Appendix 1: Population Characteristics – Accelerometry - Spirometry

Recruitment and followup: GINIplus

A detailed overview of the recruitment protocol for GINIPlus ("German Infant Study on the influence of Nutrition Intervention (Plus environmental and genetic influences) on allergy development") is available at the study's website [1] and has been published previously. [2-4] GINIplus is a population-based prospective birth cohort consisting of two arms: one interventional and one observational. GINIPlus15 is the 15-year followup.

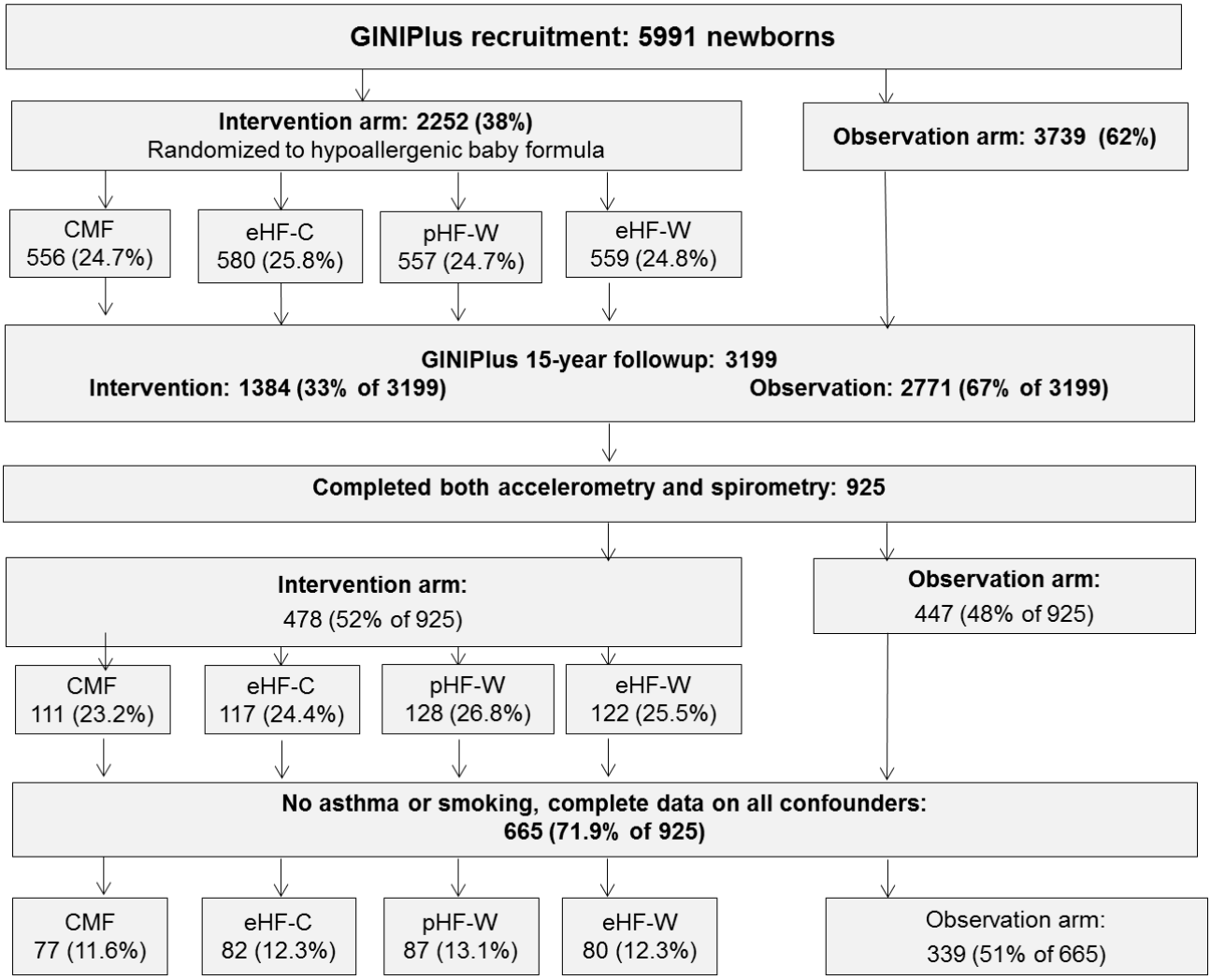
5991 healthy, full-term newborns were recruited between September 1995 and June 1998 (n=2949 in Munich, n=3042 in Wesel). Those with at least one parent or biological sibling with allergic disease were recruited for the nutritional intervention. 3739 unselected infants were recruited for the observational arm and given no formula (Figure 1a, Appendix 1). The intervention was a randomized, double-blind controlled trial of feeding with one of 3 hydrolysed formulas (partially or completely hydrolysed whey, or extensively hydrolysed casein: pHF-W, CHF-W, or eHF-C) versus cow's milk formula (CMF) during first 4 months of life (n=1165 in Munich, n=1087 in Wesel). Details on randomization and blinding have been previously published.[3-6] Following current recommendations, breastfeeding was encouraged for all families including those children enrolled in the intervention arm.

