Vitamin E Improves Clinical Outcome of Patients Affected by Glycogen Storage Disease Type Ib

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Abstract Background: It has been suggested, on a few GSD1b patients, that vitamin E improves neutrophil count and reduces frequency and severity of infections.

The main objective of the present study was to investigate the efficacy of vitamin E on the neutropenia, neutrophil dysfunction and IBD in the entire Italian caseload of GSD1b patients.

Patients and methods: Eighteen GSD1b patients, median age at the time of the study protocol 14.5 (range, 0.6–42 years), were enrolled from four Italian referral centres for metabolic diseases. For the evaluation of the efficacy of vitamin E, neutrophil count and function, frequency of infections needing hospitalization and inflammatory bowel activity were evaluated periodically all over one year before and during vitamin E therapy.

Results: Frequency (1.5 ± 0.1 vs. 6.0 ± 0.6 , $p = 0.003$) and severity of infections (2.2 ± 0.2 vs. 3.7 ± 0.4 , $p = 0.003$) were lower and mean value of neutrophil count ($1,583 \pm 668$ vs. 941 ± 809 , $p = 0.03$) higher during vitamin E supplementation. Neutrophil function results improved during vitamin supplementation. PCDAI showed a significant reduction in the inflammatory activity during vitamin E supplementation (9 ± 1.4 vs. 13 ± 1.2 , $p = 0.006$). In seven patients G-CSF requirement decreased and the dose was reduced after the end of the study.

In conclusion, our study demonstrated the efficacy of vitamin E supplementation. Vitamin E has evident advantages as compared to G-CSF, as it can be assumed orally, and it has not been associated with severe side effects.

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Introduction

Glycogen storage disease type 1 (GSD1) is an inborn error of carbohydrate metabolism. Two subtypes have been identified: GSD type 1a, caused by glucose-6-phosphatase deficiency (G6Pase, G6PC gene), and GSD type 1b, due to a glucose-6-phosphate translocase defect (G6PT, SLC37A4 gene). It is characterized by hepatomegaly, fasting hypoglycaemia, lactic acidosis, hyperlipidaemia and hyperuricaemia. GSD1b patients also show neutropenia and impaired neutrophil function, recurrent infections and inflammatory bowel disease (Melis et al. 2005; Visser et al. 2012). These complications are highly debilitating and have a significant impact on quality of life (Melis et al. 2003).

The underlying cause of GSD1b neutropenia is an enhanced neutrophil apoptosis, but patients also manifest

neutrophil dysfunction of unknown aetiology. G6PT interacts with the enzyme glucose-6-phosphatase- β (G6Pase- β) to regulate the availability of G6P/glucose in neutrophils (Guionie et al. 2003; Jun et al. 2014). A deficiency in G6PT in neutrophils impairs both their energy homeostasis and function. Their energy impairment is characterized by decreased glucose uptake and reduced levels of intracellular G6P, lactate, adenosine triphosphate, and reduced NAD phosphate, whereas functional impairment is reflected in reduced neutrophil respiratory burst, chemotaxis, and calcium mobilization (Kim et al. 2006, 2008; Chou et al. 2010; Gorman et al. 2012; Visser et al. 2012).

Actually G-CSF is considered the only therapy for either neutropenia, neutrophil dysfunction and IBD in GSD 1b patients (Visser et al. 2000). However, this treatment is associated with severe side effects (Pinsk et al. 2002; Visser et al. 2002). Moreover, it has been shown that G-CSF addition to in vitro cultures does not rescue the GSD1b neutrophils from apoptosis (Kuijpers et al. 2003). It has been shown that antioxidants, for example, a soluble analogue of vitamin E (Trolox C), reduce the neutrophil apoptosis in GSD1b neutrophils (Leuzzi et al. 2003). Other studies supported the evidence that vitamin E plays an important role in protecting the cell from apoptosis and in enhancing the action and the production of G-CSF (Singh et al. 2009; Kulkarni et al. 2012).

