CHAPTER 4.2

Beneficial effects of direct CoQ10 supplementation on mechanical performance of a fast-twitch mouse muscle

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Abstract

Background: Cystic fibrosis (CF) patients have decreased skeletal muscle performance and exercise capacity. Apart from diminished nutritional status and pulmonary function this altered performance seems to be caused by an intrinsic defect in skeletal muscle itself, possibly at the mitochondrial level. In respiratory chain diseases, boosting mitochondrial function through supplementation of micronutrients has proven successful.

Objective: To investigate the effect of direct administration of either vitamin E, vitamin B2, coenzyme Q10, or α-lipoic acid and carnitine to a fast twitch muscle in a transgenic mouse model of CF.

Design: Contractile function and energetic efficiency of 14 isolated, superfused fast-twitch EDL muscles from transgenic CFTR-deltaF508 (CF) mice and 14 wild type FVB controls (WT) were studied before and after direct supplementation of vitamin B2, vitamin E, coenzyme Q10, α-lipoic acid and carnitine.

Results: The CF mice had significantly lower bodyweights (22.5 ± 2.9 and 28.9 ± 7.6, respectively; $P<0.01$) and EDL muscle weights (6.5 ± 0.9 and 8.2 ± 1.9 mg, respectively; $P<0.01$) than the WT control mice. Specific isometric twitch force of intact EDL muscles isolated from CF mice was significantly higher than WT EDL muscles (1.3 fold; $P<0.05$). After direct supplementation of CoQ10, a significant improvement in mechanical performance (isometric twitch contractions during a series of subsequent contractions at 0.5 and 1.0 Hz) of 1.6-fold and 1.5-fold, respectively in WT and CF EDL, was found. No effect was seen after vitamins B2 or E, or a mixture of α-lipoic acid and acetylcarnitine.

Conclusions: Direct supplementation of CoQ10 has beneficial effects on mechanical performance of intact superfused fast-twitch mouse muscle of both wild type and transgenic CFTR-deltaF508. We propose that its effect is the result of directly boosting mitochondrial capacity for ATP synthesis through its role as cofactor in the respiratory chain. Future studies are needed to test this hypothesis as well as to investigate whether these beneficial effects can be maintained when CoQ10 is administered through dietary means in the intact animal.

Introduction

Cystic fibrosis (CF) patients often present with decreased exercise performance. The diminished nutritional status and decreased oxygen delivery due to restricted pulmonary function in CF have long been thought to cause this particular symptom of the disease (1,2,3). However, we have previously reported evidence for an intrinsic defect in skeletal muscle in CF (4). Specifically, we found a significantly reduced capacity for ATP free energy homeostasis during contractile work in children and adolescents with cystic fibrosis using phosphorus-31 nuclear magnetic resonance spectroscopic measurements of ATP metabolism in exercising forearm muscle. (4) In a follow-up study, we showed that pediatric CF patients have peripheral muscle weakness, reduced energetic efficiency of exercise and reduced maximal exercise performance, in addition to reduced muscle mass (5). Moser et al corroborated our findings of an intrinsic problem in muscle in CF patients (6). Importantly, their results too pointed towards abnormalities in oxygen (and thus energy) metabolism (6).

In vitro evidence for pathophysiological alterations in oxidative ATP metabolism in CF, and more specifically for abnormalities in mitochondria, have been found in skin fibroblasts of patients suggesting an anomaly in the mitochondrial NADH dehydrogenase complex (respiratory chain enzyme complex I) system (7). Similarly, studies in fibroblasts and leucocytes from CF patients reported mitochondrial abnormalities such as increased calcium content and lower NADH dehydrogenase activity compared to controls (8,9). In superfused intact fast-twitch muscles isolated from transgenic mice with the delta F508 mutation, the most common CF mutation in man, we found a relatively reduced capacity for oxygen consumption concomitant with diminished mechanical performance (Oudshoorn, this thesis, chapter 4.1).

In the treatment of a number of mitochondrial-linked diseases, especially those involving primary deficiencies of L-carnitine, coenzyme Q10, and cofactor- and vitamin-responsive enzyme defects, boosting mitochondrial function through supplementation of micronutrients has proven to be successful improving tissue function (10,11). Specifically, supplementation of respiratory chain enzyme cofactors such as riboflavin and coenzyme Q10 as well as free radical scavengers such as vitamin E and α-lipoic acid (with or without acetylcarnitine, a direct substrate for the Krebs cycle) have been tested with positive results (12-15). Vitamin B2 (riboflavin; VitB2) is a precursor of intramitochondrial flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), which normalizes the activities of flavin-dependent mitochondrial enzymes, acting in the β-oxidation(14,16). It also acts as cofactor to glutathione reductase, which keeps the antioxidant glutathione (GSH) in the reduced state (17). Coenzyme Q10 (CoQ10) in its reduced form (CoQ10red or ubiquinol-10) is an effective lipophilic antioxidant and protects directly against lipid peroxidation by scavenging radicals and indirectly by regeneration of the antioxidants vitamin E and vitamin C (18-21). Alternatively, in the oxidized form

(CoQ10ox; ubiquinone-10) it acts as an essential electron carrier in the respiratory chain in the mitochondrial inner membrane (22). Vitamin E (α-tocopherol; VitE) is a powerful antioxidant and is responsible for scavenging peroxyl radicals and inhibition of the production of superoxide (23,24). Lastly, α-lipoic acid is a mitochondrial cofactor and antioxidant (25).
Based on these considerations, we hypothesized that mitochondrial function and thereby muscle performance in CF can likewise be improved by supplementation of selected respiratory chain enzyme cofactors and protective agents. This hypothesis was tested in an experimental model of CFTR-deficient mouse muscle. Direct effects of the selected micronutrients on contractile performance and oxygen consumption of isolated superfused intact extensor digitorum longus (EDL) muscle of control and transgenic DF508 (CF) mice were studied using mechanical strain measurements and oxygen polarography. We found beneficial effect for CoQ10 for both wild type and CF EDL, but not for VitE and VitB2.

