

Efficacy and Safety of Enteral Recombinant Human Insulin for Reduction of Time-to-Full Enteral Feeding in Preterm Infants: A Randomized, Double-blind, Placebo-Controlled Trial

Naim Shehadeh MD^{1,5}, Aryeh Simmonds MD², Samuel Zangen MD³, Arie Riskin MD MHA^{4,5}, and Raanan Shamir MD^{6,7}

¹Department of Pediatrics A and the Pediatric Diabetes Unit, Rambam Health Care Campus, Haifa, Israel

²Department of Neonatology/Pediatrics, Laniado Hospital, Netanya, Israel

³Department of Pediatrics, Barzilai Medical Center, Ashkelon, Israel

⁴Department of Neonatology, Bnai Zion Medical Center, Haifa, Israel

⁵Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

⁶Institute for Gastroenterology, Nutrition and Liver Diseases, Schneider Children's Medical Center, Petah Tikva, Israel

⁷Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

ABSTRACT **Background:** Infants born very prematurely have functionally and structurally immature gastrointestinal tracts.

Objectives: To assess the safety and tolerability of administration of enteral recombinant human (rh) insulin on formula fed preterm infants and to assess whether enteral administration of rh-insulin enhances gastrointestinal tract maturation by reducing the time to reach full enteral feeding.

Methods: A phase 2, multicenter, double-blind, placebo-controlled, randomized study was conducted. Premature infants (26–33 weeks gestation) were randomized 1:1 to receive insulin 400 µU/ml mixed with enteral feeding or placebo added to their formula. The primary efficacy outcome measure was the number of days required to achieve full enteral feeding. Safety outcomes included adverse events and blood glucose levels.

Results: The study consisted of 33 infants randomized for the safety population and 31 for efficacy analysis. The mean time to full enteral feeding was 6.37 days (95% confidence interval [95%CI] 4.59–8.15) in the enteral rh-insulin treatment group (n=16) and 8.00 days (95%CI 6.20–9.80) in the placebo group (n=15), which represents a statistically significant reduction of 1.63 days (95%CI 0.29–2.97; *P* = 0.023). There was no difference in blood glucose levels between the groups and none of the participants experienced hypoglycemia. Adverse events occurred in 9/17 (53%) infants in the enteral rh-insulin group and 12/16 (75%) in the placebo group.

Conclusions: Our trial demonstrated that administration of enteral rh-insulin as supplement to enteral nutrition significantly reduced time to achieve full enteral feeding in preterm infants with a gestational age of 26–33 weeks.

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KEY WORDS: enteral feeding, gastrointestinal tract, insulin, preterm infants

It is estimated that globally 11% of all live births are premature, with 16% of these classified as very or extremely premature at gestational age ≤ 32 weeks [1]. Given the rate of gastrointestinal tract growth that occurs in the third trimester of pregnancy [2,3], premature infants are born with functionally and structurally immature gastrointestinal tracts. Very premature babies have difficulties coordinating swallowing, suckling, and breathing, which necessitate enteral feeding via an oro- or naso-gastric tube. However, due to gut immaturity, enteral feeding can be poorly tolerated and is therefore initiated in small volumes to stimulate functional and structural adaptation of the gastrointestinal tract. Consequently, fluids and nutrition need to be provided parenterally until full enteral feeding is achieved, a process that can take time [2-5].

At present, there is an unmet medical need for a direct, targeted intervention that can promote structural growth and improve functional enzymatic activity of the immature gastrointestinal tract. Such an intervention would facilitate rapid tolerance and accelerate time to full enteral feeding. In turn, accelerating time to full enteral feeding would decrease the time infants are exposed to parenteral nutrition; thereby, reducing the risk of associated complications, such as sepsis, mucosal atrophy, and cholestasis [3,4,6]. Accelerated enteral feeding would also allow more nutrition to be delivered to the gut during the first weeks of life, supporting enteral nutrition associated growth and weight gain, and facilitating hospital discharge at the earliest opportunity.

There is physiological and preclinical evidence that insulin plays a role in the structural and functional development of the gastrointestinal tract [7-9]. The presence of insulin in the amniotic fluid [10], in high concentrations of up to 2500 µU/ml in the colostrums [11], and also in mature maternal milk at concentrations of 46 µU/ml [11], support a physiologic role in the

prenatal, neonatal, and nursing periods. Preclinical studies in suckling animals have shown that insulin binds to receptors on enterocytes stimulating cell proliferation, decreasing apoptosis, and increasing mucosal enzyme activity [7,9].