We previously evaluated the efficacy of vitamin E supplementation in 7 GSD1b patients (Melis et al. 2009). Increased neutrophil counts, a significant decrease of frequency and severity of infections were detected during vitamin E supplementation. On the basis of this study, the role of vitamin E as additional therapy was proposed.

The aim of the current study was to evaluate the efficacy of vitamin E supplementation on neutrophil count, function and IBD in a larger cohort of patients and to analyse potential G-CSF adverse effects.

Patients and Methods

Patients

Eighteen GSD1b patients, median age at the time of the study protocol 14.5, (range 0.6–42 years), representing the entire Italian caseload, were enrolled from 4 Italian referral centres for metabolic diseases. Patients were coded by initials and date of birth to check for duplication. All patients with a diagnosis of GSD1b made either by enzyme studies showing the combination of deficient glucose-6-phosphatase activity in intact microsomes and (sub)normal glucose-6-phosphatase activity in disrupted microsomes or by mutation analysis of the glucose-6-phosphate transporter

gene were enrolled in the study. The patients who were considered not able to comply with the protocol and with the therapy were excluded from the study. The G-CSF treatment was not considered an exclusion criterion. The study was conducted in the centres involved in the follow-up of each patient and the schedule form with follow-up programme to be filled in was sent to each centre.

Study Design

This study was designed as a prospective study. The study protocol was in accordance with the Italian regulations on privacy protection and with the Helsinki Doctrine for Human.

Experimentation was approved by the ethical committees of the participating centres. Before inclusion in the study, patients or their legal guardians signed a written informed consent. G-CSF treatment was not considered an exclusion criterion.

The study was carried out over a 3-year period; during the first year no vitamin supplementation was prescribed; during the second year patients were treated with vitamin E (600 mg/day were given to prepubertal patients and 900 mg/day to adults); during the third year vitamin E wash-out was performed.

Clinical and biochemical parameters were monitored and the results obtained during the second year were compared to both those of the first and third year. Neutrophil counts were obtained every three weeks throughout the study. A complete clinical assessment was made every six months, with particular attention to the presence of mouth ulcers, infections and signs suggestive of IBD.

Serum vitamin E levels, neutrophil function tests and ileocolonoscopy were performed once a year.

Manifestations compatible with adverse effects of G-CSF treatment, such as splenomegaly and osteopenia, when present, were monitored every 6 months.

Methods

Clinical Examination and Assessment of Infections

Patients were routinely examined in day hospital every 6 months or at the moment of any intercurrent infection. Physical examination included evaluation of weight, height and body mass index. The presence of nose, throat, lung and/or skin infections, abdominal pain, mouth ulcers and perianal lesions was recorded and evaluated according to a severity score index; in this scoring system the site of the infection, the need for therapy and/or hospital admission for observation and the duration of the disease were considered (Melis et al. 2009).

Biochemical Investigations

Neutrophil count was measured by standard methods and neutropenia was defined as peripheral blood neutrophil count below $500 \times 10^9/L$. Neutrophil function tests included N-formyl-methionyl leucyl-phenylalanine (fMLP)-induced activation of respiratory burst and *E. coli*-induced respiratory burst and phagocytosis by flow cytometry. Serum vitamin E assay was performed by standard procedures.

Evaluation of the clinical and biochemical data associated to GSD1 and reflecting the metabolic control of the disease was also performed. In particular, the evaluation of the clinical and biochemical parameters of GSD1 included: serum glucose, triglycerides, cholesterol, lactic and uric acid levels. These parameters were expressed as mean value of all the determination obtained in each patient. Moreover, the frequency of hospital admissions for hypoglycaemia and the compliance to the dietary or medical treatment were recorded for all patients.