Methods

Animals and muscle preparation

Contractile function and energetic efficiency of 14 isolated, superfused fast-twitch (extensor digitorum longus, EDL) muscles from transgenic CFTR-deltaF508 (FVB/DF508;CF) mice and 14 sex- and age-matched wild type FVB controls (FVB;WT) were studied (both from Erasmus University laboratory, Rotterdam, The Netherlands). All experimental procedures were approved by the Committee on Animal Experiments of the University Medical Center Utrecht and complied with the principles of good laboratory animal care. Mice (aged approximately 25 weeks) were euthanized by cervical dislocation and subsequently weighed. EDL muscles of both hindlimbs were prepared free from the surrounding tissue and ligated at proximal and distal tendons with 5.0 silk suture (Ethicon, Norderstedt, Germany).

Simultaneous recording of muscle oxygen consumption and force development

EDL muscles were isolated, fixed, mounted and stimulated according to the method described by Jeneson (26). Oxygen consumption and twitch (i.e. single isometric) contraction mechanics were measured simultaneously (high-resolution oxygraph, Oroboros, Innsbruck, Austria; adjustable Harvard Apparatus 60-2995 force transducer, Harvard Instruments Limited, Edenbridge, UK; Grass S88 dual channel stimulator, Astro-med, West Warwick, RI, USA) as described by Oudshoorn et al (chapter 4.1). After 30 minutes of equilibration the measurement protocol started with 10 minutes of rest, to record basal respiratory flux, followed by 10 minutes of serial contraction stimulation at 0.5 Hz, and 10 minutes of recovery at 0.015 Hz. Subsequently, in both measuring chambers two different mixtures,

either VitE, VitB2, or CoQ10, were added and left to incubate for half an hour. Thereafter, a consecutive measuring protocol of 10 minutes each at 0.5, 1.0 and 2.0 Hz, followed by a recovery phase at 0.015 Hz for 10 minutes was performed. All measurements of muscle respiration were performed as randomized paired-experiments with simultaneous measurement of either two WT or two CF EDL muscles in a dual-chamber setup. To avoid oxygen limitation of respiration in EDL muscles at 20 ° C all measurements were performed above a PO_2 of 450 Torr (27). Chamber volume (approx. 5.3 ml) and muscle weight (blotted and tendon free) were determined after each experiment.

Direct administration of micronutrient solutions

Ringers solution (116 mM NaCl, 25.3 mM $NaHCO_3$, 4.6 mM KCl, 2.5 mM $CaCl_2$, 1.16 mM $KH2PO_4$, 1.16 mM $MgSO_4$, pH of 7.4) was prepared according to Syme (28) and placed into the measuring chambers to accommodate the EDL muscle. 0.006 ml of an α-tocopherol-TPGS (VitE) solution of 75 mg/ml (WKZ pharmacy preparation for oral administration) was added to 20 ml of Ringers solution to prepare a solution of 0.43 mg/l (0.002 mg/5.3 ml) which exceeds the plasma concentration of Vit.E in humans (7.8-33.2 μmol/l). 0.0055 gram of riboflavin (VitB2)(Fluka Biochemika, Buchs, Switzerland) was added to 10 ml Ringers to attain a solution of 0.01 mg/l (5,5 mg/5.3 ml). For the CoQ10 solution 0.0022 grams of the lipid soluble coenzyme Q10 (Sigma-Aldrich, Steinheim, Germany) was added to 4 ml ethanol 99.8% and subsequently stirred for 30 minutes to attain a solution of 10 mg/l (5.5 mg/5.3 ml). A mixture of α-lipoic acid and acetyl-L-carnitine (hereafter termed lipcar) was prepared by adding 0.00312 gram of each compound to 10 ml Ringers; solution 1.7 mg/30 ml; 0.312 mg/5.3 ml) (Sigma-Aldrich, Steinheim, Germany). Micronutrients were delivered by injection of 0.1 ml of each solution into the chambers and left to incubate for 30 minutes

Data acquisition, analysis and statistics

Oxygraph and force transducer data-acquisition was performed with LabView software (National Instruments, Woerden, The Netherlands). Mechanical performance was analyzed on a twitch-per-twitch basis with respect to five parameters: rise time, amplitude, area (tension-time integral), relaxation time and half-width (HW; in ms) using LabView subroutines. Non-linear curve-fitting analysis of the time-course of mechanical parameters during serial stimulation with respect to initial and steady-state values as well as time-constants was performed using Origin 6.0 (Microcal Software Inc., Northampton, MA, U.S.A.). Absolute muscle respiratory rates were calculated using Origin 6.0. Reported data are presented as arithmetic means ± SD. Statistical analyses were performed using a Student's unpaired t-test. Differences between CF and WT muscle were considered significant if $P<0.05$.

Table 1. Mouse characteristics of FVB/WT and FVB/DF508.

	WT (n=14)	CF (n= 14)
Age (weeks)	26.2 ± 5.4	27.7 ± 5.9
M	7	4
Bodyweight (g)	28.9 ± 7.6	22.5 ± 2.9*
EDL wet weight (mg)	8.2 ± 1.9	6.5 ± 0.9*

Mean ± SD; *P<0.01

Table 2: Mechanical characteristics of EDL muscle contraction during a single twitch, and during steady-state of serial contraction at 0.5 Hz

	WT (n=6)	CF (n= 7)
Single twitch		
rise time (ms)	11.4 ± 2.0	10.9 ± 1.9
relaxation time (ms)	61.6 ± 18.2	53.3 ± 6.8
specific force (N/g)	3.0 ± 0.6	3.8 ± 0.5†
Serial contraction		
rise time (ms)	7.7 ± 0.8	6.7 ± 1.5
relaxation time (ms)	32.3 ± 8.0	33.7 ± 7.4
half-width duration (ms)	36.9 ± 3.0	34.2 ± 4.5