The physiologic action of insulin as a locally acting growth factor on the gastrointestinal tract and the absence of insulin from infant formula [9] while present in physiologic levels in human milk [11] support a purpose for oral administration of insulin to aid gut maturation in infants born prematurely.

A formulation of insulin for enteral administration (NTRA, Nutrinia Ltd., Israel), reconstituted in infant formula, has been investigated in Phase 1 studies. Preliminary studies of NTRA have been conducted in eight preterm infants using an rh-insulin dose of 400 μ U/ml of enteral feed and in 11 infants with low birth weight at term using an rh-insulin dose of 90 μ U/ml of enteral feed and support a role for enteral rh-insulin on gut maturation with no safety alerts [7,12].

The objective of this Phase 2 study was to further evaluate the safety and tolerability of enteral rh-insulin administration mixed with infant nutrition (formula) in preterm infants and to assess whether enterally delivered insulin enhances gastrointestinal tract maturation by reducing the time to reach full enteral feeding.

PATIENTS AND METHODS

INFOSUP II was a Phase 2, multicenter, double-blind, placebo-controlled, parallel group, randomized study to assess an rh-insulin formulation (developed for enteral administration), fed concomitantly with preterm infant formula, on gut maturation of premature infants (ClinicalTrials.gov Identifier: NCT01093638). Infant recruitment was performed in neonatal intensive care units hospitals in Israel. The study was performed in accordance with the current version of the Helsinki declaration and the trial was conducted in agreement with the International Conference on Harmonisation Guidelines on Good Clinical Practice. Parents of eligible preterm infants provided written informed consent for infant participation in the study.

The study included preterm infants at 26–33 weeks of gestation, birth weight \geq 750 grams, postnatal age \leq 7 days. The infants were considered to be stable enough to tolerate oral feeds. Inclusion was restricted to infants fed with infant formula. Infants were excluded if they were not being fed enterally for any reason at study entry, were being treated with insulin, were being breast fed after day 1, were on completely oral feeds at study entry, or were fed with mother's milk, even partially. Other exclusion criteria were fraction of inspired oxygen $>$ 0.6 at enrollment, chest compressions or any resuscitation drugs given to the infant during delivery, cardiovascular instability, major congenital malformations, high index of suspicion of infection before enrollment, and development or suspicion of necrotizing enterocolitis. Maternal diabetes also merited exclusion.

The formulation of insulin investigated in this study, NTRA, was a dry powder composed of human insulin (rDNA origin) and microencapsulated within a matrix of maltodextrin. The insulin dose of 400 μ U/ml of enteral feed was based on the level of insulin that a fetus would be exposed to in utero via the amniotic fluid [10].

RANDOMIZATION, BLINDING, AND TREATMENT ADMINISTRATION

Following screening and parental informed consent, eligible infants were randomized 1:1 to receive rh-insulin 400 μ U/ml of enteral feed or a placebo via enteral consumption of NTRA mixed with infant formula. The infant formula volumes were administered according to a predefined feeding protocol. Parents, all medical teams, and the study monitors were blinded to the treatment. Study treatment was initiated within 24 hours of enrollment for 28 days or until time of discharge, whichever was sooner. Study end was 6 months after enrollment.

EFFICACY AND SAFETY MEASUREMENTS

Dietary intake (enteral and parenteral), gastric residual volume, and weight were measured daily from day 1 to day 28 or to discharge, whichever was sooner. Adverse events were recorded daily up to day 28 and reported at month 3 and month 6. Glucose levels were tested three times daily for the first 3 days and on a daily basis thereafter until achieving full enteral feeding, and then every 3 days until day 28 or discharge, whichever was sooner. Laboratory tests for blood chemistry, blood count, anti-insulin antibodies, islet antigen 2, amino acids, and stool elastase were conducted at discharge and at month 3.

The primary study endpoint was the number of days required to achieve full enteral feeding, defined as enteral consumption of 150 ml/kg/day of preterm infant formula. The secondary efficacy endpoints were number of days until gastric residuals (a measure of food tolerance and an accepted marker of gastrointestinal tract maturity [13,14], which did not exceed 2 ml/kg, number of days from birth to discharge (i.e., age at discharge), and weight gain at discharge. Safety outcomes included adverse events, hypoglycemia, and laboratory test abnormalities.