Assessment of Inflammatory Bowel Disease

Inflammatory bowel involvement was investigated by ileocolonoscopy at study entry and after one year of treatment. Inflammatory bowel activity was evaluated by the Paediatric Crohn's Disease Activity Index.

Evaluation of G-CSF Side Effects

Spleen size was evaluated by standard abdominal ultrasonography.

Bone mineral density (BMD) was measured by DEXA (Hologic QDR 1000, Hologic, Inc., Waltham, MA, USA). Measurements were taken at the L1-L4 vertebrae. Z-Scores were calculated by comparing BMD with age-matched (3–16 years) or age- and sex-matched (above 16 years) reference values according to the manufacturer's internal reference database.

Statistical Analysis

Data are expressed as mean $\pm$ SE. Statistical analysis was performed using Statistical Package for Social Science (SPSS 10 for Windows Update; SPSS Inc., Chicago, Illinois, USA). The comparisons between numerical variables were performed by Wilcoxon test. The comparison between categorical variables or associations between different parameters were performed using χ^2 test. The significance was set at 5%.

Results

Clinical Examination and Assessment of Infections

Frequency (1.5 ± 0.1 vs. 6.0 ± 0.6 , $p = 0.003$) and severity of infections (score of 2.2 ± 0.2 vs. 3.7 ± 0.4 , $p = 0.003$) were lower during the second year, when vitamin E supplementation was started. Most of them were bacterial infections. Vitamin E supplementation was the most important predictive factor of improvement of infections ($p = 0.03$). Infections' severity score results correlated with neutrophil count ($r = 0.92$, $p = 0.002$); conversely no correlation was observed between infections' severity score and parameters of metabolic control.

For 14 patients a third year of vitamin E withdrawal was available. During the vitamin E wash-out period, again the frequency and severity of infections increased (4.8 ± 0.3 , 2.9 ± 0.2 , respectively, $p = 0.01$).

Biochemical Investigations

Biochemical parameters of metabolic control were stable (Table 1) and no change in the dietary glucose requirements was recorded during vitamin E supplementation. The mean value of neutrophil count was significantly higher during vitamin E supplementation than during the period without vitamin E ($1,583 \pm 668$ vs. 941 ± 809 , $p = 0.03$) (Fig. 1).

E. coli-induced respiratory burst (0.95 ± 0.01 vs. 0.85 ± 0.009 , $p = 0.0001$), *E. coli*-induced phagocytosis (97.5 ± 0.9 vs. 73.6 ± 6.5 , $p = 0.013$) and N-formyl-methionyl leucyl-phenylalanine (fMLP)-induced activation of respiratory burst (0.16 ± 0.02 vs. 0.10 ± 0.01 , $p = 0.031$) were significantly higher during vitamin E supplementation. Mild but not significant increase of PMA-induced respiratory burst (1.98 ± 0.7 vs. 0.96 ± 0.09 , $p = 0.15$) was detected during vitamin E supplementation (Fig. 2). Neutrophil function test results correlated with biochemical parameters indicative of metabolic control; *E. coli*-induced respiratory burst inversely correlated with insulin serum levels ($r = -0.85$, $p = 0.007$), N-formyl-methionyl leucyl-phenylalanine (fMLP)-induced activation of respiratory burst correlated with glucose serum levels ($r = 0.831$, $p = 0.02$), with bicarbonate serum levels ($r = 0.827$, $p = 0.027$), inversely correlated with both triglycerides ($r = -0.87$, $p = 0.005$) and lactic acid serum levels ($r = -0.72$, $p = 0.04$); *E. coli*-induced phagocytosis correlated with bicarbonate serum levels ($r = 0.925$, $p = 0.003$) and inversely correlated with lactic acid serum levels ($r = -0.88$, $p = 0.004$).

