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CLINICAL TRIAL

The effects of 2 weeks of statin treatment on mitochondrial respiratory capacity in middle-aged males: the LIFESTAT study

Magnus Asping[1] · **Nis Stride**[1] · **Ditte Søgaard**[1] · **Tine Lovsø Dohlmann**[1] · **Jørn W. Helge**[1] · **Flemming Dela**[1,2] · **Steen Larsen**[1]

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Abstract
Background Statins are used to lower cholesterol in plasma and are one of the most used drugs in the world. Many statin users experience muscle pain, but the mechanisms are unknown at the moment. Many studies have hypothesized that mitochondrial function could be involved in these side effects.
Aim The aim of the study was to investigate mitochondrial function after 2 weeks of treatment with simvastatin (S; n = 10) or pravastatin (P; n = 10) in healthy middle-aged participants.
Methods Mitochondrial respiratory capacity and substrate sensitivity were measured in permeabilized muscle fibers by high-resolution respirometry. Mitochondrial content (citrate synthase (CS) activity), antioxidant content, as well as coenzyme Q_{10} concentration (Q_{10}) were determined. Fasting plasma glucose and insulin concentrations were measured, and whole body maximal oxygen uptake (VO_{2max}) was determined.
Results No differences were seen in mitochondrial respiratory capacity although a tendency was observed for a reduction when complex IV respiration was analyzed in both S (229 (169; 289 (95% confidence interval)) vs. 179 (146; 211) pmol/s/mg, respectively; P = 0.062) and P (214 (143; 285) vs. 162 (104; 220) pmol/s/mg, respectively; P = 0.053) after treatment. A tendency (1.64 (1.28; 2.00) vs. 1.28 (0.99; 1.58) mM, respectively; P = 0.092) for an increased mitochondrial substrate sensitivity (complex I-linked substrate; glutamate) was seen only in S after treatment. No differences were seen in Q_{10}, CS activity, or antioxidant content after treatment. Fasting glucose and insulin as well as VO_{2max} were not changed after treatment.
Conclusion Two weeks of statin (S or P) treatment have no major effect on mitochondrial function. The tendency for an increased mitochondrial substrate sensitivity after simvastatin treatment could be an early indication of the negative effects linked to statin treatment.

Keywords Human · Mitochondrial function · Side effects · Skeletal muscle · Statins

Introduction

Hypercholesterolemia is a major risk factor for development of stroke and coronary heart disease [1]. Statins (3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG-Co A reductase) inhibitors) are the first choice treatment for hypercholesterolemia. Generally, statins are well tolerated but particularly skeletal muscle adverse effects have been reported ranging from mild muscle pain to rhabdomyolysis [2], but the mechanisms behind muscle symptoms are not fully understood. It has been suggested that the adverse effects could be linked to an impaired mitochondrial function and a reduction in coenzyme Q_{10} (Q_{10}) in the electron transport chain [2, 3]. Statins have beneficial effects on heart muscle mitochondrial function (reduce the production of reactive oxygen species (ROS) and increase antioxidant capacity) [4], whereas the contrary is observed in skeletal muscle [4, 5]. Recent studies

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✉ Steen Larsen
stelar@sund.ku.dk

[1] Xlab, Center for Healthy Aging, Department of Biomedical Sciences, Faculty of Health Sciences, University of Copenhagen, Blegdamsvej 3b, 2200 Copenhagen N, Denmark

[2] Department of Geriatrics, Bispebjerg University Hospital, Copenhagen, Denmark

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have indicated that statins have a negative effect on mitochondrial respiratory capacity [5–7] and production of reactive oxygen species [4] in the skeletal muscle, which potentially could lead to muscle pain. One study reported a decrease in mitochondrial respiratory capacity after acute statin incubation (app. 2 h) in rat skeletal muscle (in vitro) and that these deleterious effects could be rescued by adding Q_{10} to the assay medium [8]. In addition to this, it was reported that suppression of Q_{10} leads to increased levels of oxidative stress [9]. These studies indicate that mitochondrial function (mitochondrial respiratory capacity, ROS production, and Q_{10} content) could be involved in the development of myalgia.

The duration to onset of muscular adverse effects seen after starting statin treatment varies from a few weeks to years [10]. An explanation for this discrepancy could be explained by the different pharmacokinetic profiles of the statins [11]. One study reported that mitochondrial function was affected differently depending on the statin used, where pravastatin (hydrophilic) did not impair mitochondrial respiratory capacity, but simvastatin (lipophilic) did [12]. This study suggests that using lipophilic statins increases the risk of developing adverse effects related to the skeletal muscle [13]. Although it has been speculated that lipophilic statins are more likely to penetrate the membrane of the myocyte, which is supported by the study by Kaufmann and colleagues [12], it has been reported that the lowest rate of rhabdomyolysis is seen with hydrophilic statins [14]. In addition, previous studies have shown a clear dose-response effect on side effects [13, 15, 16].