Followups included physical examinations and personal interviews at 1, 4, 8, 12 , 24 and 36 months, and at ages 6, 10 and 15; regular blood and urine tests for biomarkers; and questionnaires filled out by the child (age 10 and 15), the parents or both. For a detailed followup schedule see the website and previous publications. [2-4]

Attempts were made to contact each family by postal mail and telephone when the subject was 15 years old (Figure 1 main text). Of the 5991 infants recruited for GINIplus, 3199 were successfully followed up by questionnaire and/or physical examination at age 15. The current study contains 655 children from GINIplus, of whom 49% received any intervention compared with only 38% of the original cohort. Since the intervention arm was restricted to children with a family history of allergic disease, this may represent ascertainment bias, greater health-consciousness, healthcare utilization or all of these.

Appendix 1: Population Characteristics – Accelerometry - Spirometry

Figure 1a: Recruitment and followup of GINIPlus



However, the four study formulas were present in roughly equal numbers in our subsample: the largest difference was for partially hydrolysed whey in girls. 26.4% of the study population that was given an intervention got pHF-W, compared with 24.7% of all GINIplus intervention subjects. This formula was not associated with asthma development [3] and thus differential exclusion due to asthma did not take place. We find that while our sample oversamples the intervention arm of GINIplus, there was no bias toward any specific formula.

Appendix 1: Population Characteristics – Accelerometry - Spirometry

Recruitment protocol: LISAplus

A detailed overview of the recruitment and followup protocols for LISAPlus (Lifestyle-Immune System-Allergy: Influence of life-style factors on the development of the immune system and allergies in East and West Germany (Plus the influence of traffic emissions and genetics)) is available at the study's website [7] and has been published previously. [8-10] LISAplus is a prospective birth cohort in 4 regions of the former East and West Germany (Munich, Leipzig, Wesel, and Bad Honnef) instigated to examine the relationships between immune functioning and environmental and lifestyle exposures throughout life. No intervention, nutritional or other, was used in LISAplus.

3097 healthy, full-term newborns were recruited between November 1997 and January 1999 (n= 1467 from Munich, 976 from Leipzig, 348 from Wesel, and 306 from Bad Honnef) of which 1534 (50%) were followed up at age 15; of these 1107 were from Munich (930) and Wesel (177). The current study samples only these children, since accelerometry was not offered to the study centers in Bad Honnef and Leipzig (Figure 1 main text).

Followups took place regularly on a similar schedule to that for GINIplus; for details see the website and previous publications. [8-10] Questionnaires were given monthly during the first year of life, every 6 months until age 2 years and thereafter at age 4, 6, 10 and 15 years, while medical examinations took place at ages 2, 6, 10 and 15. Data on biomarkers (blood and urine) were also collected but not used in the current study.

Bias by Dropout

Dropout was very similar between GINIplus and LISAplus Munich and Wesel at all stages of the study; see Figure 1 (main text) and Tables 1b and 1c, Appendix 1. Ultimately 13% of GINIPlus and 11% of LISAPlus Munich/Wesel was included in the statistical models. While there was no strong bias towards preferential dropout in our cohort compared to the rest of the 15-year followup (Table 1b) the 15-year followup differed from the initial cohort with respect to contribution from Munich, education of the parents, and smoke exposure by the mother during and after pregnancy suggesting differential loss to followup (Table 1c.)

