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Dietary supplementation with multiple micronutrients: No beneficial effects in pediatric cystic fibrosis patients

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Abstract

Background: Cystic fibrosis (CF) patients are subjected to increased oxidative stress due to chronic pulmonary inflammation and recurrent infections. Additionally, these patients have diminished skeletal muscle performance and exercise capacity. We hypothesize that a mixture of multiple micronutrients could have beneficial effects on pulmonary function and muscle performance.
Methods: A double-blind, randomized, placebo controlled, cross-over trial with a mixture of multiple micronutrients (ML1) was performed in 22 CF patients (12.9±2.5 yrs) with predominantly mild lung disease. Anthropometric measures, pulmonary function, exercise performance by bicycle ergometry, muscular strength and vitamins A and E were determined.
Results: Analysis was performed using the paired Student *t*-test comparing the change in each parameter during ML1 and placebo. Plasma vitamin E and A levels increased during ML1 when compared to placebo. However, no significant difference between the effect of the ML1 or placebo was observed neither for FEV_1, FVC, anthropometry, nor for the parameters for muscle performance.
Conclusions: The micronutrient mixture was not superior to placebo with respect to changes in pulmonary function or muscle performance in pediatric CF patients, despite a significant increase in plasma vitamin E concentrations.

Keywords: Micronutrients; Children; Cystic fibrosis

1. Introduction

Cystic fibrosis (CF) is an autosomal recessive inherited disorder primarily characterized by chronic pulmonary infection and inflammation [1,2]. During active infection neutrophils and macrophages generate reactive oxygen species (ROS), which are necessary for bacterial killing, but may be harmful when tissue antioxidant capacity is exceeded [3]. Despite routine supplementation with fat soluble vitamins, such as the antioxidants vitamin E and carotenoids, biochemical markers for a disturbed oxidant–antioxidant balance remain present in many CF patients [4–7]. Therefore, several studies concerning micronutrient supplements have been performed in CF patients, using various amounts of α-tocopherol, retinol, ascorbic acid, and carotenoids. These studies have shown beneficial effects on

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Table 1
Composition of the micronutrient mixture ML1

		100 ml			100 ml
Energy value	kcal	80	Protein (casein:whey = 1:1)	g	6.0
				En%	30
	kJ	335	Carbohydrates (lactose,	g	7.1
			saccharose, organic acids)	En%	35
			Fat (linoleic acid 0.8)	g	3.1
				En%	35
Minerals:			Vitamins:		
Na	mg	43	A	µg RE [a]	267
K	mg	300	D	µg	2.0
Cl	mg	81	C (ascorbic acid)	mg	100
Ca	mg	126	E (α-tocopherol)	mg	215
P	mg	73	B1 (thiamine)	mg	10
Mg	mg	8	B2 (riboflavin)	mg	1.2
			B3 (pantothenate)	mg	1.6
			B5 (niacin)	mg NE [b]	8.0
			B6 (pyridoxine)	mg	2.4
			B11 (folic acid)	µg	240
			B12 (cobalamin)	µg	1.2
			Biotin (vit H)	µg	40
Trace elements:			Others:		
Fe	mg	0.4	Carnitine	mg	1200
Zn	mg	6.0	Choline	mg	40
Cu	mg	0.6	Creatine	mg	1200
Mn	mg	1.2	Taurine	mg	1200
F	mg	0.4	Coenzyme Q10	mg	60
Mo	µg	20			
Se	µg	20			
Cr	µg	13			
I	µg	40			

[a] Retinol equivalents.
[b] Niacine equivalents.

oxidant–antioxidant imbalance, but an inconsistent effect has been reported for pulmonary function [8–12].

Physical exercise programs can improve exercise tolerance and pulmonary function in CF patients, mainly as a result of increased muscle mass [13,14]. However, it turned out to be extremely difficult for the participants in these studies to maintain the increased exercise training, and just a few still complied after one year. A nutritional supplement might therefore be an important addition to exercise to optimize muscle strength. As positive effects of creatine, carnitine and taurine on muscle mass and/or performance in athletes have been described [15–17] an increased intake of these components might also be associated with an improved exercise tolerance in CF patients.