Table 1 Biochemical parameters indicative of metabolic control during vitamin E supplementation and in the year without supplementation

Parameters	Vitamin E supplementation	No vitamin	<i>p</i>
Glucose (mg/dl)	85	80	0.53
Lactic acid (mg/dl)	11.8	16.125	0.39
Uric acid (mg/dl)	5.53	5.92	0.73
Cholesterol (mg/dl)	119.66	109	0.41
Triglycerides (mg/dl)	244.26	153.06	0.09
Bicarbonate (mEq/l)	24.50	23.27	0.22
Haemoglobin (mg/dl)	10.41	10.49	0.89
ESR	41	62	0.07
CRP (mg/dl)	0.53	1.78	0.01

Fig. 1 Neutrophil count mean value during the first year, filled square (no vitamin supplementation), and second year, grey shaded square (vitamin supplementation)

Serum vitamin E levels significantly increased during vitamin supplementation ($p = 0.04$), from 750.8 ± 81.9 (first year) to 975 ± 70.3 (second year) to 784.8 ± 70 (third year). In most of the patients (10/14), vitamin E serum levels significantly decreased in the third phase. In these patients clinical features worsened during vitamin E wash-out including frequency and severity of infections, mouth ulcers and PCDAI. In the remaining patients vitamin E levels were stable. This might explain the nonsignificant reduction of vitamin E serum levels detected in the third phase.

No correlation was observed between parameters indicative of metabolic control and neutrophil count. Compliance to the dietary treatment did not correlate with neutrophil count, function and infections.

Inflammatory Bowel Disease Assessment

The presence of inflammatory bowel involvement was investigated in the seven patients followed at the Department of Pediatrics, Federico II University. Ileocolonoscopy was performed by an expert who was not aware of the treatment that patients were receiving. At study entry, ileocolonoscopy showed linear and aphthoid ulcers in three patients; histology showed severe infiltration of lymphocytes and monocytes in the lamina propria extending to submucosa and muscularis mucosa. In these patients, after one year of vitamin E supplementation, ileocolonoscopy showed an improved pattern with mild mucosal hyperaemia; histology showed foci of infiltration of lymphocytes in the lamina propria. In two additional patients, ileocolonoscopy initially showed ulcers, hyperaemia and friability of the mucosa, and histology demonstrated infiltration of lymphocytes and monocytes in the lamina propria. Normal pattern was detected both at ileocolonoscopy and histology at the end of the study.

PCDAI showed a significant reduction in the inflammatory activity during vitamin E supplementation (13 ± 1.2 vs. 9 ± 1.4 , $p = 0.006$). The abdominal pain and extra-intestinal manifestations including arthritis and mouth ulcers were the most significantly changed items. Moreover CRP levels significantly decreased during vitamin E supplementation (1.78 ± 0.5 ; 0.53 ± 0.13 , $p = 0.01$). Haemoglobin concentrations showed an increase during vitamin E supplementation; however, the changes did not reach statistical significance (10.49 ± 0.53 ; 10.41 ± 0.57 , $p = 0.89$) (Table 1).

G-CSF Side Effect Evaluation

At the study entry, splenomegaly was present in 7/18 patients and was associated with hypersplenism in two of them. These patients initially got 3–5 mg/kg G-CSF dose each day. In these patients G-CSF dose was reduced at the end of the study. In particular, five patients got every-other-day treatment; in two patients everyday treatment was changed in twice-a-week therapy. G-CSF dose reduction was combined with vitamin E supplementation. Although G-CSF dose was reduced, neutrophil count was stable and the frequency and severity of infections did not increase after 1-year follow-up.

Bone DEXA was performed in 12/18 patients and demonstrated osteoporosis in five patients and osteopenia in one patient; normal values were observed in the remaining patient.

None of the patients showed the occurrence of malignancies.

