15 for control patients.

debated [35]. Several studies have shown that mitochondrial dysfunction plays a central role in SAMS leading to skeletal muscle impairment [7–12]. These statin-induced effects are seemingly not limited to skeletal muscle [36] and may also occur in human cardiomyocytes as we have recently shown [14]. Since reports on the clinical presentations of cardiac dysfunction after statin therapy are contradictory [37–40], we studied the effects of statin use in cardiac auricles. Mitochondrial respiration was measured and compared with skeletal muscle samples from the same patients to explore whether previous observations on inhibition of oxidative phosphorylation in skeletal muscle could also be found in cardiac muscle and linked to muscle force [7–12]. Finally, statin concentrations were determined in cardiac tissue, which revealed higher intracellular concentrations as compared to unbound plasma levels. Statins inhibited cardiac fatty acid oxidation and CI- and CII-driven respiration, which, however, did not result in a loss or decline in muscle contractility. Finally, our results are in line with the previously described statin induced CIII inhibition in skeletal muscle [11], currently measured under the condition of CI and CII inhibition, which was interestingly not observed in cardiac muscle tissue.

In permeabilized muscle fibers, simvastatin inhibited CI activity and maximal ADP-stimulated respiration (with CI and CII substrates)[7,10], whereas the patients in our study mainly used atorvastatin. A study comparing skeletal muscle performance in symptomatic and asymptomatic statin users also had a higher proportion of simvastatin users, in which CII and CIV inhibition and a non-significant effect on CIII (p=0.05) was observed [12]. These findings suggest that not one particular mitochondrial complex but several complexes are affected and that this apparently depends on the type of statin [41].

Studies on the effect of statins on cardiomyocyte mitochondria are relatively scarce, sometimes contradicting the adverse effects observed in skeletal muscle cells and mainly based on animal data [36,42–45]. The few studies in human cardiomyocytes also show varying results and demonstrated that statins can improve oxidative capacity [42], reduce cellular respiration [14], and in some cases induce ferroptosis [44]. Experimental data in animals are more readily available and show that statins can disturb mitochondrial structure and function [36,45], but on the other hand are also able to attenuate mitochondrial dysfunction [43]. Many of these studies focus only on *in vitro* results and overall mitochondrial function. Here, we show in human cardiac tissue that statins may affect mitochondrial FAO and complex activity. In rat cardiomyocytes, it was previously observed that atorvastatin inhibits mitochondrial FAO [46]. Whether these effects on mitochondrial activity also result in functional loss similar to skeletal muscle has not been studied [8,12,47].

Studies on contractile properties of heart tissue are not unique [18–20], nevertheless the current study describes for the first time the effects of statins using an *ex vivo* set-up in parallel with both functional and metabolic measurements. In previous studies reduced muscle strength and mitochondrial dysfunction with loss of mitochondrial content was found in human skeletal muscle cells [8,9,12]. Intriguingly, contractile force of human cardiac tissue was unimpaired in statin users whilst mitochondrial respiration was reduced.

As myocardial ATP is more strongly related to muscle relaxation than contraction [48,49], parameters related to relaxation were assessed as