Appendix 1: Population Characteristics – Accelerometry - Spirometry

Table 1b: Study Population within GINIplus and LISAplus 15-Year Followup

	Study Population		GINIplus15		LISAplus15		P for selection (study population vs. all of 15-year followup) if <0.05	
N, % of total	895, 100		3199, 53		1107, 61		--	
Age at exam	15.2 (0.25)	15.2 (0.27)	15.3 (0.31)	15.3 (0.31)	15.1 (0.31)	15.2 (0.34)	0.002	--
Male (N, %)	401, 45		1607, 50		591, 53		<0.0001	
Height, cm	176 (7.6)	167 (6.2)	177 (7.5)	167 (6.2)	176 (7.4)	166 (6.5)	--	--
Weight, kg	64.4 (12)	58.7 (9.6)	65.5 (13)	59.2 (10)	63.5 (12)	57.5 (9.6)	--	--
BMI	20.6 (3.1)	20.9 (2.9)	20.9 (3.4)	21.1 (3.1)	20.5 (3.1)	20.7 (3.0)	--	--
GINIplus (%)	74	76	100	100	0	0	--	--
Nutritional intervention (%)[2] P for global null							--	--
CMF	7.98	9.11	11.0	9.99	0	0	**	**
s1 pHF-W	9.73	9.72	11.5	10.6	0	0	**	**
s2 eHF-W	9.73	8.30	11.1	10.7	0	0	**	**
s3 eHF-C	8.48	9.72	10.6	11.1	0	0	**	**
None (control)	64.1	63.2	55.8	57.7	100	100	**	**
FEV1 z-score	-0.55 (0.99)	-0.48 (0.91)	-0.62 (0.96)	-0.53 (0.88)	-0.46 (1.0)	-0.44 (0.92)	--	--
FVC z-score	-0.56 (0.95)	-0.43 (0.91)	-0.59 (0.93)	-0.47 (0.88)	-0.44 (1.0)	-0.39 (0.95)	--	--
FEV1/FVC z-score	-0.06 (0.94)	-0.075 (0.96)	-0.11 (1.0)	-0.10 (1.0)	-0.072 (1.0)	-0.088 (0.92)	--	--
Daily minutes MVPA	44.9 (21)	35.6 (19)	43.8 (21)	37.5 (23)	49.9 (25)	38.0 (21)	--	0.021
Any leisure sport (%)	77	80	76	79	76	81	--	--
Asthma at age 15 (%)								
Confirmed	0	0	3.38	2.54	3.88	2.33	*	*
Denied	100	100	49.8	52.9	57.6	57.2	*	*
Missing data	0	0	46.8	44.6	38.6	40.4	*	*
Smoking at age 15 (%)								
Confirmed	0	0	4.6	4.5	6.0	6.0	*	*
Denied	100	100	75	81	90	87	*	*
Missing data	0	0	20	15	4.2	6.6	*	*
Completed a physical exam	100	100	61	65	58	61	*	*
Valid accelerometry	100	100	30	36	30	34	*	*
Valid spirometry	100	100	54	60	50	52	*	*

P-value from unequal-variance T-test for normally distributed variables (spirometric z-scores); Kruskal-Wallis test for categorical variables (nutritional intervention global null hypothesis); Wilcoxon's two-tailed rank-sum test for binary and otherwise non-normal variables (all others.)
* if p-value not given because characteristic was used for inclusion,
** if pairwise comparison inappropriate (see global null),
-- if p>0.05
Moderate, vigorous and moderate-to-vigorous PA (MVPA) imputed for diaried nonwear time due to sport. Accelerometric cutpoints from [11, 12]. Spirometric z-scores from [13]

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Appendix 1: Population Characteristics – Accelerometry - Spirometry

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Table 1c: Study Population within GINIplus and LISAplus at Birth

