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Original Contribution

Effects of vitamin C, vitamin E, zinc gluconate, and selenomethionine supplementation on muscle function and oxidative stress biomarkers in patients with facioscapulohumeral dystrophy: A double-blind randomized controlled clinical trial

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ABSTRACT

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disease characterized by progressive weakness and atrophy of specific skeletal muscles. As growing evidence suggests that oxidative stress may contribute to FSHD pathology, antioxidants that might modulate or delay oxidative insults could help in maintaining FSHD muscle function. Our primary objective was to test whether oral administration of vitamin C, vitamin E, zinc gluconate, and selenomethionine could improve the physical performance of patients with FSHD. Adult patients with FSHD ($n=53$) were enrolled at Montpellier University Hospital (France) in a randomized, double-blind, placebo-controlled pilot clinical trial. Patients were randomly assigned to receive 500 mg vitamin C, 400 mg vitamin E, 25 mg zinc gluconate and 200 μg selenomethionine ($n=26$), or matching placebo ($n=27$) once a day for 17 weeks. Primary outcomes were changes in the two-minute walking test (2-MWT), maximal voluntary contraction, and endurance limit time of the dominant and nondominant quadriceps (MVC_{QD}, MVC_{QND}, T_{limQD}, and T_{limQND}, respectively) after 17 weeks of treatment. Secondary outcomes were changes in the antioxidant status and oxidative stress markers. Although 2-MWT, MVC_Q, and T_{limQ} were all significantly improved in the supplemented group at the end of the treatment compared to baseline, only MVC_Q and T_{limQ} variations were significantly different between groups (MVC_{QD}: $P=0.011$; MVC_{QND}: $P=0.004$; T_{limQD}: $P=0.028$; T_{limQND}: $P=0.011$). Similarly, the vitamin C ($P<0.001$), vitamin E as α-tocopherol ($P<0.001$), vitamin C/vitamin E ratio ($P=0.017$), vitamin E γ/α ratio ($P=0.022$) and lipid peroxides ($P<0.001$) variations were significantly different between groups. In conclusion, vitamin E, vitamin C, zinc, and selenium supplementation has no significant effect on the 2-MWT, but improves MVC_Q and T_{limQ} of both quadriceps by enhancing the antioxidant defenses and reducing oxidative stress. This trial was registered at clinicaltrials.gov (number: NCT01596803).

Abbreviations: FSHD, facioscapulohumeral dystrophy; Dux4, double homeobox 4 gene; ROS, reactive oxygen species; CuZn-SOD, copper–zinc-dependent superoxide dismutase; GSH-Px, glutathione peroxidase; GSH tot, total glutathione; GSH, reduced glutathione; GSSG, oxidized glutathione; Ab-Ox-LDL, antibodies (IgG) against oxidized LDL; MVC_{QD} or $_{QND}$, maximal voluntary contraction of the dominant or nondominant quadriceps; T_{limQD} or $_{limQND}$, endurance limit time of the dominant or nondominant quadriceps; 2-MWT, two minutes walking test

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Introduction

Facioscapulohumeral muscular dystrophy (FSHD) is the most common inherited skeletal muscle disease of adult life with a prevalence of 4/100,000 in Europe (http://www.orpha.net/orphacom/cahiers/docs/GB/Prevalence_of_rare_diseases_by_alphabetical_list.pdf). It is characterized by progressive weakness and atrophy of facial, shoulder girdle, and upper arm muscles [1,2]. Magnetic resonance imaging also revealed widespread involvement of leg muscles, particularly of the tibialis anterior and medial gastrocnemius [3]. Approximately 10% of all patients and 20% of those older than 50 years will eventually become wheelchair-dependent for outdoor activities. The pattern of muscle weakness is often asymmetrical and the rate and extent of progression may vary considerably with sudden periods of unexplained rapid disease progression [2,4].

FSHD is an autosomal dominant disease and is genetically linked to deletions in chromosome 4q35 [5] within an array of D4Z4 repeats. Each D4Z4 repeat unit includes the open reading frame of double homeobox 4 (*DUX4*) [6,7], a transcription factor that becomes derepressed in FSHD skeletal muscle cells. *DUX4* controls many genes involved in the oxidative stress response and myogenesis, potentially leading to muscle atrophy, differentiation defects, and activation of germline genes [8–12]. Recently, it was shown that mutations in the structural maintenance of the chromosomes flexible hinge domain containing 1 (*SMCHD1*) gene, which encodes a chromatin modifier of D4Z4, could act as a disease severity modifier in families affected by FSHD [13,14]. Despite major progress in the understanding of the genetic basis of FSHD, the exact mechanisms that lead to FSHD defects are not completely understood and no curative treatment is available. However, there is growing evidence that oxidative stress may contribute to FSHD pathology. The hypothesis that oxidative stress responses might be specifically altered in FSHD is supported by the deregulation of enzymes involved in oxidative stress and the consequent increased susceptibility to oxidative agents observed in primary FSHD myoblasts [15–19].