Fig. 2 Neutrophil function results during the first year, *filled square* (no vitamin supplementation), and second year, *grey shaded square* (vitamin supplementation)

Discussion

GSD1b patients' neutropenia is caused by enhanced neutrophil ER stress, oxidative stress and apoptosis arising from loss of G6PT/G6Pase-b activity (Jun et al. 2014). The G6P is the fuel for the pentose phosphate pathway that can generate NADPH by the hexose-6-phosphate dehydrogenase (Kim et al. 2008). In GSD1b neutrophils, the import of G6P into the endoplasmic reticulum is decreased, thus causing a local decrease in G6P dehydrogenase activity. This enzyme, which serves to produce NADPH, determines the cellular redox status by permitting regeneration of reduced glutathione, resulting in decreased sensitivity to direct or indirect apoptosis (Kim et al. 2006; Chou et al. 2010; Gorman et al. 2012). G-CSF treatment in GSD1b does not prevent the induction of apoptosis in circulating neutrophils (Kuijpers et al. 2003). Conversely, antioxidants reduce the neutrophil apoptosis in GSD1b neutrophils (Leuzzi et al. 2003). Moreover, it has been demonstrated that vitamin E plays an important role in protecting the cell from apoptosis and in enhancing the action and the production of G-CSF (Singh et al. 2009; Kulkarni et al. 2012).

We have previously investigated a possible role of vitamin E, a known antioxidant, on neutropenia, severity and frequency of infections and inflammatory bowel disease on a small number of patients. It is noteworthy that recurrent severe infections and inflammatory bowel disease are highly debilitating manifestations and impact on patients' quality of life and their overall health status.

In the current study, performed on the entire Italian caseload, we demonstrated that vitamin E supplementation increased the neutrophil count and improved neutrophil function. Increased and stable neutrophil counts during vitamin E supplementation correlated with significantly decreased frequency and severity of infections.

We hypothesized that vitamin E improves neutropenia by reducing the reactive oxygen species (ROS) and therefore apoptosis. Our results are in line with the observation that the specific G6PT-inhibitor S3484 increases apoptosis of neutrophils, which can be rescued by preincubation of cells with the reactive oxygen species (ROS) scavenger Trolox C or with the flavoprotein inhibitor diphenyleneiodonium (DPI).

We also detected an improvement of neutrophil function during vitamin E supplementation. Our results seem to confirm data already observed in elder patients. Both in vitro and in vivo studies demonstrated that vitamin E supplementation improves the lymphoproliferative capacity, neutrophil-mediated functions including phagocytic functions of PMN neutrophils and monocyte chemoattractant protein-1 production (Ventura et al. 1994; De la Fuente et al. 1998).

No correlation was detected among the results of biochemical parameters indicative of metabolic control and both frequency and severity of infections and neutrophil count. Conversely metabolic control seems to impact on neutrophil function; in fact increased lactic acid serum levels inversely correlated with *E. coli*-induced phagocytosis, *E. coli*-induced respiratory burst and N-formyl-

methionyl leucyl-phenylalanine (fMLP)-induced activation of respiratory burst results; surprisingly also insulin serum levels inversely correlated with *E. coli*-induced respiratory burst results. These results might suggest that both GSD1b-related metabolic disorder and excessive dietary treatment might impair neutrophil function.

We observed improved findings at ileocolonoscopy and bowel histology; a decrease of inflammatory activity was also observed by PCDAI. These effects are the most relevant for GSD1b patients and have strong implications for their quality of life and health status. Indeed, after withdrawal of vitamin E supplementation at the end of the study protocol, severe infections and low neutrophil counts were again observed and all patients asked to resume vitamin E. These results are in agreement with data observed in patients affected by ulcerative colitis (Seidner et al. 2005)

Concerning G-CSF-related side effects, splenomegaly was recorded in 7/18 patients and was associated with hypersplenism in two of them. During vitamin E supplementation, in these seven patients G-CSF dose was reduced after the end of the study and no significant reduction of neutrophil count neither increase of frequency and severity of infections were observed.

In conclusion, our study suggests the efficacy of vitamin E supplementation on improving clinical outcome of GSD1b patients.