Mean ± SD; †P<0.05

Table 3: Mechanical characteristics of EDL muscle single twitch contraction: FVB WT versus C57BL/6 WT

	FVB WT (n=6)	C57BL/6 WT (n= 6)
specific force (N/g)	3.0 ± 0.6	4.2 ± 0.8†
rise time (ms)	11.4 ± 2.0	12.7 ± 0.3
relaxation time (ms)	61.6 ± 18.2	97.1 ± 25.3†
half-width duration (ms)	36.9 ± 3.0	45.7 ± 2.2*

Mean ± SD; *P<0.01; †P<0.05

Results

Anatomical and mechanical properties of mice and EDL muscles

CF and WT mouse characteristics including age, sex, body and muscle mass are presented in table 1. The FVB/CF mice had significantly lower bodyweights (22.5 ± 2.9 and 28.9 ± 7.6, respectively; $P<0.01$) and EDL muscle weights (6.5 ± 0.9 and 8.2 ± 1.9 mg, respectively; $P<0.01$) than the FVB/WT control mice. Specific isometric twitch force (i.e., isometric force (N) produced in a single twitch per g muscle) of intact EDL muscles isolated from CF mice was significantly higher than WT EDL muscles (1.3 fold; $P<0.05$). No differences were found between CF and WT in either rise or relaxation time of a single twitch or of HW at steady-state during serial contraction at 0.5 Hz (Table 2). Table 3 shows twitch contraction parameters for WT FVB and WT C57BL/6 mice for comparison since our previous study in a CF mouse model was done using the latter strain (Oudshoorn, this thesis (chapter 4.1)). Data for that strain were taken from that study (Oudshoorn, this thesis (chapter 4.1)). Specific force (N/g) of isometric twitch contraction was lower for WT FVB mice than WT C57BL/6 mice. With respect to the kinetics, we found that only the rise time was the same in both groups. Both the relaxation time of a single twitch and the steady-state HW attained during serial contraction at 0.5 Hz were significantly shorter in WT FVB mice.

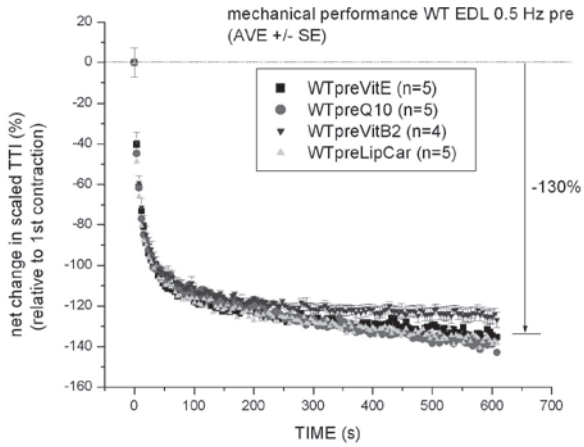
Effects of direct micronutrient supplementation on mechanical performance at 0.5 Hz

Figure 1 shows the net change in scaled tension-time-integral (sTTI) of serial twitch contractions at 0.5 Hz for WT before (pre; figure 1a) and after (post; figure 1b) micronutrient supplementation for each of the tested compounds. Figure 1a shows the previously described steep drop in sTTI directly after onset of stimulation towards a steady-state value attained within 100 s (Ter Veld et al, Pflugers Archives, in press). As can be seen, this effect was the same in each group and highly reproducible between groups. The net drop in sTTI was 130% compared to the first contraction. Figure 1b shows the same data after acute supplementation of the various tested compounds. For the VitB2, VitE and lipcar groups no effect on sTTI was found. However, for the CoQ10 group a substantial improvement in mechanical performance was found: the net drop in sTTI compared to the first contraction was 1.6-fold less than before addition of CoQ10. The same result was observed in the CF groups (Figures 2a and b). Figure 2a shows the time-course of net change in sTTI for the four CF groups before addition of micronutrients. Similarly as in WT, a net drop in sTTI of 120 to 170% compared to the first contraction was found. After supplementation, a 1.5-fold improvement in mechanical performance was found only for the CoQ10 group (Figure 2b).

Effects of direct micronutrient supplementation on twitch half-width (HW)

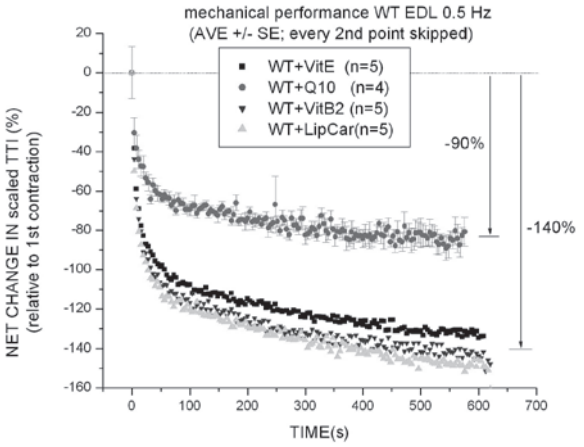
Figure 3 shows the time-course of HW of twitch contraction during serial stimulation at 0.5 Hz before and after addition for each of the micronutrient supplementation experiments

Figure 1a. The time-course of the net change in scaled tension-time integral (TTI) of twitch contractions of wild type EDL muscle stimulated at 0.5 Hz prior to the direct supplementation of vitamin E (VitE), coenzyme Q10 (Q10), vitamin B2 (VitB2), and a-lipoic acid/acetylcarnitine (lipcar) for each of group. The net change was computed by each twitch TTI scaled to the steady state TTI determined at 0.5 Hz (50-100s) and this value was subtracted from the first contraction.