The aim of the present study was to investigate if short-term use (2 weeks) of statins impairs mitochondrial respiratory capacity and content in human skeletal muscle and if there are differences between lipophilic (simvastatin) and hydrophilic (pravastatin) statins. We hypothesized that mitochondrial respiratory capacity was impaired after 2 weeks of statin treatment and that the effect was more pronounced when a lipophilic statin (simvastatin) was used.

Method

Participants

Twenty healthy male subjects were recruited to participate in the study. Subjects were randomized to either simvastatin (S; 80 mg/day) or pravastatin (P; 40 mg/day) treatment for 14 days. Participants were included in the study if they were between 40 and 50 years of age and had a BMI between 25 and 35 kg/m^2. Participants were excluded from the study if they had any signs of heart disease, family history of diabetes, or used any medication that could affect the results.

The ethics committee of the municipality of Copenhagen and Frederiksberg in Denmark approved the study protocol (H-4-2009-095). Oral and written consent was obtained from each participant in accordance with the Helsinki Declaration.

Experimental protocol

Participants reported to the laboratory between 8:00 and 9:00 AM after an overnight fast (10 to 12 h) on three separate days (one screening day and two experimental days). Participants were instructed to abstain from alcohol and strenuous exercise the day before each visit. On the first day (screening day), a medical history was obtained including a measurement of physical activity using the International Physical Activity Questionnaire (IPAQ) [17], followed by an electrocardiogram recording. This was done to exclude individuals with signs of coronary ischemia. Thereafter, a measurement of body composition was obtained using a dual-energy X-ray absorptiometry scan (Lunar Prodigy Advance, Lunar, Madison, Wisconsin), and finally, an incremental cycling test was performed to determine the maximal oxygen uptake (VO_{2max}) (Jaeger ER 800, Erich Jaeger, Würzburg, Germany). VO_{2max} was achieved when a respiratory exchange ratio higher than 1.15, a maximal heart rate (220—age), or a leveling off or decline in VO_2 was present. The screening day was always placed at least 4 days before the first experimental day, to avoid any influence of the maximal oxygen uptake (from the screening day) test on the muscle biopsy. The second and third experimental days were identical. The participants reported to the laboratory in the fasted state and after a 15-min rest, blood pressure and resting metabolic rate (Oxycon Pro, Jaeger, Würzburg, Germany) was measured. Then, a basal blood sample was drawn and a muscle biopsy obtained under local anesthesia (lidocaine; 5 mg/ml) using the Bergstrom needle modified for suction. No adverse effects were seen with the biopsy procedure. This was followed by measuring the hand grip strength (average of three measurements) (Takei, A5401, Physical Company, High Wycombe, UK). Thereafter, an incremental cycling test was performed to determine the maximal fat oxidation and maximal oxygen uptake as described elsewhere [18].

After the first experimental day, the participants were randomized to either 2 weeks of simvastatin (S) (80 mg/day) or pravastatin (P) (40 mg/day) treatment and this was blinded for the researchers and the participants. The two statins were given to the participants so that they could not identify the tablets. The treatment period of 2 weeks was chosen because it has been reported that side effects are seen already after weeks [10] and we wanted to make sure that the participants complied to the intervention. All 20 participants that were recruited finished the study. The participants were asked at the last visit (second experimental day) about any side effects noted during the 2 weeks of statin treatment. None of the participants was using any medication, and they reported that they

were all following a normal diet and not using any over-the-counter drugs.

Analytical procedures

All analytical procedures are described in detail in the Supplementary material that is available online.

Side effects

After the intervention, at the final visit (experimental day two), the participants were asked about any side effects during the treatment period.

Statistics and calculations

Data are presented as means with 95% confidence intervals, except for Table 1 with subject characteristics and figures where data is presented as means ± SEM. Statistical analysis of differences in mitochondrial respiratory capacity between the groups was carried out with a two-way ANOVA for repeated measures. The following restrictive assumptions—normality and equal variance—were checked before the statistical analysis was conducted. Significant main effects or interactions were further analyzed by the Holm-Sidak post hoc test. If normality and equal variance test failed, the data were transformed and reanalyzed and this is stated in the figure legend. Differences between the two groups pre-treatment were evaluated using a one-way ANOVA. All statistical analysis was performed using the software program SigmaPlot 12.5 (Systat Software, San Jose, CA, USA). $P < 0.05$ was considered significant. If not stated in the legends, figures, or tables, it means that data from all participants were available for the particular analysis.