Moreover, we recently reported that reduced physical performance in patients with FSHD is associated with important redox unbalance and oxidative stress in blood [20]. Specifically, patients had higher levels of oxidized DNA and significantly more elevated lipid peroxides levels compared to the control group. The ratio between reduced (GSH) and oxidized glutathione (GSSG) was also strongly decreased in all blood samples from patients with FSHD compared to controls. Although no significant difference was found between FSHD and control blood samples concerning superoxide dismutase (CuZn-SOD) and glutathione peroxidase (GSH-Px) levels, patients had significantly lower levels of zinc (a SOD cofactor), selenium (a GSH-Px cofactor involved in the elimination of lipid peroxides), and vitamin C. Hence, we hypothesized that insufficient intake of antioxidant vitamins and minerals may reduce the body capacity to regulate free radical insults, leading to a condition known as oxidative stress that could affect muscle function performance in patients with FSHD. We thus conducted a pilot randomized double-blind placebo-controlled trial to test whether oral administration of vitamins and minerals could improve the physical performance (2-minute walking test, maximal voluntary contraction, and endurance limit time test of quadriceps of patients with FSHD. Vitamin E (as alpha tocopherol), vitamin C, selenium, and zinc were chosen for this supplementation due to their physiological role in antioxidative processes [21].

Materials and methods

Study design and patients

For this randomized double-blind placebo-controlled study, we recruited adults with FSHD at the Clinical Physiology Department, Montpellier University Hospital (France), between May 2010 and April 2012. Inclusion criteria were between four and nine D4Z4 repeat units and positive family history for FSHD; age between 18 and 60 years; no HIV and/or hepatitis. Exclusion criteria included confinement to a wheelchair, smoking, concomitant comorbidity (cardiac or pulmonary diseases, diabetes, etc.), or being on medication (including mineral or vitamin supplement and /or other antioxidants). The study was approved by the institutional review board and by ANSM (French Health Products Safety Agency). The trial objectives, study design, risks, and benefits were explained and written informed consent was obtained from all participants.

Supplementation

The supplement choice was based on our previous finding [20] that most patients with FSHD have higher levels of oxidative damage (specifically higher lipid peroxide and oxidized DNA levels), significantly lower GSH/GSSG ratio values (as a consequence of GSSG accumulation), and significantly lower concentrations of vitamin C and essential elements (particularly selenium and zinc) than healthy controls. Vitamin C, the major water-soluble antioxidant, and vitamin E (a lipid-soluble vitamin found in cell membranes and circulating lipoproteins) were selected due to their protective effects against lipid peroxidation in humans [22–24]. Alpha tocopherol is the vitamin E form that is preferentially absorbed and accumulated in humans [25]. Zinc and selenium were selected because lower than normal selenium and zinc levels have been detected in most patients with FSHD and to protect against oxidative stress [20]. The importance of maintaining adequate levels of zinc and selenium is emphasized by studies indicating that low antioxidant status may be associated with increased risk of developing various diseases [26–28]. Selenium was given as selenomethionine because its bioavailability is nearly twice that of selenium as selenite [29]. Zinc gluconate was used because it is absorbed more efficiently [30].

Safe dose ranges were determined based on the recommended dietary allowance (RDA) and the tolerable upper intake levels (UL) for vitamins E and C and selenium [31]. The daily dose of zinc gluconate (25 mg zinc gluconate providing 3.5 mg elemental zinc) was based on the internationally recommended daily intake [32] and it is below the RDA recommendations, because it is difficult to determine the level of zinc intake due to the lack of sensitive indicators of zinc nutritional status in humans.

Randomization and masking

Patients were randomly allocated (1:1) to receive 500 mg vitamin C, 25 mg zinc gluconate, 200 μg selenomethionine, and 400 mg *dl*-alpha tocopheryl-acetate per day or matching placebo (microcrystalline cellulose). Both were given in encapsulated form (120 capsules per bottle) to ensure that they were indistinguishable and both were provided by CRID PHARMA (France), which was responsible for the quality control of all clinical product batches. All patients were instructed to take one vitamin C capsule at breakfast, one zinc capsule 1 h after the vitamin C, one selenium capsule 3 h after the vitamin C (to avoid interactions between antioxidants), and one vitamin E (as alpha tocopherol) capsule after dinner. Pills were taken with food to avoid potential stomach irritation.

Patients' randomization was computer generated with permuted blocks of four and stratified based on the baseline maximal voluntary contraction of the dominant quadriceps (MVC_{QD}) ($>$ or ≤ 5 kg). Individual sealed envelopes containing the treatment allocation were given to the trial pharmacist to allow disclosing the treatment group in case of clinical emergency. All personnel