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Improved lung function at age 6 in children born very preterm and fed extra protein post discharge

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Abbreviations:

BPD:	bronchopulmonary dysplasia, need of respiratory assistance with oxygen at least until PMA 36 weeks
BW:	birth weight
CA:	corrected age
FeNO:	fractional exhaled nitrogen oxide
FHM:	fortified human milk
GA:	gestational age
PF:	preterm formula
PMA:	post menstrual age

Ppb: parts per billion
Rocc: airway occlusion with interrupter technique
SGA: small for gestational age
sRaw: specific airways resistance
Reff: effective airway resistance
UHM: unfortified human milk
VPI: very preterm born infants
Rapid growth: weight growth with an increase in weight z-score > 1 SD from PMA 34 to 2 month CA
Abstract page

Toftlund L, Halken S, Agertoft L, Zachariassen G

Improved lung function at age 6 in children born very preterm and fed extra protein post-discharge

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Background: In very preterm-born children alveolar maturation is challenged and lung function is often compromised during childhood. So far, very few studies have focused on type of early nutrition and lung function in children born preterm.

Methods: This study is a six years follow-up of 281 very preterm-born infants (VPI) with a gestational age (GA) < 32+0 weeks. Infants breastfed at discharge from hospital were randomized to unfortified (UHM) or fortified (FHM) mother's (human) milk, whereas those not breastfed received a preterm formula (PF). The intervention lasted until 4 months corrected age. At six years of age fractional exhaled nitric oxide (FeNO), airway resistance and occlusion measurements with reversibility were performed. Data on predisposition to asthma and allergy as well as possible allergic symptoms of the child were obtained with questionnaires.

Results: Outcome data was fully or partially available on 160 (66.9%) of 239 children. This included 49 (30.6%) children fed UHM, 58 (36,3%) fed FHM, and 53 (33,1%) fed PF. Successful FeNO measurements were obtained in 119 (74.4%) children and airway resistance measurements in 160. FeNO results were not significantly different between feeding groups. Children fed a protein enriched diet (FMH/PF) had the lowest, i.e. best, airway resistance; FHM-fed had lower values than UHM-fed (p=0.042) before, and PF-fed had significantly lower values than UHM-fed after beta-2-agonist inhalation (P=0.050). The tendency of lower airway resistance when protein enriched were the same in gender specific analyses. In SGA children the same tendency was found between PF- and UHM-fed (P=0.007 before and P=0.046 after beta-2-agonist inhalation). All values were within reference limits.

Conclusions:
Lung function in very preterm-born children may improve when fed a protein enriched nutrition post-discharge.

Key words: FeNO, follow-up, children, lung function, nutrition, preterm, sRaw, 6-years,

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Introduction

Lung growth accelerates during the third trimester of pregnancy, and alveolar growth continues until term birth, why very preterm birth affects lung maturation(1). In fetal sheep intra-uterine-growth-retardation results in decreased alveolarization and pulmonary vascular growth(2). Lung function in preterm-born children depends on the degree of immaturity and the treatment provided in the neonatal intensive care unit (NICU). Since the 1990's, the survival rate of very preterm-born infants (VPI) has increased significantly due to use of surfactant and improved ventilatory-treatments(3, 4).

Very preterm birth often leads to a need of respiratory assistance, which can cause lung damage such as bronchopulmonary dysplasia (BPD) and impaired lung function(5). At school age very preterm-born children may have impaired lung function(6), more frequently suffer from wheezing than term-born children(7), and have increased incidence of asthma (8, 9). When being born very preterm and small for gestational age (SGA), asthma is more common, compared to children born at term or large for gestational age(10).

Type of nutrition may affect lung development(11), and rapid weight gain may increase the risk of asthma(12). Though studies of preterm-born children focus on the risk of developing asthma(13-15), only few studies involve early nutrition as an intervention(15, 16), and fewer studies have focused on the consequences of type of nutrition given after hospital discharge(17). In preterm-born infants with BPD, no influence of increased intake of energy on respiratory outcomes has been found(18). Among non-BPD preterm-born infants, wheezing at one year of age was not associated with post-discharge nutrition (19).

Measurement of fractional exhaled nitric oxide (FeNO) is a non-invasive method to screen for airway inflammation in asthma. FeNO is synthesised and expressed by airway epithelial cells as an inflammatory response, and the value can be increased two-three fold in patients with asthma(20).