Since CF is characterized by both oxidative stress and poor exercise tolerance, we hypothesized that children with CF might benefit from a mixture of micronutrients with antioxidant and muscle fortifying action. It was anticipated that initial effects of this mixture, such as improvement of oxidant–antioxidant balance and muscle performance, could be observed within the study period of three months [11,15,16], while for other parameters, such as FEV_1, a favorable trend might become detectable within the same timeframe.

2. Methods

2.1. Subjects and study design

Thirteen boys and sixteen girls with CF aged 9.8–18.9 years (mean 13.3 yrs) with predominantly mild pulmonary disease were recruited from the CF centre of the Wilhelmina Children's Hospital, University Medical Centre Utrecht. Included were children aged 9 to 18 years with a stable clinical condition (*i.e.*, no need for oral or IV antibiotic treatment other than chemoprophylaxis in the two months prior to testing), absence of musculoskeletal disorders and an FEV1 of ≥ 70% predicted. The study protocol was approved by the UMC Medical Ethics Committee, and each participant and/or the parents gave informed consent. In a cross-over design each participant received once daily 100 ml of a liquid micronutrient mixture (ML1) for three months, or 100 ml placebo with a wash-out period of three months. The composition of ML1 is described in Table 1.

The placebo tasted the same, contained identical amounts of protein, carbohydrates and fats, but lacked the micronutrients. Four female and two male participants (three on placebo and three on ML1) dropped out of the study during the first few weeks because of unpleasant taste of the mixture. One boy did not complete the entire study due to the emotional impact of a non-study CF related complication. One participant could not perform the bicycle tests at the measurements after both ML1 and placebo, first because of a soccer injury and secondly because of a broken leg.

Because of small stature two participants performed a standardized treadmill test (endurance) instead of the bicycle tests [18]. Finally, 22 patients, with a mean age of 12.9±2.5 years, were available for analysis. The clinical characteristics of these 22 subjects are described in Table 2.

Table 2
Baseline clinical characteristics of the 22 remaining CF patients[1,2]

	Subjects (n=22)
Male : Female	10: 12
Age (years)	12.9±2.5
Height (m)	1.51±0.12
(in SDS)	(−0.9±1.0)
Weight (kg)	42.2±12.6
(Weight-for-Height in SDS)	(−0.01±1.0)
BMI (kg/m^2)	18.0±3.1
(in SDS)	(−0.3±1.2)
FFM (kg)	28.8±7.8
Skinfolds (sum of 4; in mm)	34.6±18.4
FEV_1 (ml)	2130±584
FEV_1 (% predicted)	87.2±17.0
FVC (ml)	2745±680
Isometric muscle force (sum of 6; in N)	1244±241

[1]Values given as mean±SD.
[2]BMI; body mass index, FFM; fat free mass calculated from four standard skinfold measurements, FEV_1; forced expiratory volume in one second, FVC; forced vital capacity, isometric muscleforce; summed maximal force in six muscle groups.

Table 3
Baseline results for anaerobic and aerobic performance[1,2]

	Subjects (n=22)
Anaerobic performance	
Ppeak (Watt)	538±236
Ppeak/FFM (Watt/kg LBM)	15.0±3.5
Pmean (Watt)	312±129
Pmean/FFM (Watt/kg LBM)	8.7±1.7
Aerobic performance	
Wmax (Watt)	139±41
VO_2max (ml/min)	1581±433
VO_2max (ml/kg/min)	38±6

[1]Values given as mean±SD.
[2]Ppeak; peak power measured by WAnT, Pmean; mean power measured by WAnT, LBM; lean body mass, Wmax; cycling maximal workload, VO_2max; maximal oxygen consumption during bicycle ergometry.