STATISTICAL ANALYSIS

A sample size of 76 infants was considered to yield a reasonably precise estimate in this exploratory Phase 2 study as there was no precedent to inform sample size. Interim analysis was planned after approximately one-half of the infants had been enrolled. The significance level was adjusted using the Pocock method to control the overall type I error rate below 5%. Following this adjustment, the interim analysis and final analysis significance levels were set to $\alpha = 0.03$ for the primary endpoint and $\alpha = 0.05$ for the secondary endpoints.

Primary and secondary efficacy endpoints were analyzed by a mixed model (combining the singleton population as a subset and twins as a paired subset) to estimate the number

of days to full enteral feeding (primary outcome), the number of days until last gastric residual above 2 ml/kg, and the number of days from birth to discharge (secondary efficacy outcomes) for each group, as well as the difference between each group. All other parameters are reported as descriptive statistics. Missing data were not imputed, only observed data were used for the analyses.

The efficacy analysis population included all randomized infants, with the exclusion of those with major protocol or entry violations [2] likely to affect the efficacy outcome. Whether a deviation was major was decided by a physician blinded to the treatment prior to database lock. The safety analysis population included all infants who initiated study treatment.

RESULTS

PATIENTS

The study was conducted between August 2010 and February 2013 at three centers in Israel. The current sponsor of the clinical program (ElganPharma, Israel) recently released the data for publication. The cohort description is shown in Figure 1. The interim analysis included the first 33 infants who had been

randomized: 17 had been administered enteral rh-insulin and 16 had received placebo with nutrition. All 33 infants were included in the safety analysis and 31 infants, 16 in the rh-insulin group and 15 in the placebo group, were included in the primary efficacy analysis. One infant was excluded from the primary efficacy analysis due to violation of inclusion criteria (infant was terminally ill on enrollment and should not have been included) and one infant was excluded due to suspected necrotizing enterocolitis necessitating cessation of enteral feeding prior to receiving any study treatment (see the Safety section).

Baseline characteristics of all randomized infants are shown in Table 1. There were no relevant differences between the treatment groups. The mean exposure time to placebo was 23.3 ± 7.2 (range 2–29) days and to enteral rh-insulin was 25.6 ± 3.6 (range 18–28) days.

EFFICACY OUTCOMES

The results of the mixed model analysis showed the mean time to full enteral feeding was 6.37 days (95%CI 4.59–8.15) in the insulin group and 8.00 days (95%CI 6.20–9.80) in the placebo group [Figure 2]. The 1.63 days (95%CI 0.29–2.97) difference between the groups was statistically significant (*P* = 0.023) and represented a 20% decrease in time to full enteral feeding for in-

Figure 1. Cohort description

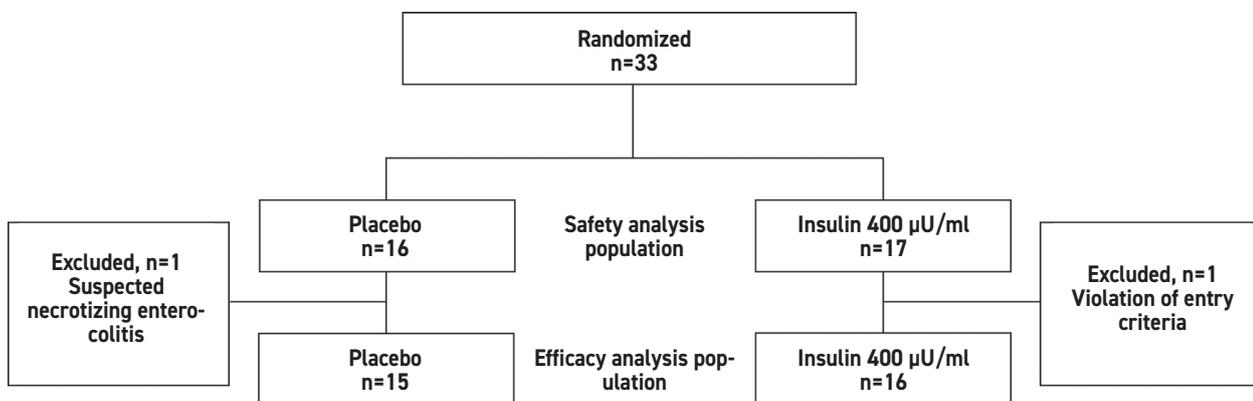
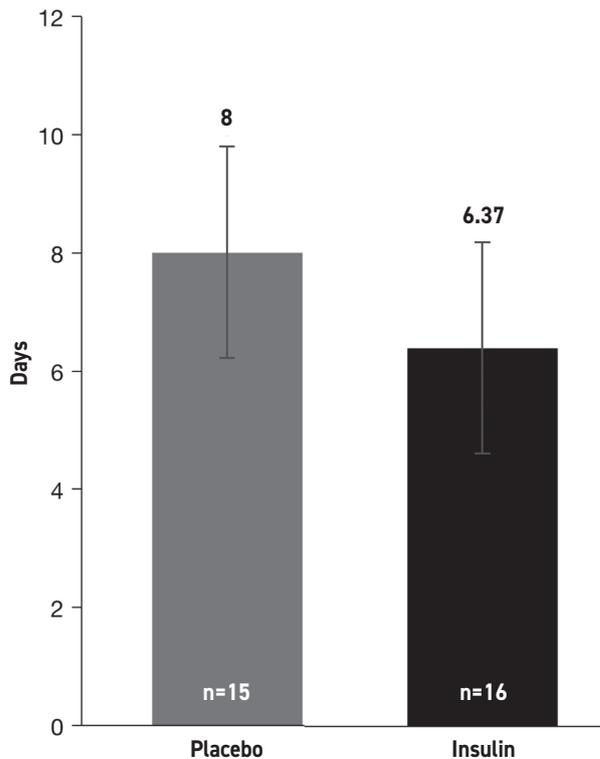