A whole-body plethysmography is a non-invasive procedure to measure specific (sRaw) and effective (Reff) airway resistance by simultaneous recording of airflow and plethysmographic volume during tidal breathing(21). Plethysmographic airway resistance measurement is accepted as a useful method for discriminating lung disease in young children(22).

Since the type of nutrition may affect lung development our hypothesis was that protein enrichment of post-discharge feeding (preterm formula (PF) or enriched mother's milk (FHM)) might improve lung function in preterm-born children.

Thus, this study intended to determine whether the type of post-discharge nutrition affects lung function in 6-year-old children born very preterm by measuring airway resistance and FeNO.

Method

From 2004-2008 VPI's (n=320) with gestational age (GA) ≤32+0 weeks from 4 neonatal units in Denmark were included in a prospective, randomized clinical trial on nutrition from discharge to 4 months corrected age (Figure 1). During hospital stay all infants received mother's own milk or donor bank milk until they reached post-menstrual age (PMA) of at least 30 weeks. In addition, all children received fortification according to existing national standards. From PMA = 32 weeks children continued receiving mothers own milk if breastfed, or if breastfeeding was not sufficient or possible, the infant received a preterm formula (not randomized) (68 kcal, 2 g protein/100 ml). Before discharge the infants fed human milk were randomized to supplementation with human milk fortification (17.5 kcal, 1.375 g protein/ 5 packets (daily supplementation)) or not. The 3 nutrition groups were 1) unfortified human milk (UHM), 2) fortified human milk (FHM), and 3) preterm formula (PF). Children with BPD (n=9) and diseases influencing nutritional status such as necrotizising enterocolitis, chromosomal anomalies and intra ventricular haemorrhage were excluded(23). No children were excluded due to changes of feeding regimen.

At 6 years corrected age (CA) a follow-up program including 1) a questionnaire based interview with clinical data as regards predisposition to asthma and allergy, respiratory and possible allergic symptoms, environmental exposures including parental tobacco smoking, and social status, 2) FeNO measurements and 3) airway resistance measurements. FeNO was measured using a single breath NiOX MINO (Aerocrine AB, Stockholm, Sweden) and a few tests were performed using Exhaled Breath Analyzer (Aerocrine AB, Stockholm, Sweden) due to replacement of equipment.

Two persons carefully performed the measurements according to a standard procedure. If the child did not succeed another attempt was offered later. Furthermore, airway resistance was measured by means of specific airway resistance (sRaw) effective airway resistance (Reff) and occlusion technique (Rocc) using whole-body plethysmography (Jaeger Masterscreen Bodybox, Carefusion, Würzburg, Germany, with JLAB version 5.21 software), in accordance with ERS/ATS recommendations(22). The value of Reff is calculated from multiple points throughout the breathing cycle, whereas sRaw is measured between points of maximum plethysmographic pressure (24). The children were thoroughly instructed by one of two persons and according to a standard procedure. The breathing frequency of the child was aimed for 30 to 45 breath/min. Computer-generated technically acceptable loops were used to calculate median sRaw and median Reff from 10 loops with similar configurations and inclinations. An occlusion was applied during expiration to measure the occlusion or interrupter resistance (Rocc). When measuring Rocc, children were instructed to keep hands on their cheeks, and the body-box was opened.

Reversibility test was performed using a β_2-agonist with four doses of Salbutamol (0.1 mg/dose) administered by a spacer (OptiChamber advantage) with a mouthpiece. The plethysmographic procedures were repeated 15-20 minutes after inhalation of β_2-agonist. The best outcome value (sRaw best and Rocc best) was calculated by the plethysmographic software. All airway resistance measurements were performed using the same equipment at Hans Christian Andersen Children's Hospital, Odense University Hospital.

Social status was evaluated according to the parent's education and employment.

Early rapid weight growth was defined as weight growth with an increase in weight z-score > 1 SD from PMA 34 to 2 months CA, and correlated to airway resistance.