Moreover, during vitamin E supplementation, G-CSF doses or frequency of administration can be reduced with consequent reduction of G-CSF-related side effects. Vitamin E has evident advantages as compared to G-CSF, as it can be assumed orally, and it has not been associated with severe side effects.

The control of the metabolic disorder and the strict adherence to dietary prescription is highly recommended.

The efficacy of the vitamin E should also be evaluated in other forms of congenital neutropenias in which increased apoptosis has been reported, such as cyclic neutropenia, myelokathexis and congenital dysgranulopoietic neutropenia.

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Take-Home Message

Vitamin E supplementation improves clinical outcome of GSD1b patients and allows a G-CSF dose reduction with consequent reduction of G-CSF-related side effects.

Compliance with Ethics Guidelines

Conflict of Interest

We underline that: there are no prior publications or submissions with any overlapping information; the work is not and will not be submitted to any other journal while under consideration by JIMD; there are no potential conflicts of interest, real or perceived; Dr. D. Melis wrote the first draft of the manuscript and gave substantial contributions to conception and design, acquisition of data and analysis and interpretation of data; and neither an honorarium or grant or other forms of payment were given to anyone to produce the manuscript. All the authors listed on the manuscript take full responsibility for the manuscript; moreover, Dr. D. Casa, G. Minopoli, F. Balivo, G. Parenti, S. Paci and C. Dionisi-Vici gave substantial contributions to conception and design, acquisition of data and analysis and interpretation of data, revised the manuscript critically for important intellectual content, approved the submission of this version of the manuscript and take full responsibility for the manuscript. Dr. Marcolongo and Prof. Benedetti performed the biochemical investigation, namely, the neutrophil function test; gave substantial contributions to conception and design, acquisition of data and analysis and interpretation of data; revised the manuscript critically for important intellectual content; approved the submission of this version of the manuscript; and take full responsibility for the manuscript. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Prof. Generoso Andria and Giancarlo Parenti gave substantial contribution to conception and design, acquisition of data and analysis and interpretation of data and critically revised the manuscript.

Daniela Melis, Giorgia Minopoli, Francesca Balivo, Paola Marcolongo, Rossella Parini, Sabrina Paci, Carlo Dionisi-Vici, Roberto Della Casa, Angelo Benedetti, Generoso Andria and Giancarlo Parenti declare that they have no conflict of interest.

References

- Chou JY, Jun HS, Mansfield BC (2010) Neutropenia in type Ib glycogen storage disease. *Curr Opin Hematol* 17:36–42
- De la Fuente M, Ferrández MD, Burgos MS, Soler A, Prieto A, Miquel J (1998) Immune function in aged women is improved by ingestion of vitamins C and E. *Can J Physiol Pharmacol* 76:373–380