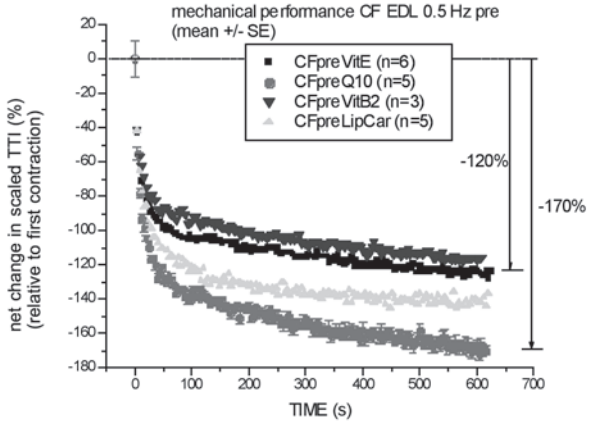
The time-course of the pre-vitB2 group is given as mean±SE and is representative for the SE of the other groups.

Figure 1b. The time-course of the net change in scaled tension-time integral (TTI) of twitch contractions of WT EDL muscle stimulated at 0.5 Hz after the direct supplementation of micronutrients for each of group. Net change in scaled TTI for WT after various additions at 0.5 Hz



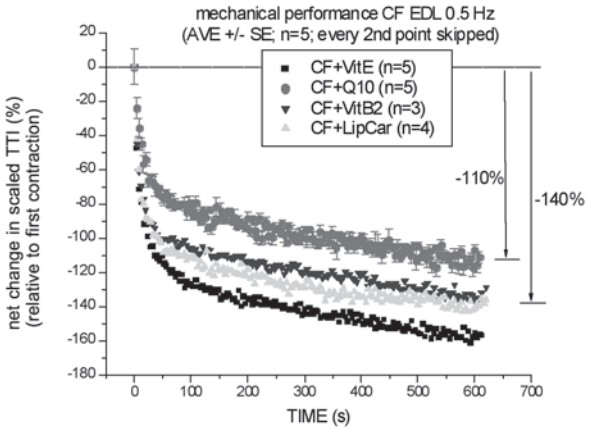
The time-course of the post-CoQ10 group is given as mean±SE and is representative for the SE of the other groups. For clarity every second point is skipped.

Figure 2a. The time-course of the net change in scaled tension-time integral (TTI) of twitch contractions of transgenic CF EDL muscle stimulated at 0.5 Hz prior to the direct supplementation of vitamin E, coenzyme Q10, vitamin B2, and a-lipoic acid/acetylcarnitine for each of group. The net change was computed by each twitch TTI scaled to the steady state TTI determined at 0.5 Hz (50-100s) and this value was subtracted from the first contraction.



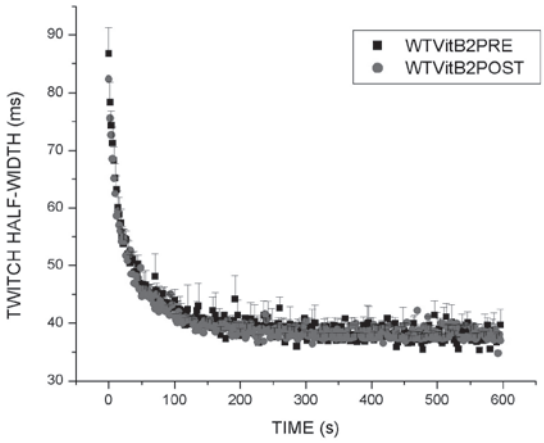
The time-course of the pre-Q10 group is given as mean±SE and is representative for the SE of the other groups.

Figure 2b. The time-course of the net change in scaled tension-time integral (TTI) of twitch contractions of CF EDL muscle stimulated at 0.5 Hz after the direct supplementation of micronutrients for each of group. Net change in scaled TTI for WT after various additions at 0.5 Hz



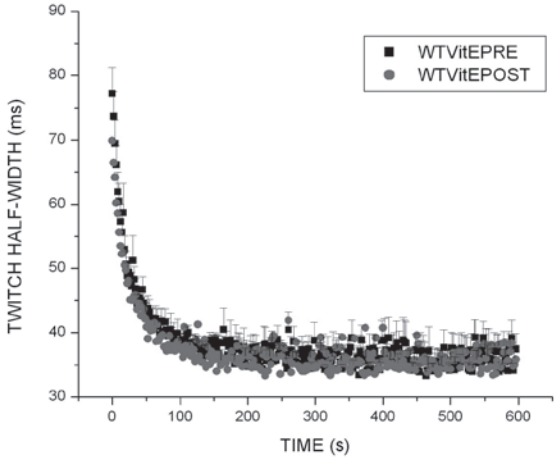
The time-course of the post-Q10 group is given as mean±SE and is representative for the SE of the other groups. For clarity every second point is skipped.

Figure 3a. The time-course of twitch half-width (in ms) of twitch contractions of WT EDL muscle during serial stimulation at 0.5 Hz prior and post direct supplementation of VitB2.



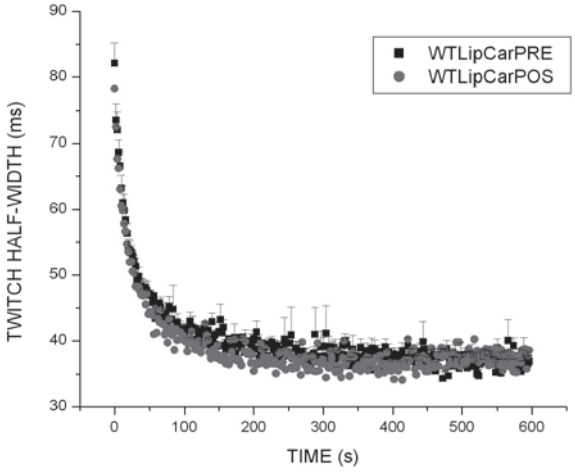
The time-course of the pre-VitB2 group is given as mean + SE and is representative for the - SE of the pre-VitB2 group and the ±SE of the post-VitB2 group.