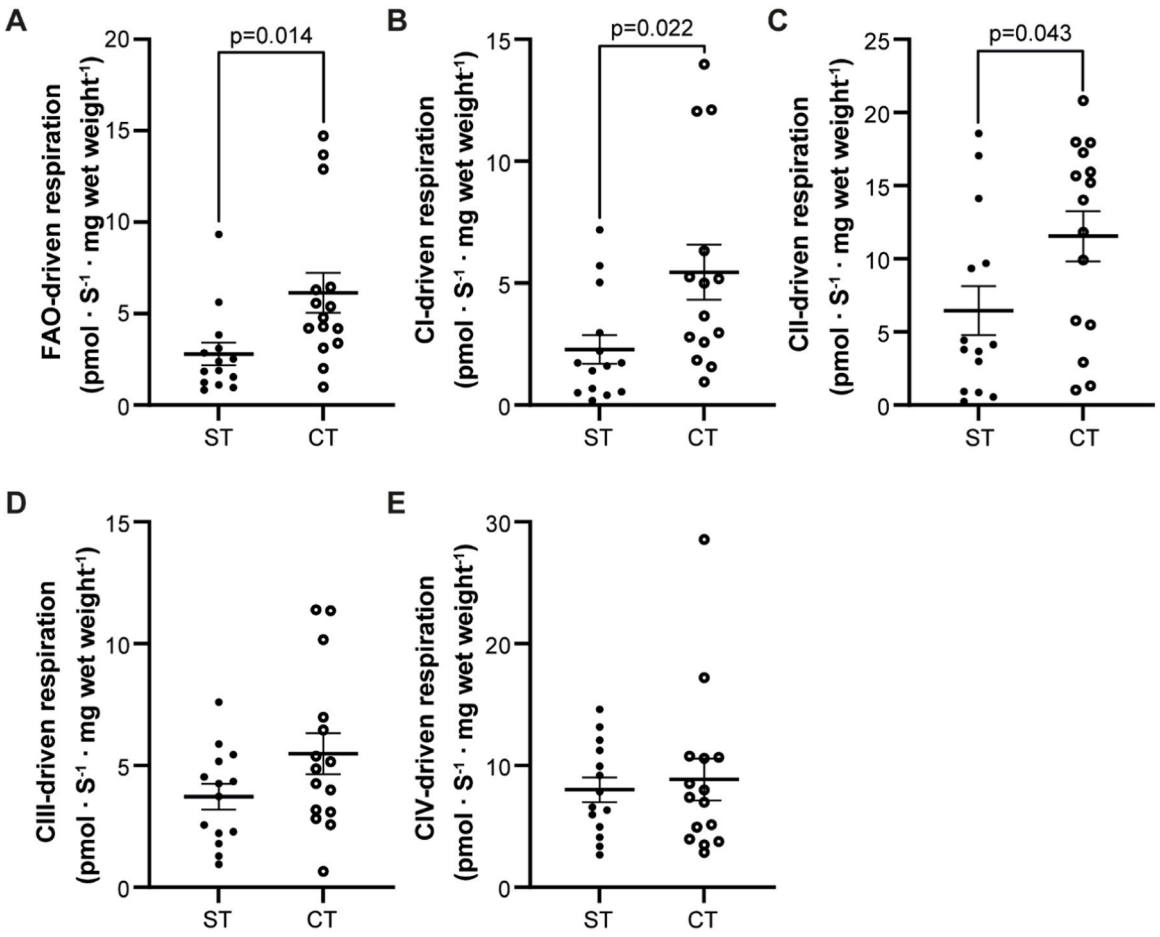


**Fig. 3.** Effect of statins on cardiac muscle respiration. Enzyme activities as measured by the Oxygraph 2k in cardiac muscle tissue from patients receiving statin treatment (ST) and control patients (CT). Fatty acid oxidation (A) and respiration driven by complex I-IV (B-E) was calculated after stimulating and inhibiting one complex after the other. Every dot represents the mean of two replicates from one patient. Unpaired *t*-test with Welch's correction when variances were significantly different according to F-test, significant if $p < 0.05$, mean ± SEM, n=14 for statin patients, n=14–15 for control patients.

well and found to be insignificantly different. A possible explanation is the metabolic flexibility of the heart, resulting in its ability to easily shift towards more glycolysis [50–52]. The current contractile measurements were performed under a continuous flow of fresh glucose providing sufficient resources for an alternative metabolism independent of mitochondrial respiration. Moreover, previous studies have argued whether FAO is inhibited in the diseased heart [52,53], suggesting that there is no direct correlation between FAO and contractile force. However, reduced mitochondrial oxidation does precede cardiac hypertrophy and heart failure, and could diminish cardiac contraction under circumstances of sickness (e.g. type 2 diabetes and inadequate metabolic alterations)[54,55].