Table 1. Patient characteristics at baseline

Characteristic	Placebo n=16	Insulin 400 µU/ml n=17
Mean age ± SD, days	4.4 ± 2.1	4.6 ± 2.3
Male, n (%)	8 (50)	7 (41.2)
Singletons, n (%)	6 (37.5)	6 (35.0)
Twin/triplets, n (%)	10 (62.5)	11 (65.0)
Mean gestational age ± SD, weeks	30.6 ± 2.1	30.9 ± 1.5
Mean birth weight ± SD, grams	1446.8 ± 364.8	1470.7 ± 299.7

SD = standard deviation

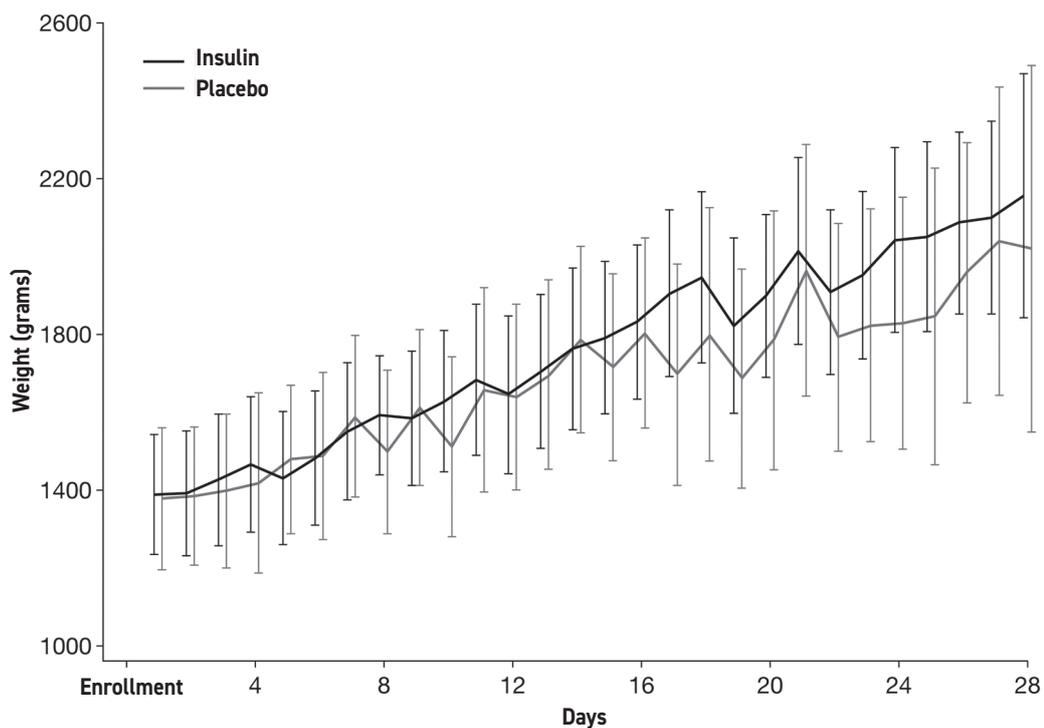
Figure 2. Primary efficacy outcome: time to complete enteral feeding



fants treated with enteral rh-insulin. As the *P* value reached was less than the significance level declared for the interim analysis ($\alpha = 0.03$), the trial was considered to show a clear benefit in favor of enteral rh-insulin treatment and was terminated at that point per the study protocol.