Figure 3b. The time-course of twitch half-width (in ms) of twitch contractions of WT EDL muscle during serial stimulation at 0.5 Hz prior and post direct supplementation of VitE.



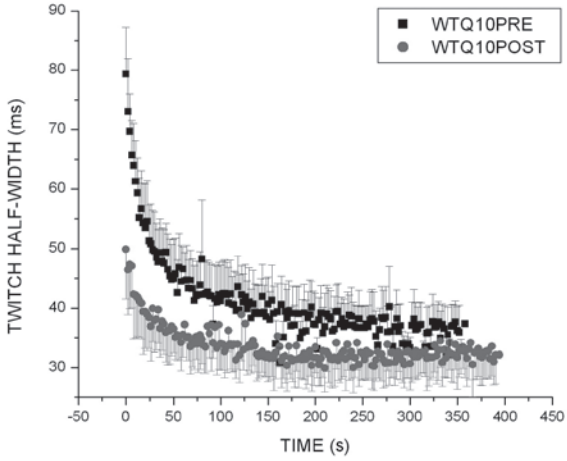
The time-course of the pre-VitE group is given as mean + SE and is representative for the - SE of the pre-VitE group and the ±SE of the post-VitE group.

Figure 3c. The time-course of twitch half-width (in ms) of twitch contractions of WT EDL muscle during serial stimulation at 0.5 Hz prior and post direct supplementation of a-lipoic acid/ acetylcarnitine.



The time-course of the pre-lipcar group is given as mean + SE and is representative for the – SE of the pre-lipcar group and the ± SE of the post-lipcar group.

Figure 3d. The time-course of twitch half-width (in ms) of twitch contractions of WT EDL muscle during serial stimulation at 0.5 Hz prior and post direct supplementation of Q10 (WT group).



For clarity the time-course of the pre-Q10 group is given as mean + SE and the post-Q10 group as - SE.

Figure 4a. The time-course of twitch half-width (in ms) of twitch contractions of CF EDL muscle during serial stimulation at 0.5 Hz prior and post direct supplementation of vitamin E.

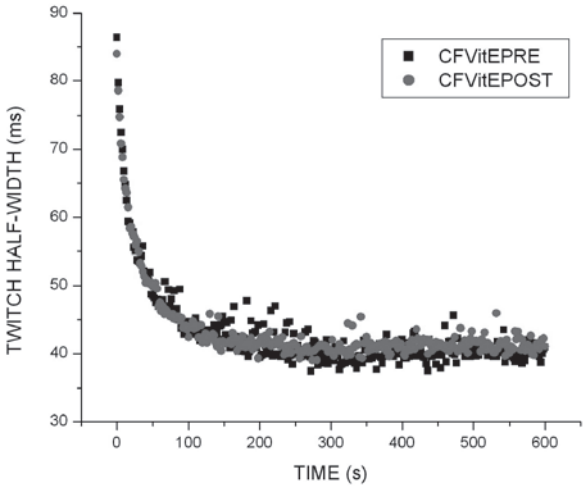
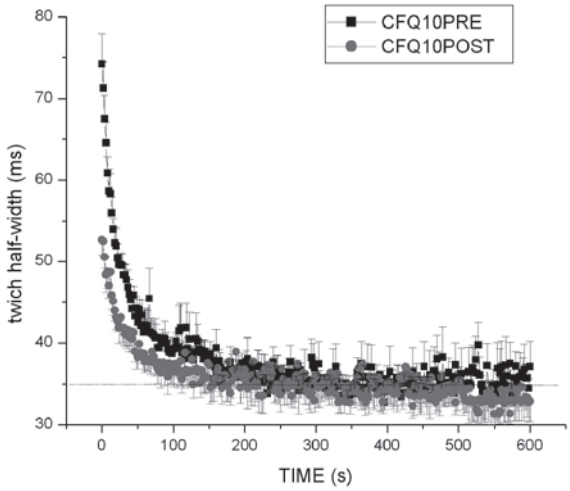


Figure 4b. The time-course of twitch half-width (in ms) of twitch contractions of CF EDL muscle during serial stimulation at 0.5 Hz prior and post direct supplementation of coenzyme Q10.



For clarity the time-course of the pre-Q10 group is given as mean + SE and the post-Q10 group as - SE.

in the WT groups. No effect was found for VitE, VitB2, or lipcar (figures 3a-c). CoQ10 did have a clear and significant effect (figure 3d). The HW of the first contraction (initial HW) post-supplementation of CoQ10 was 1.3-fold shorter than pre (50 ± 17 versus 79 ± 16 ms (mean ± SD; n=4), respectively; $P<0.05$). The same result was found in paired comparison of pre- and post-supplementation for each muscle (data not shown). Twitch HW at steady state was not significantly different between pre- and post (37 ± 9 versus 32 ± 8 ms (mean ± SD; n=4), respectively). The time-course of this parameter during serial stimulation followed a bi-exponential decay function characterized by two time constants (tau1 and tau2) (r2>0.9) and was faster post-supplementation (tau1 8.5 ± 2.8; tau2 62.4 ± 9.8 ms (mean ± SE of regression)) than pre (11.1 ± 1.4 and 106 ± 16 ms (mean ± SE of regression), respectively).
In the CF groups the result was the same. Figure 4a shows an identical time-course of twitch HW pre- and post-supplementation of VitE. A similar negative result was found for VitB2 and lipcar (data not shown). Figure 4b shows the result for supplementation of CoQ10. Analogous to the findings in WT, the HW of the first contraction post-supplementation of CoQ10 was 1.3-fold shorter than pre (53 ± 4 versus 74 ± 8 ms (mean ± SD; n=5), respectively; $P<0.01$). Twitch HW at steady state was not significantly different between pre- and post (35 ± 2 versus 33 ± 3 ms (mean ± SD; n=5), respectively). The kinetics of attaining steady-state with respect to this parameter were only slightly faster post-supplementation (tau1 23.4 ± 3.6; tau2 81.5 ± 6.5 ms versus 12.4 ± 1.2 and 132 ± 43 ms (mean ± SE of regression); post versus pre, respectively).