The effects of statins are also related to their ability to accumulate in muscle tissue over time [56]. It was previously shown that statins accumulated in skeletal muscle cells of patients who developed myopathy, for this reason a 100-fold higher concentration relative to the calculated steady-state plasma concentration was used in the *in vitro* studies [11]. Data on statin accumulation in cardiomyocytes is limited to *in vitro* experiments [14]. The present study shows that statins reach similar concentrations in cardiac tissue as in skeletal muscle cells and higher intracellular concentrations as compared to the unbound concentration in plasma. However, there is no observed difference in the total plasma concentration, which could be explained by similar binding of statins to intracellular proteins resulting in an equilibrium between the total plasma and the free and bound concentration in myocardial cells. Interestingly, patients with statin-associated muscle symptoms do not experience cardiac symptoms [4–6]. This could be a consequence of pharmacokinetic, morphological, physiological and metabolic differences between skeletal and cardiac muscles, as described previously [13].

Limitations of the current study are that only asymptomatic statin users were included, whereas symptomatic patients are more likely to experience a reduction in mitochondrial function [12]. Also, it seemed that statin users had significantly more comorbidities based on the higher number of drug prescriptions, possibly affecting cellular respiration and contractile properties. In addition, no information was available on the duration of statin treatment. Also, no data were collected regarding daily physical activity, which can be of influence on mitochondrial function of skeletal muscle [57–59]. A limited number of patients was eligible for this study due to the high rate of comorbidities or (slightly) reduced ventricular function within the surgical population. Atrial auricles and pectoral muscle samples were chosen because these were easily accessible during routine cardiac surgery and generally disease free [18]. Pectoral muscle was chosen because other skeletal muscle tissue could not be harvested without introducing an additional invasive procedure. Since CIII is dependent on electron transfer from coenzyme Q (CoQ10) and CoQ10 receives its electrons from CI and CII, it is possible that CIII is not fully stimulated as it was measured after both CI and CII were inhibited, and could therefore be underestimated. Although we used the glycerol-3-phosphate shuttle that is known for its alternative electron routing excluding CI and CII [60,61], it is unknown whether overstimulation of this shuttle fully compensates for the loss of function of CI and CII. Since this was the case for both the statin and control group, we expect that it only minimally affected the observed