The time to reach a gastric residual < 2 ml/kg was 1.67 days (95%CI -1.42–4.76) in the rh-insulin group and 5.09 days (95%CI 1.92–8.26) in the placebo group. The difference between the treatment groups was 3.42 days (95%CI 0.12–6.96) ($P = 0.056$). An exploratory analysis of the time to wean-off parenteral nutrition showed 3.5 days in the rh-insulin group and 5.9 days in the placebo group. Although there was 2.4 days difference between averages this did not reach statistical significance. Age at hospital discharge was 34.6 days in the enteral rh-insulin group and 39.5 days in the placebo group ($P = 0.36$). Since twins are predominantly discharged together in Israel, an analysis of age at discharge of singletons only was also performed, with 34.9 days in the enteral rh-insulin group and 44.0 days in the placebo group; however, the result was non-significant due to the small sample size ($n=13$, $P = 0.418$). During the first 28 days, there was a mean increase in weight of 768.9 grams among those receiving enteral rh-insulin and 643.6 grams among the infants receiving placebo, representing a 19% increase in weight among infants in the group receiving enteral rh-insulin compared with the placebo group [Figure 3].

Figure 3. Weight measurement over first 28 days following birth



SAFETY

Adverse events throughout the study and follow-up period were reported in nine infants (53%) in the rh-insulin group and 12 infants (75%) in the placebo group. Adverse events were hematemesis (n=1), supraventricular tachycardia (n=1), gastroenteritis (n=2), inguinal hernia (n=4), low hemoglobin (n=1), sepsis (n=6), acute otitis media (n=1), upper respiratory tract infection (n=2), respiratory distress (n=1), upper lobe pneumonia (n=1), neutropenia (n=1), hypothyroidism (n=2), gastroesophageal reflux (n=1), diaper dermatitis (n=1), skin irritation (n=1), ulcerated hemangioma (n=1), apnea (n=2), fever (n=1), and conjunctivitis (n=1).

The majority of adverse events were mild to moderate in nature. Serious adverse events were reported in three infants in the rh-insulin group, one of these infants experienced several serious adverse events reported as fever, gastroenteritis, gastroesophageal reflux, and viral infection. One infant experienced serious recurrent apnea and upper lobe pneumonia as serious adverse events and congenital hypothyroidism was reported in one infant as a serious adverse event. In the placebo group, sepsis was reported as a serious adverse event in two infants. All serious adverse events were resolved and none of the adverse events were considered by the investigators to be related to study treatment.

One patient who was randomized but did not receive study treatment died during the course of the study. This infant was terminally ill at enrollment and was considered by the investigator to have violated entry criteria. One infant who was randomized but did not receive study treatment developed necrotizing enterocolitis.

Hypoglycemia was not observed in any infant, and blood glucose levels between the insulin and placebo groups were comparable: mean \pm SD glucose levels for the rh-insulin and placebo groups were 92.5 ± 17.3 mg/dl (n=17) and 100.8 ± 128.73 mg/dl (n=16) on day 3 and 73.3 ± 10.9 mg/dl (n=6) and 76.5 ± 14.4 mg/dl (n=4) on day 28. There was no clinically relevant difference between the treatment groups with regards to laboratory tests (data not shown). All patients, regardless of treatment group, who had data at discharge and month 3 had insulin autoantibodies < 7 μ U/ml and islet antigen 2 values of < 0.75 μ U/ml (no infant developed insulin antibodies).

DISCUSSION

This Phase 2 study demonstrates that enteral administration of enteral rh-insulin to premature infants significantly reduced time to achieve full enteral feeding in preterm infants with a gestational age of 26–33 weeks compared to placebo. This administration has not raised any safety concerns.