Prior to the study, a power calculation was performed on the primary outcome (mitochondrial respiratory capacity). From the literature, we anticipated a 15–20% difference in mitochondrial respiratory capacity. With the known standard error of the measurement, 10 participants in each group are sufficient.

The calculation of substrate sensitivity (C_{50}) and maximal respiratory capacity (V_{max}) has been described previously [19].

We have used Cohen s d formula to calculate the effect size on the primary outcome of the study which was mitochondrial respiratory capacity. The following d values were used to quantify the effect size: very small—0.01; small—0.20; medium—0.50; large—0.80; very large—1.20; and huge—2.00.

Results

No differences were present at baseline between S and P in regard to weight, BMI, fat percentage, and blood pressure, and these parameters did not change after treatment (Table 1). The maximal oxygen uptake per body weight was different between groups at baseline and after treatment, but the treatment did not change this parameter (Table 1). Resting and maximal whole body fat oxidation were similar between groups and were not affected by the statin treatment (data not shown). Furthermore, there were no differences in physical activity level between the two groups measured with the IPAQ (data not shown).

Side effects

Ten of the 20 participants reported side effects after treatment. The side effects reported can be categorized into three: soreness after physical activity (mild myalgia), tiredness, and

Table 1 Participant characteristics before and after 2 weeks of simvastatin or pravastatin treatment

	Simvastatin (S)		Pravastatin (P)	
	Pre	Post	Pre	Post
Age (years)	44.3 ± 2.11	–	46.8 ± 1.44	–
Weight (kg)	82.7 ± 2.12	81.9 ± 2.14	87.6 ± 3.35	87.8 ± 3.27
BMI (kg/m^2)	24.5 ± 0.48	24.2 ± 0.49	25.1 ± 0.86	25.4 ± 0.82
Body fat (%)	20.4 ± 1.49	–	25.1 ± 2.21	–
LBM (kg)	62.9 ± 1.26	–	62.2 ± 2.50	–
Sys BP (mmHg)	136 ± 4	130 ± 3	129 ± 5	130 ± 4
Dia BP (mmHg)	83 ± 3	80 ± 2	77 ± 2	81 ± 2
VO_{2max} (mlO_2/min/kg)	45.1 ± 1.91*	44.7 ± 1.59*	38.3 ± 2.72	36.6 ± 2.28
VO_{2max} (lO_2/min)	3.70 ± 0.12	3.64 ± 0.12	3.38 ± 0.32	3.22 ± 0.25
Handgrip (kg)	52.9 ± 2.14	52.1 ± 2.27	48.0 ± 1.69	46.8 ± 1.83

Data are means ± SEM

BP blood pressure, *LBM* lean body mass, VO_{2max} maximal oxygen uptake

*$P < 0.05$ S vs. P (pre or post)

stomachache. The side effects reported were equally distributed between S and P participants (data not shown).

Blood analyses (Table 2)

Alanine transaminase (ALAT) increased with S, but not with P. Total cholesterol and LDL decreased in both groups (more in S compared with those of P). In addition, triglyceride decreased in S with the intervention. Creatine kinase (CK) and lactate were comparable at baseline between S and P and were not affected by the treatment. Parameters related to glucose homeostasis (glucose, insulin, quantitative insulin sensitivity check index (QUICKI), and adiponectin) did not differ between the groups and did not change with the intervention.

Muscle analysis (Table 3)

Citrate synthase (CS) and β-hydroxy-acyl-CoA-dehydrogenase (HAD) activity was comparable before treatment in S and P and was not change after treatment. Complex I–IV enzyme activity was not changed after treatment. No differences were seen in the proteins analyzed (complexes I–V, voltage-dependent anion channel (VDAC), mnSOD, and catalase). Q_{10} did not change after treatment in S (45 ± 5 vs. 45 ± 5 μg/g of tissue, respectively). Mitochondrial respiratory capacity was not different between S and P at baseline and was not affected after treatment when complex I and complex I + II-linked respiratory capacity and electron transport system (ETS) capacity were analyzed (Fig. 1a, c, e). When analyzing complex II-linked respiratory capacity alone, no differences were seen at baseline between S and P, but there was a tendency ($P = 0.067$) for an increase after treatment in S (Fig. 1b). Cytochrome c oxidase (COX) activity was also similar at baseline between S and P, and a tendency for a decrease was seen in both S ($P = 0.062$) and P ($P = 0.053$) (Fig. 1d) after treatment. Lipid-linked respiratory capacity was comparable at baseline between groups, and a tendency for a decrease was seen in S ($P = 0.070$) after treatment (Fig. 1f). When mitochondrial respiratory capacity was normalized to CS activity, no differences were seen in any of the different respiratory states (Fig. 2a–f). The effect size (Cohen d) that was calculated on different substrate combinations (CI_P; CII_P; CI + II_P; COX; ETS; ETF_P) was ranging from 0.03 to 0.71, indicating a very small to medium effect size. Delta changes with 95% confidence interval on mitochondrial respiratory capacity are shown in Supplementary Table 1.