Statistics:

Student's t-test was used for comparison if data were continuous and chi^2 test if data were categorical. A multiple regression model was used to perform further analyses with adjustment for possible confounders; gender, birth weight (BW), GA, social status, parental tobacco smoking and predisposition to asthma and allergy. Airway resistance outcomes were analysed according to a reference of healthy term born children(25). Outcome variables were tested for normal distribution. Rocc data were not normal distributed and were therefore logarithmically transformed. The regression models were tested for co-linearity and outliers. One outlier was found in FeNO

measurements and was removed in analyses. Analyses were calculated as intention to treat. All statistical tests were performed by the use of STATA 14.

Ethics:

The primary study was approved by the Danish Ethical Committee (J. Nr.VF20030208) and several amendments have been approved during the 6 years follow-up. Moreover, approval from the Danish Data Protection Agency has been obtained, latest with a renewal in 2016 (J. Nr. 2016-41-4921). The study has been reported to clinicaltrial.gov and accepted for release with journal number SDU-2014-LHT. All parents gave informed consent before examinations.

Results

A total of 281 children were eligible for the 6 years examination, and a total of 239 (85%) participated, 160 (66,9) children performed the body plethysmography and 119 (49,8) performed the FeNO measurement.

Baseline characteristics of the children were similar within groups except from numbers of multiple births and age at follow-up being lower in the UHM-fed group, and the numbers of SGA born children were higher in the PF-fed group. Further, parents to PF-fed children were more often tobacco smokers, PF-fed children were more predisposed to asthma, and finally, PF-fed children belonged to lower social status (table 1). Dropouts were significantly more frequent among boys and those with a low social status (table 1).

The FeNO measurement was difficult for some of the children and 143 of 239 children failed the procedure first time. By a second attempt 4-6 months later another 23 succeeded (figure 1). The median FeNO was similar in the groups. Removal of one outlier, FeNO = 70 ppb, in the FHM group, did not affect median FeNO. PF-fed boys had significantly higher FeNO as compared to FHM-fed boys (P=0.021). A total of 38 children were born SGA and they had very similar FeNO values, but we found a slightly higher median FeNO when PF-fed as compared with UHM fed (p 0.05). Only 4 children had FeNO values above normal limit (20 ppb), all UHM-fed girls (table 2).

The specific airway resistance measurements showed that children receiving extra protein (formula or fortification) had lower mean sRaw before as well as after (figure 2, 3) inhalation of β_2-agonist compared to exclusively breastfed. This was significant when comparing UHM- versus FHM-fed

before inhalation (p=0.042), and comparing UHM- with PF-fed after inhalation of β_2-agonist (p=0.050). FHM- and PF-fed had similar mean values both before and after inhalation of β_2-agonist. All feeding groups were within reference limits before reversibility, and all groups had lower mean sRaw values after reversibility. UHM-fed girls had significantly higher sRaw after β_2-agonist compared to PF-fed girls (0.024). Similar to the analyses of the entire groups, protein enriched SGA born children had lower mean sRaw compared to exclusively breastfed SGA children, this being significant between UHM- versus PF-fed before (P=0.007) as well as after inhalation of β_2-agonist (P=0.046) (table 2).

Results from Reff measurements showed similar tendencies with lower values (less resistance) when fed protein enrichment. Significantly lower mean values were found in PF-fed compared to UHM-fed children when analysing SGA children both before and after, and in girls after inhalation of β_2-agonist.

Measuring resistance with occlusion technique, showed no difference between feeding groups, neither before nor after inhalation of β_2-agonist. Also, in subgroup analyses regarding SGA and gender no difference was found according to nutrition. All nutrition groups had values of sRaw, Reff, and Rocc within reference limits and lower values after the inhalation of β_2-agonist (table 2).

A positive reversibility test defined as a reduction of more than 25% in sRaw after inhalation of β_2-agonist was found in a high proportion of children in each feeding group, highest in UHM-fed (UHM: 53%, FHM: 41%, PF: 43%), but without significant differences (table 3).

The number of children with rapid weight growth (n=81) was significantly lower in the UHM-fed group compared to the FHM- and PF-fed groups (p=0.034, p=0.046) (UHM: 35%, FHM: 53%, PF: 62%). More children in the PF-fed group (25%) had both rapid growth and a positive reversibility test as compared to UHM-fed (14%) and FHM-fed (16%), though not significant (table 3).

Predisposition to asthma and allergy did not affect any of our outcomes. Also, parental tobacco smoking did not affect the outcomes of the regression analyses.