2.2. Nutritional assessment

Anthropometric measurements were acquired prior to exercise testing. Bodyweight was measured using a platform beam balance (Mettler, Greifensee, Switzerland). Height was measured with a stadiometer (Holtain, Crymich, UK). Body mass index (BMI) was calculated as weight/height^2. Body composition, i.e. fat-free mass (FFM), was determined by calculation from the sum of four standard skinfold-thickness measurements [19–21].

2.3. Pulmonary function tests

Pulmonary function tests were performed after administration of 800 μg of salbutamol, in order to rule out important bronchial hyperreactivity. Forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1) were obtained from maximal expiratory flow–volume curves (Masterscreen, Jaeger/Viasys, Hochberg, Germany). Values are expressed as percent of predicted (pred) values as compared to normal values for age and gender [22].

2.4. Peripheral muscle strength

Isometric muscle force was measured for six muscle groups on the non-dominant side of the patient, using a hand-held dynamometer (Penny and Giles, Christchurch, UK). Maximal voluntary force of the shoulder abductors, elbow flexors, wrist extensors, hip and knee extensors, and ankle flexors were measured as described by Backman et al [23]. Each muscle group was tested three times, and the highest value obtained was reported. Results for peripheral muscle force are presented as the total maximal force (*i.e.*, summed maximal force in six muscle groups).

2.5. Exercise testing

Subsequent anaerobic and aerobic exercise tests were performed on an electronically controlled resistance cycle ergometer (Lode Examiner, Lode, Groningen, The Netherlands) as described by Klijn et al [24,25]. The Wingate anaerobic test (WAnT) was used to assess short-term anaerobic power. The WAnT comprises two parameters: peak power (Ppeak; highest power during the test) and mean power (Pmean; power averaged over 30 s). The subjects rested for at least 45 min before aerobic fitness was assessed by a standard progressive incremental exercise test (Wmax; endurance). All subjects were familiar with the test equipment. Verbal encouragement was given throughout the tests to stimulate maximal performance.

2.6. Laboratory assays

2.6.1. Malondialdehyde

In the fasting state, prior to exercise testing, a venous blood sample was drawn for malondialdehyde, vitamin E (α-tocopherol) and A (retinol) measurement.

Malondialdehyde (MDA) levels were determined fluorimetrically (Fluostar Galaxy, BMG, Germany) by using the TBARS (Thio Barbituric Acid Reactive Substances) method according to Wasowics et al [26]. Serum concentrations of α-tocopherol and retinol were determined simultaneously by the standard reversed phase HPLC method used in our hospital laboratory.

2.7. Data analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS, version 11.0 for Windows, Chicago, IL). Since a cross-over design was used, and the results were normally distributed, a simple paired Student *t*-test was used. Data are presented as mean±SD.

3. Results

Baseline values of both groups were comparable. No seasonal nor carry-over effect was seen in the groups starting

Table 4
Effect of micronutrient mixture (ML1) on clinical characteristics[1,2]

	Changes during ML1	Changes during placebo	P value
BMI (kg/m^2)	0.3±0.6	0.2±0.9	0.46
Sum of 4 skinfolds (mm)	1.6±4.6	2.8±9.8	0.65
FFM (kg)	0.9±1.0	0.6±1.3	0.42
FEV_1 (ml)	−76±244	52±192	0.11
FEV_1pred. (%)	−5.7±11.1	−0.1±9.1	0.15
FVC (ml)	−62±242	35±203	0.14
Isometric muscle force (nm)	−10±135	−46±135	0.41

[1]Values given as mean±SD.
[2]BMI; body mass index, FFM; fat free mass calculated from four standard skinfold measurements, FEV_1; forced expiratory volume in 1 s, FVC; forced vital capacity, isometric muscleforce; summed maximal force in six muscle groups.