The significant reduction in time to full enteral feeding in the enteral rh-insulin group compared with placebo provides evidence that orally administered insulin supports gut maturation

in premature infants. This finding is further supported by the trend of an earlier time to enteral tolerance in those receiving rh-insulin as evidenced by the decreased time to gastric residuals < 2 ml/kg in the rh-insulin group compared with placebo. The findings of the current study are supported by data from two small pilot studies that showed that enterally administered insulin promoted enteral tolerance and supported accelerated enteral feeding in premature infants [9]. In addition to these studies with supplemented insulin, IGF-1 that shares its receptors with insulin was studied in a randomized trial from the Netherlands. Other studies [8] compared infant formula supplemented with insulin-like growth factor-1 with standard infant formula, and found no difference between the groups on the primary endpoints of number of days to full enteral feeding, number of days to regain birth weight, or growth rate [8]. The group speculated it was not insulin-like growth factor-1 alone but either another growth factor or combination of trophic factors and hormones, found in human milk, which was responsible for the thriving of milk-fed infants. Our results suggest that insulin maybe that missing factor.

Accelerating the time to full enteral feeding among premature babies has short-term clinical benefits and long-term clinical implications. In the short term, it means a decrease in exposure to parenteral feeding and associated complications. It also enables the provision of optimal nutrition, allowing a premature infant to achieve a rate of growth comparable to that in utero and facilitate earlier hospital discharge. Although not statistically significant there was a 19% difference in the first 28-day weight gain between the treatment groups in the present study. This difference may indicate a greater rate of catch-up growth following enteral rh-insulin administration, which in turn enabled a shorter hospital stay, although this shorter duration did not reach statistical significance.

In the long-term, catch-up growth in the post-natal period is important to prevent the long-term consequences on growth and neurodevelopmental outcomes [3,4,15-17]. The rate of growth in the first few weeks of life has been observed to have a major impact on neurodevelopmental and psychomotor outcomes in later infancy [15]. This study highlights the importance of utilizing the window of opportunity that exists between birth and hospital discharge to facilitate growth and weight gain and avoid the potential neurodevelopmental impairments and deficits in learning, behavior, and memory that have been observed in children born prematurely [4].

There was no incidence of hypoglycemia and glucose blood levels were comparable between the treatment groups. Additionally, there was no stimulation of insulin antibodies and islet antigen 2. Taken together, these data support a lack of systemic insulin exposure following enteral administration in premature infants. The incidence of adverse events was comparable between the treatment groups and there were no discontinuations, demonstrating that enteral rh-insulin administration was well tolerated.

LIMITATIONS

This exploratory Phase 2 study had a planned interim analysis that was powered accordingly with an $\alpha = 0.03$. The protocol statistical design included a study stop at interim if significance level of less than $\alpha = 0.03$, thus study was terminated post-interim analysis. Unfortunately, the small number of study participants whose data were available at the interim analysis stage limited the conclusions that could be reached on the secondary outcome measures. A good example is the large difference in time to hospital discharge in singletons (8.9 days earlier in the insulin group) that did not reach statistical significance due to the low number of infants included. Finally, our study was limited by the inclusion criteria restricting recruitment to infants being fed solely on infant formula. While necessary ethically, this tended to exclude extremely premature infants who are more likely to be fed human milk.

CONCLUSIONS

Enteral administration of insulin in very premature or premature infants can accelerate the time to full enteral feeding. The results of further studies on the role of enteral insulin using other nutrition sources such as human milk and in studies in extremely premature infants are needed to verify and extend our findings.

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The study sponsor collaborated in the design of the study, which was funded by the Clinical Research Associate Duet Medical Consulting Ltd and Techo STAT Ltd. to conduct the statistical analyses of the study. In collaboration with the authors, the study sponsor was involved in the interpretation of the data.

CONFLICTS OF INTEREST

Dr. Shehadeh and Dr. Shamir were medical consultants for Nutrinia Ltd and held shares in Nutrinia Ltd.

Correspondence

Dr. N. Shehadeh

Dept. of Pediatrics A, Rambam Health Care Campus, Haifa 3109601, Israel

Phone: (972-4) 854-2646; (972-4) 777-1606

Fax: (972-4) 854-2441

email: n_shehadeh@rambam.health.gov.il; n_shehadeh@rhc.gov.il

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There is no such thing as a self-made man. We are made up of thousands of others. Everyone who has ever done a kind deed for us, or spoken one word of encouragement to us, has entered into the make-up of our character and of our thoughts.

George Matthew Adams (1878–1962), American newspaper columnist

I'd rather see a sermon than hear one any day; I'd rather one should walk with me than merely tell the way.

Edgar Guest (1881–1959), British-born American poet who became known as the People's Poet