Discussion

Several studies have found type of nutrition to affect lung development in both preterm-born animals and children. In a study by Kelly et al, preterm-born guinea pigs were given full or restricted access to nutrition(26). Restricted access resulted in decreased protein synthesis, but also an unchanged protein breakdown, why in terms of growth and repair capacity of the lungs; they consider protein to play an important role. A review by Bhatia and Parish stated that correct

postnatal supply of several nutrients is required for optimal lung development(27). Protein supply has been shown to improve lung function when given during hospital stay(28). We found lung function, expressed by airway resistance (sRaw, Reff and Rocc), to be within normal range regardless of feeding group. Meanwhile, those receiving protein supplementation to human milk or preterm formula had non-significantly better lung function compared to non-enriched children. As the lung function in FHM-fed (protein-enriched) children is only slightly and not significantly better than UHM-fed, one could speculate that the amounts of fortifier used were too small or the influence of these nutritional differences post-discharge were clinically insignificant. Though, since all results are within normal range(25), the explanation could also be that protein supplementations during hospital stay might be more important than post-discharge supplementation.
Hyper reactivity of the airways is known to occur frequently in children born very preterm(1) though primarily in children with BPD, and it can be demonstrated by a positive reversibility test with a decrease in sRaw $\geq$ 25% after inhalation of a β_2-agonist(21). We found that almost half of the children had a positive reversibility test. This hyper reactivity may indicate that there can be an increased risk for development of lung disease in a cohort of very preterm-born children without BPD or other severe complicating conditions.

In preterm-born children (GA<30 weeks) Kriemler(29) showed a decrease in lung function measured at 5-7 years of age, and in a meta-analysis Sonnenschien(30) found that low GA (<37 weeks) was associated with asthma. These studies demonstrated that preterm birth may have a negative impact on lung function, but they did not focus on nutrition before or after discharge. Our results show that SGA born children have lung function similar to the lung function of the non-SGA-born children. The tendency of lower airway resistance in PF-fed was similar in SGA and non-SGA children. They all seemed to benefit from protein enrichment during hospitalization or post-discharge.

In our cohort, PF-feeding was associated with rapid growth during hospital stay and lower mean sRaw. A correlation of rapid growth and improvement in lung function compared to not rapid growth has been shown in a study of Ehrenkranz RA et al including 18-22 months old preterm-born children(31). On the contrary, increased weight gain during the first year of life has been correlated with asthma in a recent meta-analysis (12). In our cohort, 81 infants had rapid growth, and 29 of those also had hyper-reactive airways. Meanwhile, metabolic abnormalities have also been

correlated to early rapid weight growth(32), and a balance may be needed to improve growth and lung development without increasing risk of later diseases.

Plethysmographic measurements has become a useful tool for identifying airway obstruction and reversibility in children from 2 years of age(21). In our study, some of the children were unable to succeed with the plethysmographic and FeNO measurements at the first attempt, but succeeded later on. These findings are not unexpected given the complexity of the tests and the age of the children studied.

In spite of the PF children being more predisposed to asthma and more often had parents who were tobacco smokers, and had lower social status, their mean lung resistance was low. This could be caused by a higher protein intake as compared to the other groups. Meanwhile the clinical relevance is unknown.

The strength of this study is the prospective randomized design allowing infants to continue breastfeeding. Also, children suffering from BPD were excluded leaving only lung function in healthy children to be analysed according to nutrition. Thus, it can also be regarded as a limitation that the nutritional impact on those having chronic lung diseases is omitted. Other limitations of the study are the relatively small number of participants, stratified into 3 groups. Therefore, significant difference would be difficult to achieve.

Conclusions

Lung function as measured by airway resistance (sRaw, Reff and Rocc) in children fed preterm formula or fortified mother's milk were not significantly different compared to those exclusively breastfed. This was also the case for SGA-born children. All lung function results remained within the normal reference ranges. Therefore, post-discharge fortification may not be necessary if protein enrichment and growth rates before discharge are optimal. A much larger study will be necessary to confirm this suspicion. In spite of airway resistance within normal ranges, hyper reactivity was very common in the entire study population. Long-term follow-up studies are needed to gain more knowledge about development of lung function in relation to early nutrition and growth in very preterm-born children after the age of six years.