- Gorman AM, Healy SJ, Jäger R, Samali A (2012) Stress management at the ER: regulators of ER stress-induced apoptosis. *Pharmacol Ther* 134:306–316
- Guionie O, Clottes E, Stafford K, Burchell A (2003) Identification and characterization of new human glucose-6-phosphatase isoform. *FEBS Lett* 551:159–164
- Jun HS, Weinstein DA, Lee YM, Mansfield BC, Chou JY (2014) Molecular mechanisms of neutrophil dysfunction in glycogen storage disease type 1b. *Blood* 123:2843–2853
- Kim SY, Nguyen AD, Gao JL, Murphy PM, Mansfield BC, Chou JY (2006) Bone marrow-derived cells require a functional glucose-6-phosphate transporter for normal myeloid functions. *J Biol Chem* 281:28794–28801
- Kim SY, Jun HS, Mead PA, Mansfield BC, Chou JY (2008) Neutrophil stress and apoptosis underlie myeloid dysfunction in glycogen storage disease type 1b. *Blood* 111:5704–5711
- Kuijpers TW, Maiani NA, Tool AT, Smit GP, Rake JP, Roos D, Visser G (2003) Apoptotic neutrophils in the circulation of patients with glycogen storage disease type 1b (GSD1b). *Blood* 101:5021–5024
- Kulkarni SS, Cary LH, Gambles K, Hauer-Jensen M, Kumar KS, Ghosh SP (2012) Gamma-tocotrienol, a radiation prophylaxis agent, induces high levels of granulocyte colony-stimulating factor. *Int Immunopharmacol* 14:495–503
- Leuzzi R, Bănhegyi G, Kardon T, Marcolongo P, Capecchi PL, Burger HJ, Benedetti A, Fulceri R (2003) Inhibition of microsomal glucose-6-phosphate transport in human neutrophils results in apoptosis: a potential explanation for neutrophil dysfunction in glycogen storage disease type 1b. *Blood* 101:2381–2387
- Melis D, Parenti G, Della Casa R, Sibilio M, Berni Canani R, Terrin G, Cucchiara S, Andria G (2003) Crohn's-like ileo-colitis in patients affected by glycogen storage disease 1b: two years' follow-up of patients with a wide spectrum of gastrointestinal signs. *Acta Paediatr* 92:1415–1421
- Melis D, Fulceri R, Parenti G, Marcolongo P, Gatti R, Parini R, Riva E, Della Casa R, Zammarchi E, Andria G, Benedetti A (2005) Genotype/phenotype correlation in glycogen storage disease type 1b: a multicentre study and review of the literature. *Eur J Pediatr* 164:501–508
- Melis D, Della Casa R, Parini R, Rigoldi M, Cacciapuoti C, Marcolongo P, Benedetti A, Gaudieri V, Andria G, Parenti G (2009) Vitamin E supplementation improves neutropenia and reduces the frequency of infections in patients with glycogen storage disease type 1b. *Eur J Pediatr* 168:1069–1074
- Pinsk M, Burzynski J, Yhap M, Fraser RB, Cummings B, Ste-Marie M (2002) Acute myelogenous leukemia and glycogen storage disease 1b. *J Pediatr Hematol Oncol* 24:756–758
- Seidner DL, Lashner BA, Brzezinski A, Banks PL, Goldblum J, Fiocchi C, Katz J, Lichtenstein GR, Anton PA, Kam LY, Garleb KA, Demichele SJ (2005) An oral supplement enriched with fish oil, soluble fiber, and antioxidants for corticosteroid sparing in ulcerative colitis: a randomized, controlled trial. *Clin Gastroenterol Hepatol* 3:358–369
- Singh VK, Brown DS, Kao TC (2009) Tocopherol succinate: a promising radiation countermeasure. *Int Immunopharmacol* 9:1423–1430
- Ventura MT, Serlenga E, Tortorella C, Antonaci S (1994) In vitro vitamin E and selenium supplementation improves neutrophil-mediated functions and monocyte chemoattractant protein-1 production in the elderly. *Cytobios* 77:225–232
- Visser G, Rake JP, Fernandes J, Labrune P, Leonard JV, Moses S, Ullrich K, Smit GP (2000) Neutropenia, neutrophil dysfunction, and inflammatory bowel disease in glycogen storage disease type 1b: results of the European Study on Glycogen Storage Disease type I. *J Pediatr* 137:187–191
- Visser G, Rake JP, Labrune P, Leonard JV, Moses S, Ullrich K, Wendel U, Smit GP (2002) European Study on Glycogen Storage Disease Type I. Consensus guidelines for management of glycogen storage disease type 1b -European Study on Glycogen Storage Disease Type 1. *Eur J Pediatr* 161(Suppl 1):S120–S123
- Visser G, de Jager W, Verhagen LP, Smit GP, Wijburg FA, Prakken BJ, Coffey PJ, Buitenhuis M (2012) Survival, but not maturation, is affected in neutrophil progenitors from GSD-1b patients. *J Inher Metab Dis* 35:287–300