Effects of direct micronutrient supplementation on mechanical performance at 1 Hz
Figure 5a shows the net change in sTTI of serial twitch contractions at 1 Hz for WT post- micronutrient supplementation for each of the tested compounds. As found for the mechanical performance at 0.5 Hz (Figure 1b), the CoQ10 group outperformed the other groups. The net drop in sTTI after 10 min of contraction at 1 Hz compared to the first contraction in the series was 1.5-fold less in the CoQ10 group than any of the other groups (Figure 5a).
Figure 5b shows the results for the CF groups. A similar result was found as in WT, although the scatter in the CoQ10 group data was large: after six min of contraction at 1 Hz the net drop in sTTI was less for the CoQ10 group than for any of the other groups (Figure 5b).
Figure 5c shows a comparison of the net change in sTTI during 10 min of serial contraction at 1 Hz for the WT and CF VitE supplementation groups. In absence of any measurements prior to micronutrient delivery at this stimulation frequency, this particular comparison provided the best possible information on differences in performance during serial contraction at 1 Hz between WT and CF muscles since supplementation of VitE did not have any effect on net mechanical performance in either group (Figures 1-4). WT EDL muscles outperformed CF muscles 1.5-fold, both with respect to net drop in sTTI after 600 contractions as well as the rate of fatigue after 90 contractions (30% versus 45% and -1.5 versus -2.3 %/min, respectively; Figure 5c).

Figure 5a. The time-course of the net change in scaled tension-time integral (TTI) of twitch contractions of wild type EDL muscle stimulated at 1.0 Hz after the direct supplementation of vitamin E, coenzyme Q10, vitamin B2, and a-lipoic acid/acetylcarnitine for each of group. The net change was computed by each twitch TTI scaled to the steady state TTI determined at 0.5 Hz (50-100s) and this value was subtracted from the first contraction.

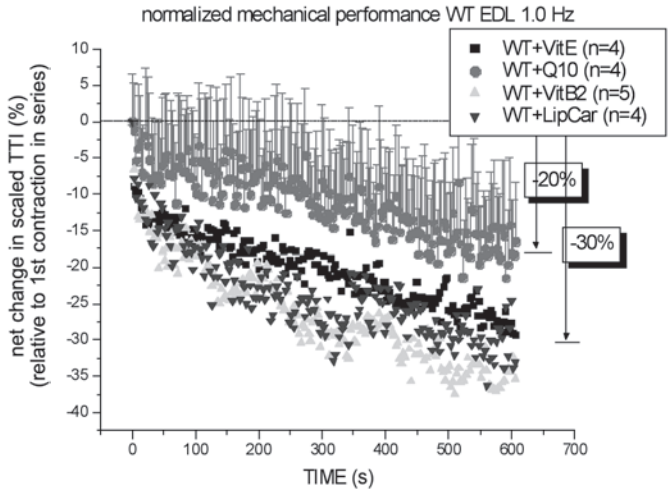


Figure 5b. The time-course of the net change in scaled tension-time integral (TTI) of twitch contractions of transgenic CF EDL muscle stimulated at 1.0 Hz after the direct supplementation of the different micronutrients for each of group. Scaled mechanical performance (TTI) for CF after various additions at 1.0 Hz

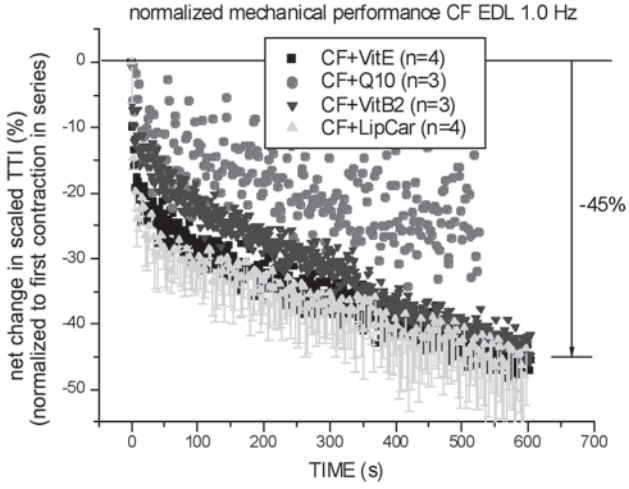


Figure 5c. The time-course of the net change in scaled tension-time integral (TTI) of twitch contractions of wild type and transgenic CF EDL muscle stimulated at 1.0 Hz after the direct supplementation of vitamin E.