Table 5
Effect of micronutrient mixture (ML1) on anaerobic and aerobic performance[1,2]

	Changes during ML1	Changes during placebo	*P* value
Anaerobic performance			
Ppeak (Watt)	52±100	104±160	0.16
Pmean (Watt)	32.6±49.6	50.3±109.3	0.29
Ppeak/FFM (Watt/kg LBM)	1.0±2.8	2.3±3.7	0.13
Pmean/FFM (Watt/kg LBM)	0.6±1.1	1.1±2.4	0.25
Aerobic performance			
Wmax (Watt)	−3.2±13.8	4.2±17.7	0.19
VO_2 max (ml/kg/min)	−1.5±3.5	0.3±3.9	0.10

[1]Values given as mean±SD.
[2]Ppeak; peak power measured by Wan T, Pmean; mean power measured by WAnT, LBM; lean body mass, Wmax; cycling maximal workload, VO_2max; maximal oxygen consumption during bicycle ergometry.

with either ML1 or placebo. The clinical characteristics of the 22 children with CF who completed the study are presented in Table 2. All patients were adolescents with an FEV_1 pred of 87.2±17% (mean±SD). Baseline results for anaerobic and aerobic performance are presented in Table 3.

At the end of the three months intervention period the plasma level of vitamin E had increased with 16.9±11.6 μmol/l (from 11.7±7.4 μmol/l to 28.6±12.8 μmol/l), while in the placebo group vitamin E levels had dropped 2.3±7.9 μmol/l (from 13.2±5.9 μmol/l to 11.0±6.1 μmol/l). This difference in change between intervention and placebo period is significant ($P<0.001$). The vitamin A levels too had increased after the three months intervention period: from 1.01±0.3 μmol/l to 1.09±0.4 μmol/l, but were slightly lower at the end of the placebo period (from 1.16±0.3 μmol/l to 1.09±0.4 μmol/l). This change of 0.09±0.17 μmol/l in the intervention period versus −0.06±0.23 μmol/l in the placebo period was not significant ($P=0.06$).

However, for all other parameters no significant difference was found. The change in nutritional parameters, BMI, FFM and skinfolds, was essentially identical in the intervention and the placebo period (Table 4). Also no significant differences were seen in the change of any of the pulmonary function tests under ML1 and placebo. Similar results were seen for peripheral muscle strength, aerobic and anaerobic muscle performance (Table 5). However, for the pulmonary function tests FEV_1, FVC, as well as for the aerobic Wmax, and VO_2max, and for the anaerobic Ppeak of WAnT a trend towards significance was found for the placebo. Similarly we found that plasma MDA concentrations decreased during placebo (−0.263 μmol/l±0.66 μmol/l), but marginally increased during ML1 (0.023 μmol/l±0.61 μmol/l) ($P=0.15$). During the intervention period six pulmonary infections were reported (one needing IV antibiotics), as opposed to eleven pulmonary infections in the placebo period (four needing IV antibiotics). This difference was not significant.

4. Discussion

In this study a mixture of multiple micronutrients, with either muscle fortifying or antioxidant action, showed no beneficial effects on either pulmonary function or muscle performance in CF patients as compared to placebo.

Isometric muscle strength, anaerobic power (WAnT) and endurance (Wmax, VO_2max) were similar in the micronutrient and placebo group. A similar lack of effect has been reported for triathlon athletes receiving antioxidant supplementation [27]. Creatine, however, seems to improve muscular performance, but most effectively in combination with high-intensive training [28,29]. Also in these studies much higher doses are used: initial loading dose of 20 g/d for 7 d, and a maintenance dose of 3–5 g/d versus 1.2 g/d in our