Mitochondrial substrate sensitivity was not affected by treatment although there was a tendency ($P = 0.092$) for an increased sensitivity with glutamate in S (Table 4). Furthermore, there was a difference between S and P at baseline with glutamate as a substrate (Table 4). Although it was

Table 2 Blood analyses

	Simvastatin			Pravastatin		
	Pre	Post	Δ change (95% CI)	Pre	Post	Δ change (95% CI)
ALAT (U/l)	21.9 (17.8; 26.1)	27.3* (21.5; 33.1)	5.40 (2.85; 7.95)	24.0 (18.6; 29.4)	26.3 (18.8; 33.9)	−0.300 (−7.55; 6.95)
ASAT (U/l)	23.6 (19.8; 27.4)	28.3 (24.5; 32.1)	4.7 (1.96; 7.44)	25.3 (22.9; 27.7)	25.7 (18.5; 32.8)	−2.20 (−9.75; 5.35)
Cholesterol (mM)	4.76 (4.26; 5.26)	3.08*,# (2.71; 3.45)	−1.68 (−2.07; −1.29)	5.20 (4.28; 6.12)	4.15* (3.54; 4.76)	−1.05 (−1.62; −0.48)
HDL (mM)	1.68 (1.21; 2.15)	1.61 (1.21; 2.01)	−0.07 (−0.25; 0.11)	1.50 (1.17; 1.83)	1.57 (1.24; 1.90)	0.07 (−0.01; 0.15)
LDL (mM)	2.65 (2.11; 3.19)	1.20*,# (0.92; 1.48)	−1.45 (−1.82; −1.09)	3.17 (2.41; 3.93)	2.13* (1.57; 2.69)	−1.04 (−1.48; −0.60)
TG (mM)	0.843 (0.716; 0.970)	0.563*,# (0.401; 0.725)	−0.280 (−0.402; −0.158)	1.198 (0.795; 1.601)	0.898 (0.771; 1.025)	−0.300 (−0.633; 0.033)
CK (U/l)	159 (83.1; 234)	225 (143; 307)	59.6 (−11.3; 131)	129 (78.8; 179)	152 (100; 204)	23.1 (−22.0; 68.2)
Lactate (mM)	0.948 (0.657; 1.24)	0.934 (0.654; 1.22)	−0.012 (−0.309; 0.285)	1.073 (0.882; 1.26)	0.997 (0.735; 1.26)	−0.076 (−0.321; 0.169)
Glucose (mM)	5.63 (5.45; 5.80)	5.58 (5.32; 5.83)	−0.08 (−0.34; 0.18)	5.47 (5.33; 5.61)	5.44 (5.14; 5.74)	−0.028 (−0.257; 0.201)
Insulin (pM)	26.9 (17.6; 36.1)	27.0 (16.4; 37.6)	−0.28 (−9.04; 8.48)	30.3 (16.6; 44.0)	26.0 (14.9; 37.1)	−4.28 (−11.3; 2.76)
QUICKI	0.395 (0.366; 0.434)	0.395 (0.363; 0.415)	0.001 (−0.023; 0.024)	0.392 (0.367; 0.413)	0.403 (0.349; 0.431)	0.011 (−0.002; 0.023)
Adiponectin (μg/ml)	24.2 (15.6; 32.8)	25.6 (14.9; 36.4)	0.804 (−1.40; 3.00)	26.6 (21.6; 31.6)	25.8 (20.0; 31.6)	−0.746 (−3.49; 2.00)

Data are means with 95% confidence intervals

ALAT alanine transaminase, *ASAT* aspartate aminotransferase, *CK* creatine kinase, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *TG* triglyceride, *QUICKI* quantitative insulin sensitivity check index.