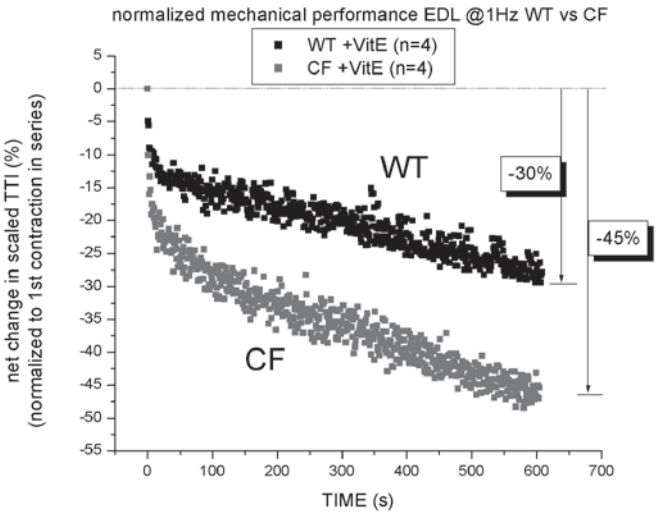
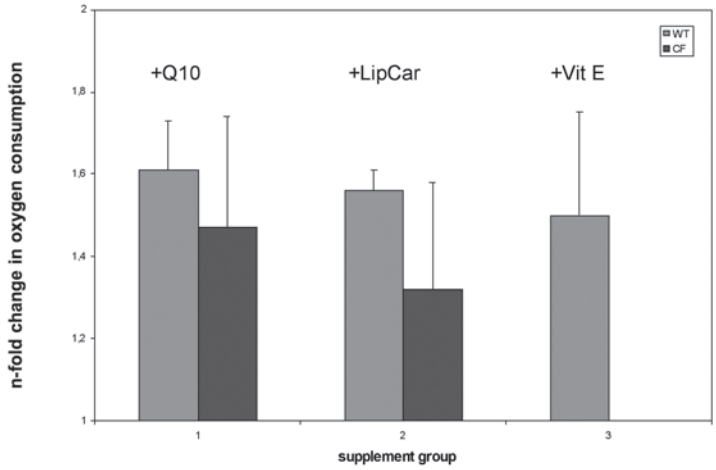


Figure 6. The net change in oxygen consumption after doubling of the serial contraction frequency from 0.5 to 1 Hz after the direct supplementation of coenzyme Q10, a-lipoic acid/ acetylcarnitine, and vitamin E for each group of WT and CF.



CoQ10 supplementation groups: WT n=2, CF n=2
Lipcar supplementation groups: WT n=3, CF n=3
Vitamin E supplementation group: WT n=2

Effects of direct micronutrient supplementation on mechanical performance: oxygen consumption

The oxygen consumption rate during serial contraction at 0.5 Hz at steady-state prior to any supplementation of micronutrient was the same in WT and CF (2.27 ± 0.54 versus 2.17 ± 0.51 nmol/s/g above basal rate (mean ± SD; n=10), respectively). Figure 6 shows the net change in oxygen consumption after doubling of the contraction frequency from 0.5 to 1 Hz for the various micronutrient supplementation groups for WT and CF. In the WT groups, oxygen consumption increased 1.6-fold after doubling of the contraction frequency (Figure 6). This result was the same as we previously found (Oudshoorn, this thesis (chapter 4.1)). For the CF groups, oxygen consumption data at 0.5 and 1 Hz were only available for two micronutrient supplementation groups (i.e., CoQ10 and lipcar). The difference observed as compared to WT was NS (Figure 6).

Discussion

We found that direct supplementation of CoQ10, but not vitamins B2 or E, nor a mixture of α-lipoic acid and acetylcarnitine, improves mechanical performance of serially stimulated superfused intact fast-twitch muscle isolated from both wild-type FVB mice and a transgenic strain with the DeltaF508 CF mutation. This result together with its mechanistic basis and possible implications for clinical management of human CF patients presenting with reduced exercise performance are discussed.

Mouse model and experimental design of the study

The present study was done in a different mouse strain than our previous study (FVB versus C57BL/6, respectively; Oudshoorn, this thesis, Chapter 4.1). Although we found the same abnormalities in the FVB CF phenotype with respect to body weight (Table 1) and EDL muscle weight and specific force (Table 2) that we first reported for the C57BL/6 CF phenotype (Oudshoorn, this thesis, Chapter 4.1), there were a number of phenotypic strain differences for the WT FVB and C57BL/6 EDL muscles (Table 3) indicating that EDL muscle in FVB mice is faster than in the C57BL/6 strain. Specifically, the rate of EDL relaxation following isometric twitch contraction (and, as such, half-width duration of a twitch contraction) for the FVB strain was 1.3-fold faster than for C57BL/6; rise time was unaltered (Table 3). Surprisingly, specific force of FVB EDL muscle was 1.3-fold lower than for C57BL/6. These findings suggested that in FVB EDL muscle either total calcium released upon a single stimulation is reduced or calcium buffering and/or resequestration is increased compared to C57BL/6. These apparent phenotypic strain differences influenced the present study as FVB EDL muscles were found to have a significantly more limited operational range of

mechanical performance compared to C57BL/6. Whereas WT EDL muscles of the latter strain show significant fatigue only at stimulation frequencies of 2 Hz and higher (Ter Veld et al., Pflugers Archives, in press), WT FVB EDL muscle already showed significant fatigue at 1 Hz (Figure 5c). This was even more evident in the CF group. As such, the experimental design used in our previous study, measuring EDL mechanical and concomitant mitochondrial performance during two consecutive doublings of stimulation frequencies between 0.5 and 2 Hz (Oudshoorn, this thesis, chapter 4.1) could not be used here. At 1 Hz, the high rate of fatigue (and resulting drop in ATP utilization rate) in the CF group subdued any ancillary activation of mitochondrial respiration in the vitamin B2 and E groups, if present at all (Figure 6).
The design of the present study – i.e. to test the effects of direct supplementation of micronutrients to isolated EDL muscles, rather than dietary administration in intact animals, similar to the approach taken in human patients (Oudshoorn, this thesis), was chosen for the following reason: we have previously found significant, acute effects of supplementation of various oxidative substrates (i.e., glucose, lactate and pyruvate) on mitochondrial respiration and cytosolic ATP free energy potential in unstimulated mouse EDL and SOL muscles using this exact same experimental set-up and design of compound delivery (Wiseman RW, Jeneson JAL and Kushmerick MJ, unpublished results). The significant, direct effects of CoQ10 supplementation on TTI and HW in both the WT and CF groups found in the present study confirmed that these small molecules are indeed rapidly taken up by the muscle-cells, reach the mitochondrial compartment and exercise their molecular function. Therefore, the lack of significant effects of the other compounds tested should be more likely attributed to lack of any significant metabolic effect than to a failure for these molecules to reach the mitochondria. As we now have determined the direct effects, future studies should test if the observed effects for CoQ10 can also be found after dietary administration.