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Table 1: Characteristics and comparison of children and dropouts, N=160

	UHM (N=49)	FHM (N=58)	PF (N=53)	P (UHM vs FHM)	P (UHM vs PF)	P (FHM vs PF)
GA at birth median (min-max)[a]						
- Days	207	214	208	0.29	0.34	0.82
- Min-max	(180-224)	(171-224)	(186-221)			
- Weeks+days	29+4	30+4	29+5			
Birth weight grams median[a] (min-max)	1273 (590-2255)	1335 (690-2024)	1294 (612-2030)	0.41	0.70	0.16
SGA n/N (%)[b]	13 (33)	9 (17)	11 (38)	0.06	0.70	0.031
Boys n/N (%)[b]	22 (45)	25 (43)	31 (58)	0.85	0.17	0.11
Multiple birth, n/N (%)[b]	10 (20)	25 (43)	21 (40)	0.013	0.035	0.71
Height at 6 years cm, mean (SD)[a]	121 (5.7)	120 (5.2)	119 (5.3)	0.14	0.40	0.50
Age at follow-up years, mean (SD)[a]	6.5 (0.5)	6.2 (0.4)	6.3 (0.1)	0.008	0.09	0.34
Social status at follow up n/N (%)[b]						
Low (3-5)	24 (49)	23 (40)	35 (66)	0.33	0.08	0.005
Maternal smoking n/N (%)[b]	5 (11)	9 (16)	18 (36)	0.43	0.004	0.021
Mothers age at child's birth, years Median (min-max)[a]	31 (20-39)	30 (23-42)	30 (19-46)	0.67	0.98	0.68
Fathers smoking n/N (%)[b]	12 (28)	15 (28)	14 (30)	0.99	0.79	0.77
Fathers age at child's birth, years Median (min-max)[a]	32 (20-45)	33.5 (27-54)	32 (21-54)	0.10	0.89	0.09
Familiar predispositions n[b]						
- Asthma	14 (30)	9 (16)	17 (35)	0.08	0.66	0.027
- Allergy	21 (46)	30 (54)	14 (29)	0.43	0.09	0.010
Characteristics and comparison of dropouts						
	UHM (n=40)	FHM (n=39)	PF (n=42)	Dropouts (N=121)	Study group (N=160)	*(P)*
GA, days median (min-max)	207 (180-224)	213 (169-224)	207 (175-223)	206	207	0.57
BW g median (min-max)	1315 (548-2058)	1258 (535-1953)	1226 (630-2140)	1300	1305	0.91
SGA n/N (%)						
Boys n/N (%)	28 (70)	23 (59)	25 (60)	76 (63)	78 (49)	0.019
Social status at follow up n/N (%) - Low	27 (68)	28 (72)	24 (57)	79 (65)	82 (51)	0.018
Multiple birth n/N (%)	11 (28)	15 (38)	19 (45)	45 (37)	56 (35)	0.71

[a] t-test if continuous variables, [b] chi^2-test if categorical variables. Social status low=3-5, high=1, 2. No difference between feeding groups were found in dropouts.