*$P < 0.05$ Pre vs. post

#$P < 0.05$ S vs. P (pre or post)

Table 3 Skeletal muscle analysis

	Simvastatin			Pravastatin		
	Pre	Post	Δ change (95% CI)	Pre	Post	Δ change (95% CI)
Protein content (AU)						
Catalase	1.00 (0.53; 1.47)	1.05 (0.54; 1.57)	0.05 (−0.44; 0.55)	0.89 (0.36; 1.42)	1.09 (0.61; 1.56)	0.19 (−0.62; 1.01)
MnSOD	1.00 (0.76; 1.24)	1.04 (0.78; 1.29)	0.04 (−0.03; 0.10)	0.90 (0.71; 1.09)	0.91 (0.68; 1.14)	0.01 (−0.11; 0.14)
VDAC	1.00 (0.62; 1.38)	1.02 (0.65; 1.40)	0.02 (−0.23; 0.27)	0.99 (0.55; 1.43)	1.05 (0.69; 1.41)	0.07 (−0.32; 0.46)
Complex I	1.00 (0.49; 1.51)	0.96 (0.47; 1.46)	−0.03 (−0.33; 0.25)	0.81 (0.45; 1.17)	0.80 (0.43; 1.17)	−0.01 (−0.29; 0.27)
Complex II	1.00 (0.71; 1.29)	1.11 (0.75; 1.47)	0.11 (−0.14; 0.37)	0.91 (0.72; 1.10)	0.87 (0.69; 1.05)	−0.04 (−0.22; 0.14)
Complex III	1.00 (0.52; 1.48)	1.01 (0.49; 1.54)	0.01 (−0.34; 0.36)	0.71 (0.43; 0.98)	0.70 (0.53; 0.88)	−0.01 (−0.29; 0.28)
Complex IV	1.00 (0.47; 1.53)	1.12 (0.54; 1.70)	0.12 (−0.15; 0.39)	0.78 (0.50; 1.07)	0.75 (0.52; 0.99)	−0.03 (−0.29; 0.23)
Complex V	1.00 (0.66; 1.34)	1.10 (0.84; 1.35)	0.10 (−0.30; 0.49)	0.91 (0.65; 1.16)	0.89 (0.75; 1.03)	−0.02 (−0.22; 0.18)
Enzyme activities (μmol/g/min)						
CS	178 (144; 212)	170 (140; 203)	−6.44 (−19.4; 6.50)	151 (128; 174)	139 (117; 161)	−12.1 (−37.2; 13.1)
HAD	122 (95.0; 149)	114 (90.0; 139)	−5.42 (−13.9; 3.08)	111 (95.5; 126)	103 (83.2; 122)	−7.90 (−27.8; 12.0)
Complex I	12.9 (4.77; 20.9)	11.6 (6.40; 16.7)	−1.29 (−7.55; 4.97)	8.66 (1.66; 15.67)	8.08 (1.83; 14.3)	0.216 (−4.34; 4.77)
Complex II	23.9 (15.7; 32.0)	22.3 (18.3; 26.2)	−1.61 (−10.2; 6.93)	20.3 (11.2; 29.5)	18.8 (12.8; 24.8)	−0.601 (−11.8; 10.6)
Complex III	59.7 (30.0; 89.4)	57.1 (36.5; 77.7)	−2.61 (−28.3; 23.0)	53.8 (34.5; 73.2)	50.7 (29.2; 72.2)	0.503 (−24.2; 25.2)
Complex IV	97.9 (43.8; 152)	92.7 (72.1; 113)	−5.20 (−47.5; 37.1)	72.6 (38.7; 107)	72.3 (47.2; 97.4)	3.64 (−28.5; 35.8)

Data are means with 95% confidence intervals

AU arbitrary units, *CS* citrate synthase, *HAD* β-hydroxy-acyl-CoA-dehydrogenase, *MnSOD* manganese superoxide dismutase, *VDAC* voltage-dependent anion channel

not significant, V_{max} decreased approximately 20% in S after treatment using succinate as a substrate (Table 4).

Discussion

Two weeks of statin treatment (simvastatin and pravastatin) elicit major decreases in total cholesterol and LDL, but do not induce major changes in mitochondrial function. Furthermore, relevant enzyme activities or protein content remained unchanged. Mitochondrial respiratory capacity per muscle weight tended to be reduced with complex IV substrates (COX activity) with both S and P, but the Cohen d s effect size was very small to medium indicating that mitochondrial respiratory capacity is not affected after statin treatment for 2 weeks.

No differences in whole body fat oxidation at rest or during exercise were observed at baseline or after the intervention. This is in line with a previous study in young healthy subjects where no difference was seen after simvastatin treatment on fat oxidation during exercise [20] and supported by unchanged levels of adiponectin in our participants before or after the intervention in the present study [21].