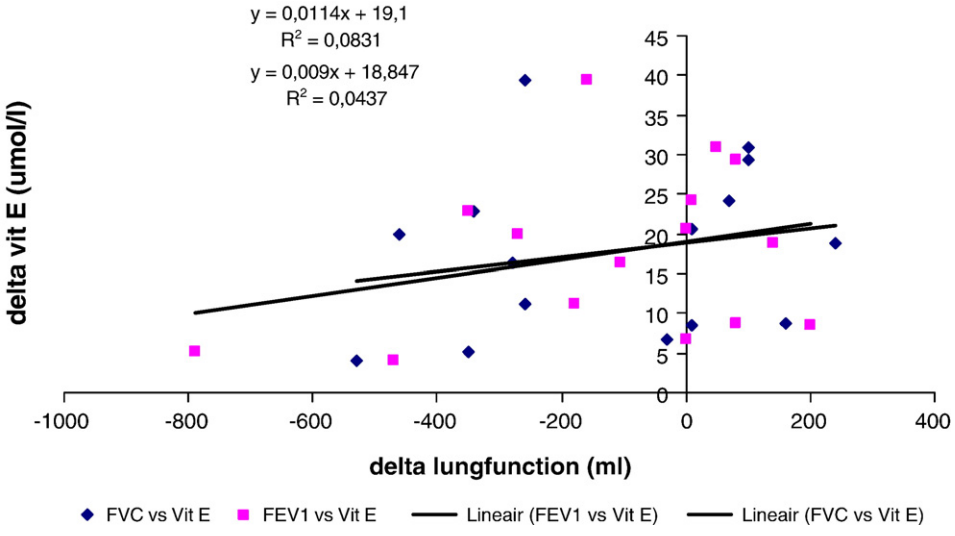

Fig. 1. Correlation between change in vitamin E concentration and FEV_1 and FVC during ML1. Delta FEV_1, changes in forced expiratory volume in 1 s; delta FVC, changes in forced vital capacity. Data are nonparametric and were analyzed by using Spearman's rank correlation.

study [28]. An open-label pilot study in 18 adolescent CF patients showed a significant increase in maximal isometric muscle strength, but no change in lung function after creatine supplementation [30]. In this study also higher doses were used (initial loading dosage 12 g/d first 7 d, maintenance dose 6 g/d for 11 weeks). Taurine has been described as an effective free radical scavenger and reportedly reduces oxidative damage [31,32]. A positive correlation between change in taurine concentration and changes in exercise time to exhaustion and maximal workload (Wmax) was reported in healthy young men after taurine supplementation [17]. Unfortunately, this intervention study lacked a control group, so taurine might not be as effective as suggested. Supplementation of L-carnitine has been reported to normalize decreased levels induced by high-intensity physical exercise [33,34]. So carnitine supplementation may enhance performance in athletes, but healthy, essentially sedentary persons and untrained individuals might not benefit from supplementation.

We did not find any significant changes in pulmonary function. A study by Wood et al. showed comparable results after giving an antioxidant mixture for two months [12]. In this study and ours, interestingly, a non-significant trend towards the opposite was seen, as the decline in FEV_1 and FVC in the intervention group was higher. Moreover, in our study plasma MDA concentration rose in the micronutrient mixture group, and declined in the placebo group, suggesting a shift to a pro-oxidative state when giving the micronutrient combination. Negative effects of antioxidant supplementation, especially vitamin E, have been described by others, and may play a role in our study [18,35–38]. Although Wood et al. [12] did describe a correlation between an increase in β-carotene plasma levels and an improvement in FVC, as well as a correlation between an increase in plasma selenium concentrations and an increase in FEV_1, this does not imply that exogenous administration of these substances does have the desired beneficial effect on pulmonary function. In our study too an increase in plasma vitamin E correlated with increase in pulmonary function ($P=0.04$, Fig. 1).

Both the present report and the study by Wood et al. [12] showed a trend towards a negative effect for pulmonary function when administering a micronutrient mixture consisting either partially or totally of antioxidants. Although it has been thoroughly documented that low levels of antioxidant molecules such as vitamin E and β-carotene can be normalized in CF patients [8–10], so far no hard evidence has been brought forward that boosting plasma levels of these antioxidants reduces pulmonary deterioration. A study by Renner et al. [11] showed that high dose β-carotene supplementation does normalize plasma MDA levels. However no effect on FEV_1 was seen, although the number of days on antibiotics was less in the intervention group.

In conclusion, in the current study we found no significant effects of a micronutrient mixture on either muscle performance or pulmonary function. Therefore, we believe that before conducting further studies with multiple micronutrients, carefully designed interventions with single antioxidants, including determination of the optimal amount to normalize parameters of oxidative stress, are warranted.

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