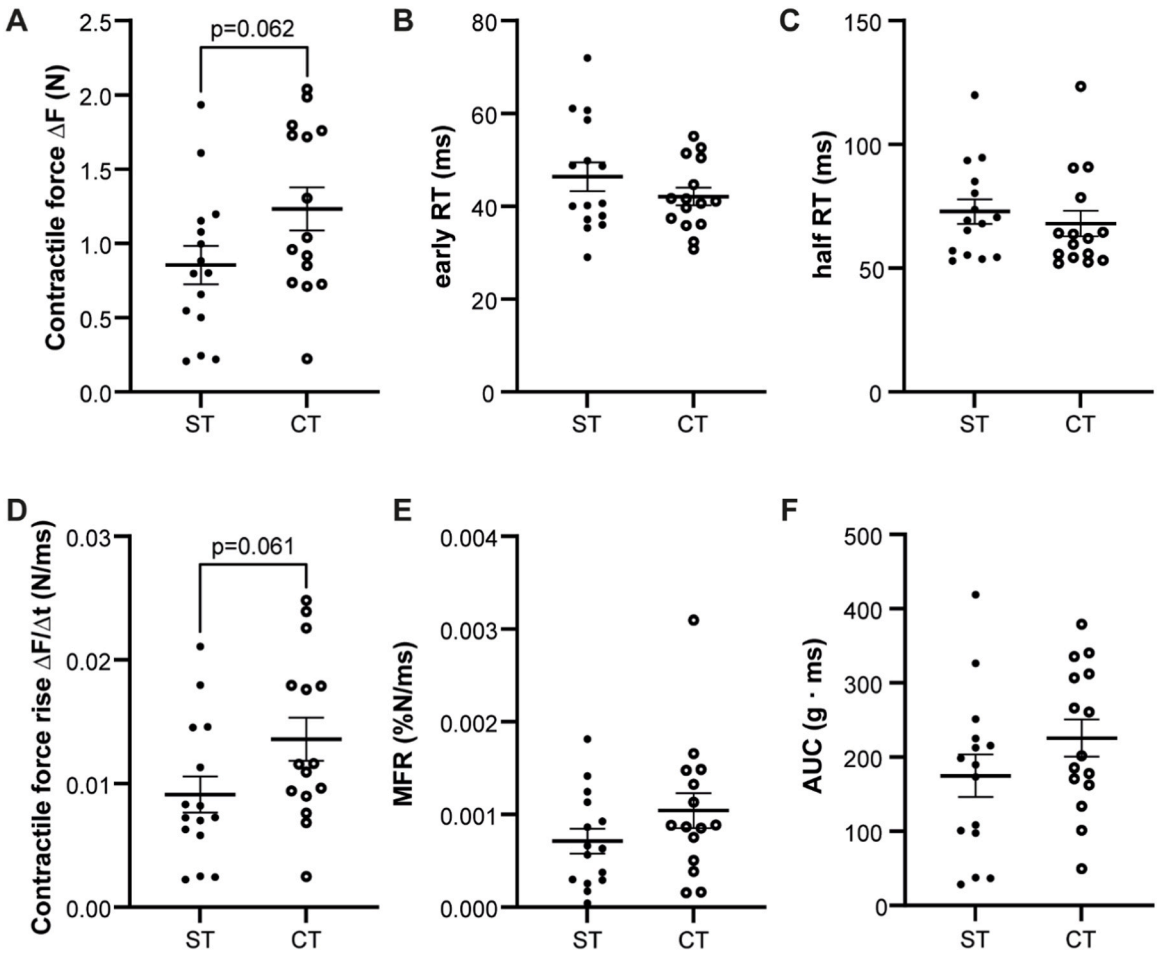


**Fig. 4.** Effect of statins on cardiac muscle contraction. Results of the different contraction parameters in patients using statins (ST) and in control patients (CT). RT = relaxation time; MFR = maximal rate of force rise; AUC = area under the curve. Every dot represents the mean of two replicates of an auricle from one patient. Unpaired *t*-test with Welch's correction when variances were significantly different according to F-test, significant if $p<0.05$, mean ± SEM, n=15 for statin patients, n=15 for control patients.

differences. Furthermore, statins have shown to reduce circulating CoQ10 levels in blood, but data regarding CoQ10 in muscle tissue are much more variable as are the effects of CoQ10 suppletion on statin-induced muscle pains [62–64]. The observed results on reduced CIII in statin users might thus also be partially explained by statin induced CoQ10 inhibition. Since *in vitro* studies with cultured cardiomyocytes cannot fully represent the situation in adults or elderly [65], *ex vivo* human cardiac tissue remains the best model. It is noteworthy that respiration rates of pectoral and cardiac tissue were similar, whereas previous studies showed significantly higher respiration rates for cardiac muscle [66]. Finally, the number of patients was not powered to calculate sex differences.

Future research should therefore focus on including patients who experience muscle symptoms due to statin use. Symptomatic statin users show a more pronounced decline in respiration and higher intracellular statin concentrations as compared to asymptomatic users [11,12]. Presumably these effects are less prevalent in asymptomatic users, as in this study, than in symptomatic patients. Furthermore, the inclusion of elderly and patients with compromised mitochondrial function, although underrepresented in the clinic, could provide valuable insights into whether the observed effects are evident due to reduced mitochondrial content or function [67–69].

## 5. Conclusions

Statin use reduces mitochondrial respiration in both skeletal and cardiac muscle tissue and fatty acid oxidation in cardiac muscle tissue alone without significantly affecting cardiac contractility. Future research in symptomatic statin users should provide insight into whether possible cardiac effects do occur in these patients and could support more personalised statin therapy.

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## CRediT authorship contribution statement

**Petra H.H. van den Broek:** Writing – review & editing, Methodology, Investigation. **Tom J.J. Schirris:** Methodology, Conceptualization. **Frans G.M. Russel:** Writing – review & editing, Supervision, Resources. **Margit C.M. Janssen:** Writing – review & editing, Software, Methodology, Investigation. **Wim J. Morshuis:** Writing – review & editing. **Renee G.C. Maas:** Writing – review & editing. **Jan W. Buikema:** Writing – review & editing. **Sailay Siddiqi:** Writing – review & editing, Supervision. **Tim Somers:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data Availability

Data will be made available on request.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2024.117492.

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