Minor differences in mitochondrial respiratory capacity were observed after the intervention in the two groups. There was a tendency for a reduction in mitochondrial respiratory capacity when activating COX. This is supported by the observation in two patients in statin treatment, in whom complexes I and II + III were within the reference range but complex IV (COX activity) was significantly reduced [22]. In a cross-sectional design without age-matching, Sirvent and colleagues [7] reported similar findings of reduced COX activity in statin (various types)-treated patients with myalgia. A reduced COX activity may indicate a mitochondrial volume reduction [23], but the CS activity data do not support this notion.

It has previously been reported that statin treatment decreases mitochondrial respiratory capacity [4, 5] and mitochondrial content [3, 4], but this was not seen in the present study. Paiva and colleagues reported a significant reduction (app. 36–49%) in several enzymes in the respiratory chain after 8 weeks of simvastatin treatment, but not with atorvastatin [3]. In the present study, we were not able to detect significant differences in enzyme activities in the respiratory chain after 2 weeks of statin treatment, with no differences between S and P. Length of the intervention and differences in age may explain the discrepancy between the former [3] and the present study.

Recently, studies have indicated that statin treatment increases the production of reactive oxygen species

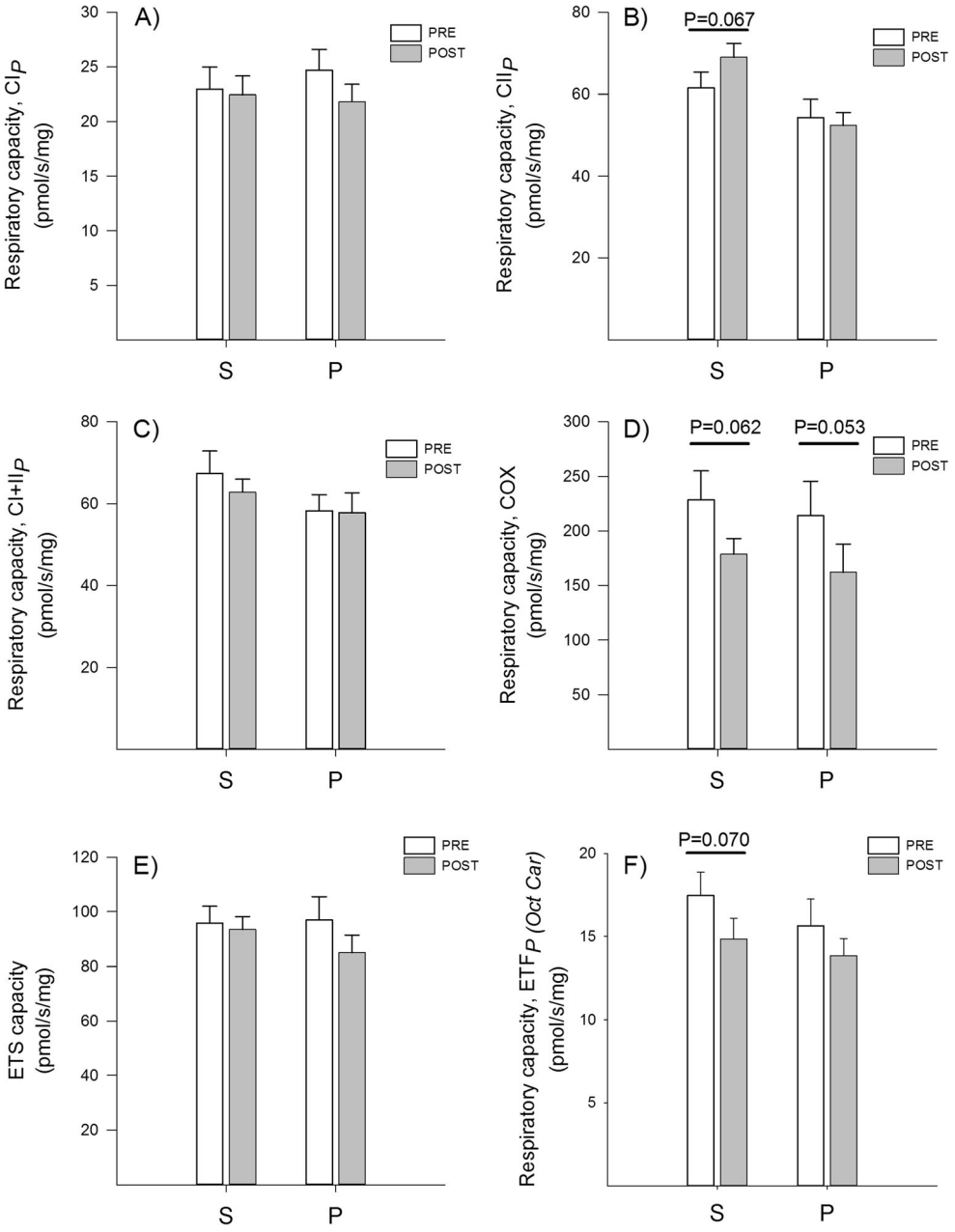

Fig. 1 Mitochondrial respiratory capacity (measured as O_2 flux rates per milligrams muscle tissue). *White bars* are before and *gray bars* are after statin treatment. The protocols are described in the Method section. *COX* cytochrome c oxidase, *ETS* electron transport system, *S* simvastatin, *P* pravastatin. Data are means ± SEM