Table 2: Results from airway examinations according to nutrition

	N in regression	UHM	FHM	PF	P (UHM-FHM)	P (UHM-PF)	P (FHM-PF)
FeNO, ppb median (min-max)	98	8 (2-16)	8 (5-35)[a]	9 (5-20)	0.40	0.045	0.22
- Boys		8 (5-12)	7 (5-15)	9 (5-20)	0.41	0.13	0.021
- Girls		8 (2-16)	8 (5-35)	8 (5-19)	0.30	0.30	0.91
- SGA		9 (6-12)	7 (5-14)	9 (5-19)	0.57	0.05	0.22
sRaw before β_2 kPa*s mean (SD)	160	1.20 (0.3)	1.07 (0.3)	1.08 (0.3)	0.042	0.09	0.79
- Boys		1.24 (0.33)	1.13 (0.8)	1.16 (0.25)	0.32	0.52	0.68
- Girls		1.17 (0.30)	1.02 (0.28)	0.98 (0.35)	0.07	0.08	0.88
- SGA		1.3 (0.27)	1.1 (0.27)	1.0 (0.29)	0.39	0.007	0.07
sRaw after β_2 kPa*s mean (SD)	154	0.86 (0.2)	0.80 (0.2)	0.78 (0.2)	0.12	0.050	0.60
- Boys		0.9 (0.24)	0.85 (0.22)	0.86 (0.20)	0.38	0.50	0.82
- Girls		0.83 (0.22)	0.76 (0.23)	0.67 (0.16)	0.18	0.024	0.28
- SGA		0.9 (0.22)	0.9 (0.33)	0.7 (0.17)	0.68	0.046	0.12
Reff before β_2 kPa*s mean (SD)	159	1.03 (0.28)	0.91 (0.27)	0.93 (0.30)	0.07	0.17	0.71
- Boys		1.06 (0.29)	0.97 (0.26)	1.01 (0.24)	0.37	0.74	0.53
- Girls		1.00 (0.27)	0.87 (0.28)	0.82 (034)	0.93	0.10	0.89
- SGA		1.12 (0.25)	0.99 (0.26)	0.80 (0.28)	0.38	0.007	0.08
Reff after β_2 kPa*s mean (SD)	154	0.68 (0.17)	0.64 (0.22)	0.61 (0.20)	0.33	0.07	0.38
- Boys		0.71 (0.16)	0.69 (0.21)	0.69 (0.18)	0.77	0.78	0.99
- Girls		0.67 (0.19)	0.61 (0.22)	0.51 (0.17)	0.25	0.024	0.21
- SGA		0.72 (0.19)	0.73 (0.30)	0.53 (0.19)	0.67	0.035	0.10
Rocc before β_2 kPa*L^{-1}*s^{-1} median (min-max)	159	1.00 (0.64-1.66)	0.98 (0.69-1.96)	1.01 (0.44-1.66)	0.85	0.79	0.64
- Boys		0.99 (0.64-1.23)	0.92 (.69-1.96)	1 (0.44-1.35)	0.82	0.74	0.54
Girls		1.02 (0.79-1.66)	1.00 (0.7-1.73)	1.06 (0.51-1.66)	0.94	0.95	1.00
- SGA		1.08 (0.85-1.4)	1.06 (0.81-1.42)	0.95 (0.44-1.66)	0.64	0.17	0.06
Rocc after β_2 kPa*L^{-1}*s^{-1} median (min-max)	155	0.85 (0.55-1.56)	0.80 (0.61-1.74)	0.83 (0.56-1.13)	0.93	0.78	0.70
- Boys		0.83 (.55-10.01)	0.78 (0.61-1.74)	0.79 (.56-1.11)	0.80	0.49	0.65
- Girls		0.86 (0.6-1.19)	0.83 (0.64-1.49)	0.9 (0.69-1.13)	0.96	0.88	0.91
- SGA		0.86 (0.71-1.05)	0.84 (0.7-1.15)	0.84 (0.59-.1)	0.83	0.39	0.26

Regression analyses adjusted for nutrition, GA, BW, height at 6 year follow up, gender, parents smoking. Predispositions to allergy and asthma did not influence the results. [a]Range of 5-71 including one outlier on 71, outlier removed from the regression model. Reference value of mean sRaw = 1.3 kPa*s(21)

Table 3: Rapid growth and hyper reactive airways according to nutrition groups

	UHM N=49	FHM N=58	PF N=53	P (UHM vs FHM)	P (UHM vs PF)	P (FHM vs PF)
Rapid growth n (%) (N=160)	17 (35)	31 (53)	33 (62)	0.034	0.046	0.96
Hyper reactivity n (%) (N=154)	26 (53)	24 (41)	23 (43)	0.38	0.53	0.83
Combined n (%) (N=154)	7 (14)	9 (16)	13 (25)	0.51	0.13	0.36

Regression analysis adjusted for nutrition, gestational age, gender, birth weight, predispositions to allergies and asthma, parents tobacco smoking, combined=children with rapid weight growth AND hyper reactive airways

Figure legends

Figure 1: Flowchart
CP: cerebral palsy, ---- indicates beginning of 6 years follow-up

Figure 2: Airway resistance test (sRaw) according to mean height and reversibility test
Normal reference approximate 1-1.75 kPa/s, increasing height leads to a small decrease in sRaw (25)

Figure 3: Box plot of mean sRaw before and after reversibility according to nutrition
Normal reference approximate 1-1.75 kPa/s, increasing height leads to a small decrease in sRaw (25)

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