Direct effects of micronutrient supplementation on EDL mechanical performance during serial stimulation

The main finding of the present study was that direct supplementation of CoQ10, but not vitamins B2 or E, nor a mixture of α-lipoic acid and acetylcarnitine, improved mechanical performance of serially stimulated superfused intact fast-twitch muscle isolated from both wild-type FVB mice and a transgenic strain with the DeltaF508 CF mutation. This conclusion was based on the observation that the net drop in TTI of isometric twitch contractions during a series of subsequent contractions relative to TTI of the first contraction in the series was 25% less after supplementation of CoQ10 (Figures 1d and 2b). This effect was absent for each of the other tested compounds (Figures 1a-c and Figure 2a).
Further analysis of individual twitch contractions with respect to amplitude and rise- and relaxation kinetics revealed the major cause of this improved maintenance of TTI. It was found that after CoQ10 supplementation, the typical biphasic, dramatic acceleration of muscle relaxation during the first two minutes of serial stimulation reflected in a biphasic

drop of isometric twitch duration from 80 to 45 ms (e.g., Figures 3 and 4, pre-supplementation groups) was considerably blunted. Specifically, the initial twitch HW post-CoQ10 supplementation was on average 30 ms shorter than prior to supplementation in both the WT and CF groups (Figures 3d and 4b). Furthermore, curve-fit analysis of the HW time-course with respect to the time constants of the bi-exponential decay showed that a steady-state with respect to this contraction parameter post-CoQ10 supplementation was more swiftly attained than pre. This was more pronounced in the WT group than in CF (Figures 3d and 4b).

We have only once before made a similar observation. In a previous study of intact EDL muscles isolated from transgenic creatine kinase knock-out (CK k.o.) mice in a C57BL/6 strain, it was likewise found that the net drop in TTI after the first contraction in a series was heavily blunted compared to WT (Ter Veld et al., Pflugers Archives, in press). We analyzed the time course of twitch HW during serial stimulation at 0.5 Hz for these muscles and found that initial HW in the CK k.o. group was 50 ms and remained constant throughout the entire series of stimulations, whereas in WT initial HW was 65 ms and typically dropped to 45 ms (data not shown). This suggests that identification of a common factor in the remodeled CK k.o. EDL muscle and the CoQ10-supplemented EDL muscle could lead us to the mechanistic basis for the faster EDL relaxation observed in the latter group.

It has been well documented that fast-twitch skeletal muscle of CK k.o. mice has an almost twofold higher mitochondrial density than WT and, as such, a twofold higher Vmax of respiration (29). In addition, it has been reported that chronic CoQ10 supplementation to human patients diagnosed with muscle disease caused by mitochondrial dysfunction likewise increased Vmax of respiration and ATP synthesis (30). Taken together, an explanation of the observed effects of CoQ10 supplementation on EDL relaxation after contraction in the present study could be the following: CoQ10 increases Vmax of respiration, resulting in a higher 'gain' of mitochondrial ATP utilization sensing (through detection of alterations in cytosolic ADP concentration)(31). During a twitch, one and the same amount of ADP produced by ATP hydrolysis by the actomyosin ATPase and sarcoplasmic reticular calcium pumps (SERCa) therefore results in a higher ATP synthesis rate supporting faster SERCA calcium recovery and thereby faster relaxation of the muscle. A consequence of this mechanism is that CoQ10 would need to function as an electron donor rather than free radical electron scavenger. CoQ10 is found in the inner membrane of the mitochondria first tightly bound to NADH (complex I), where four protons are released in a reductive cycle, before electrons are transferred to a second loosely bound CoQ10 to form CoQ10red. This can travel through the lipid in the membrane to complex III where the CoQ10red is oxidized again via an oxidation-reduction cycle, allowing four protons to cross the membrane for each CoQ10red oxidation cycle (19).

There is an important implication with respect to striated muscle physiology of this hypothesis for the mechanistic basis of the measured acute CoQ10 effect. In smooth muscle, it has been argued that cation pumps such as the sarcolemnal Na-K-ATPase and SERCa are

preferentially driven by ATP derived non-oxidatively from glycogenolysis (32). It has been further argued that such compartmentation should also be physiologically relevant in striated muscle. However, the proposed mechanistic explanation for the faster muscle relaxation following CoQ10 supplementation entails that SERCa pumps are driven by mitochondrial ATP. If proven correct, this would refute any preferential non-oxidative ATP supply of the SERCa pumps. Testing of this proposed mechanism for faster calcium recovery following CoQ10 supplementation can be performed by determining whether the beneficial effect of CoQ10 can be reversed by inhibition of mitochondrial respiration at complex III or IV using standard inhibitors such as antimycin and cyanide, respectively. The oxygen consumption measurements in the present study unfortunately did not provide data of sufficient quality to test this hypothesis by means of comparing oxygen consumption rate pre and post-CoQ10 supplementation. The ratio of the steady-state rates measured during serial stimulation at 0.5 and 1 Hz (Figure 6) likewise did not provide any information on this question. Although the ratio of 1.6 for the WT oxygen consumption rates at 1 versus 0.5 Hz was identical to our previous study (Oudshoorn, this thesis, chapter 4.1), as a result of the high fatigue rate of FVB CF EDL muscles stimulated at 1 Hz (see above) there was insufficient oxygen consumption data to draw any conclusion regarding the effects of micronutrient supplementation on mitochondrial function.

In summary, we have found a direct effect of CoQ10 supplementation on mechanical performance of intact superfused fast-twitch mouse muscle. We propose that this beneficial effect is the result of directly boosting mitochondrial capacity for ATP synthesis through its role as enzyme cofactor in the respiratory chain. Future studies are needed to test this hypothesis as well as to investigate whether these beneficial effects can also be obtained through dietary administration.

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