(ROS) [4, 6], which also has been linked to impaired glucose tolerance in insulin-resistant subjects [24] and hypercholesterolemic patients [5, 25]. ROS production was not measured here, and in contrast to others [4], we found no difference in the amount of antioxidant proteins.

In line with previous studies in patients with type 2 diabetes [19] and in simvastin-treated patients [5], we found a tendency for increased sensitivity for glutamate (complex I-linked substrate) after simvastatin treatment. These groups are all characterized by an impaired glucose tolerance and elevated ROS. A high sensitivity for glutamate would be considered beneficial because it counteracts ROS elevation by facilitating oxidative phosphorylation.

It has been suggested that statin treatment reduces coenzyme Q_{10} in the electron transport system thereby reducing the electron transport capacity [2]. In the present study, however, we found no differences in skeletal muscle Q_{10} content after simvastatin treatment, which is in agreement with some [26] but not all studies [3, 5, 27].

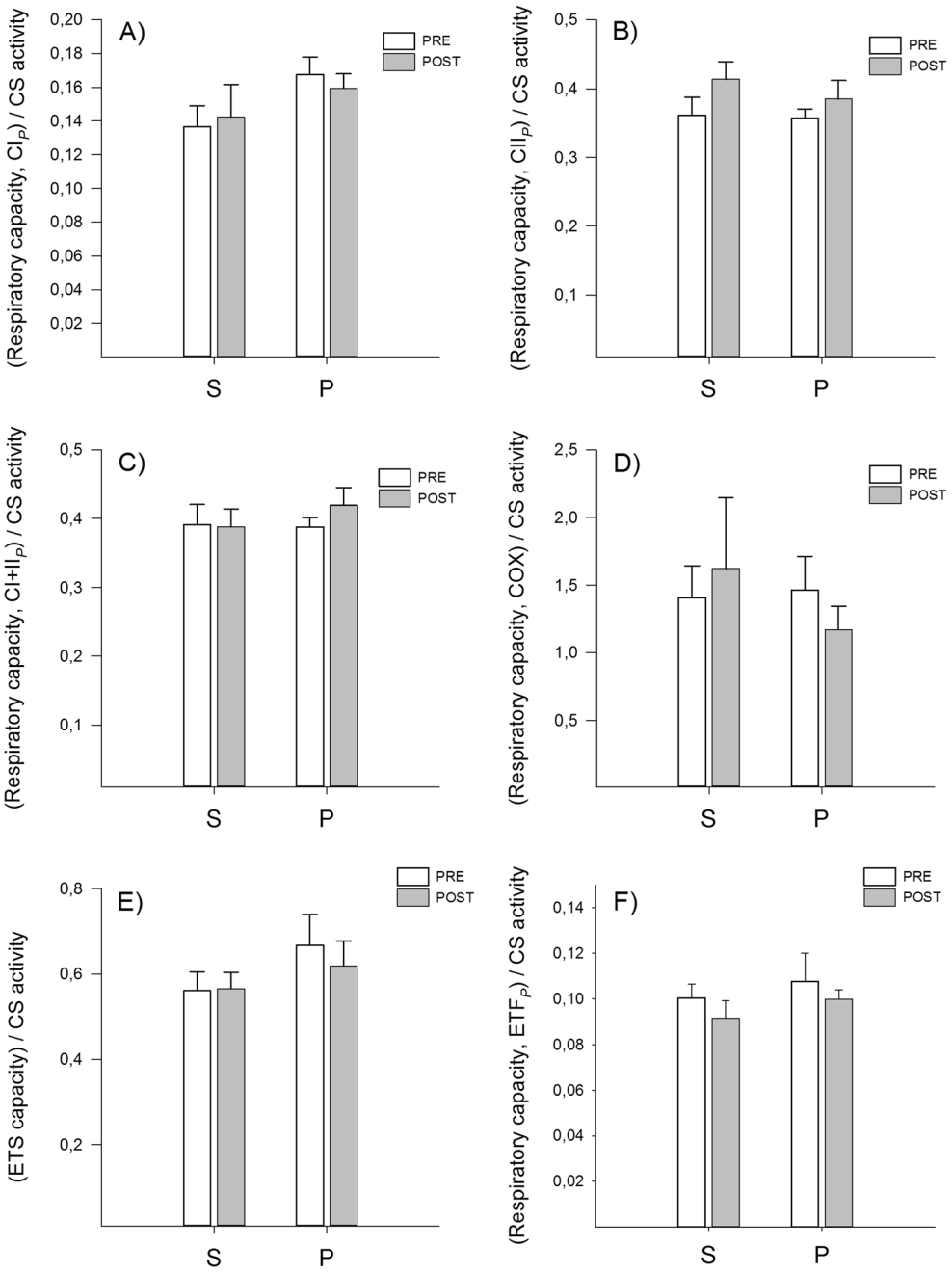

Fig. 2 Mitochondrial respiratory capacity normalized to CS activity. *White bars* are before and *gray bars* are after statin treatment. The protocols are described in the Method section. *COX* cytochrome c oxidase, *ETS* electron transport system, *S* simvastatin, *P* pravastatin. Data are means ± SEM

Fifty percent of subjects in the present study reported side effects already after 2 weeks of statin treatment, ranging from muscle soreness/myalgia after exercise to tiredness and stomachache, with no difference between S and P. This indicates that the myalgic side effects are present before they can be measured at the mitochondrial level, or that these side effects are not linked to the mitochondria (or not due to the statin treatment). Further investigations are needed to answer this.

In conclusion, we found that 2 weeks of statin treatment (simvastatin and pravastatin) did not change mitochondrial function (mitochondrial respiratory capacity, mitochondrial content, antioxidant capacity, or Q_{10} content) in healthy middle-aged subjects, but there was a tendency for a reduction in COX activity with both statins. Simvastatin may increase substrate sensitivity with complex I-linked substrates.

Table 4 Mitochondrial substrate sensitivity measurements (C_{50} and V_{max})

	C_{50}			V_{max}		
	Pre	Post	Δ change (95% CI)	Pre	Post	Δ change (95% CI)
Simvastatin						
Glutamate	1.64# (1.28; 2.00)	1.28* (0.99; 1.58)	−0.357 (−0.855; 0.141)	25.5 (20.8; 30.3)	28.9 (24.0; 33.8)	3.33 (−3.43; 10.1)
Succinate	3.41 (2.71; 4.10)	3.64 (2.85; 4.43)	0.233 (−0.744; 1.21)	52.7 (42.6; 62.8)	39.6 (31.1; 48.1)	−13.1 (−27.4; 1.24)
Oct car	35.2 (23.5; 46.9)	45.3 (28.7; 61.8)	9:05 (0.396; 17.1)	11.5 (8.56; 14.5)	9.80 (6.94; 12.7)	−1.57 (−3.76; 0.626)
Pravastatin						
Glutamate	1.17 (0.725; 1.61)	1.12 (0.851; 1.40)	0.074 (−0.339; 0.487)	26.4 (20.7; 32.1)	25.7 (21.1; 30.4)	−0.694 (−8.17; 6.78)
Succinate	4.47 (3.50; 5.45)	3.69 (2.25; 5.14)	−0.776 (−2.02; 0.464)	42.0 (29.7; 54.2)	38.9 (23.2; 54.5)	−3.13 (−22.9; 16.6)
Oct car	44.6 (21.6; 67.5)	39.0 (27.5; 50.4)	−5.59 (−33.8; 22.7)	10.1 (6.27; 14.0)	9.83 (8.13; 11.52)	−0.280 (−4.08; 3.52)

The protocols for the different mitochondrial substrate measurements are described in the Method section. C_{50} half maximal substrate concentration. C_{50} is reported in millimolar for glutamate and succinate and in micromolar for octanoyl-carnitine (oct car), V_{max} is reported in picomoles per milligram per second

Data are means with 95% confidence intervals

*$P = 0.09$ pre vs. post; #$P < 0.05$ S vs. P (pre or post)

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Author contribution NS, JWH, FD, and SL designed the study. MA, NS, DS, TLD, FD, and SL collected data and undertook analysis. MA, NS, DS, TLD, JWH, FD, and SL wrote and accepted the finale version of the manuscript.

Compliance with ethical standards The ethics committee of the municipality of Copenhagen and Frederiksberg in Denmark approved the study protocol (H-4-2009-095). Oral and written consent was obtained from each participant in accordance with the Helsinki Declaration.

Conflict of interest The authors declare that they have no conflict of interest.

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