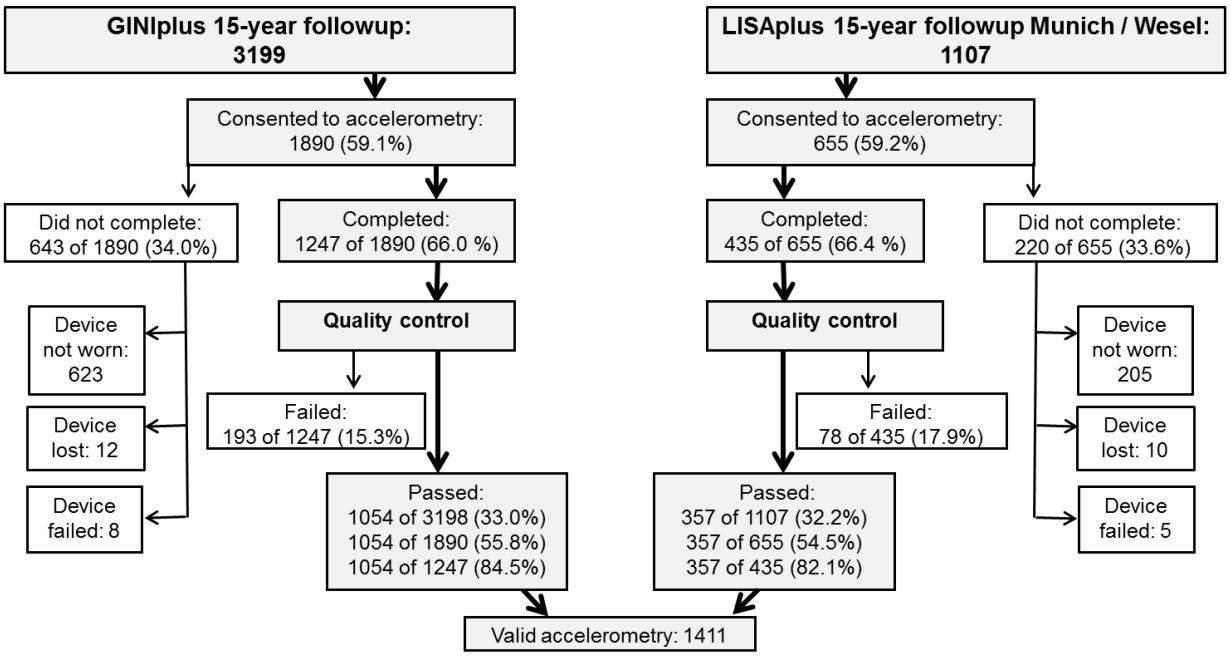
	Study population		GINIplus: Munich and Wesel		LISAplus: Munich and Wesel		P for selection (study population vs. all of GINILISA Munich and Wesel at birth) if <0.05	
	Boys	Girls	Boys	Girls	Boys	Girls	Boys	Girls
N	895		5991		1812		--	--
Male (N, %)	401, 45		2991, 51		954, 53		<0.0001	
Birthdate	3 July 1997	7 May 1997	18 Nov 1996	21 Nov 1996	2 Aug 1998	3 Aug 1998	<0.0001	0.033
Parents highly educated[1] (%)	68	70	59	63	76	74	0.033	0.025
Study center Munich (%)	61	54	49	48	81	81	0.041	--
Nutritional intervention (%)[2] P for global null							<0.0001	<0.0001
CMF	7.98	9.11	9.80	9.26	0	0	**	**
s1 pHF-W	9.73	9.72	9.70	9.40	0	0	**	**
s2 eHF-W	9.73	8.30	9.70	9.48	0	0	**	**
s3 eHF-C	8.48	9.72	10.0	9.86	0	0	**	**
None	64.1	63.2	60.8	62.0	100	100	**	**
Birthweight (g)	3526 (443)	3422 (451)	3,544 (478)	3,393 (439)	3,513 (437)	3395 (428)	--	--
Exclusively breastfed (%) P for global null							<0.0001	<0.0001
Never	34.7	35.2	48.3	45.1	38.6	38.3	**	**
Months 1-4 only	10.2	9.11	9.05	10.8	11.9	11.3	**	**
Past month 4	55.1	55.7	42.7	44.1	49.4	50.3	**	**
Mother smoked tobacco when pregnant	9.47	10.3	17.3	18.2	15.5	16.2	<0.0001	<0.0001
Tobacco smoke at home up to age 6	30.9	30.4	35.4	34.3	42.5	44.1	0.071	0.0021

GINIplus = German Infant Study on the influence of Nutrition Intervention (Plus environmental and genetic influences) on allergy development; LISAplus = Lifestyle-Immune System-Allergy: Influence of life-style factors on the development of the immune system and allergies in East and West Germany (Plus the influence of traffic emissions and genetics.)
For details see paper or [2-4] for GINIplus, [7, 8, 10] for LISAplus.
1) Parents highly educated= at least one parent entered university
2) CMF=cows' milk formula, pHF-W= partially hydrolysed whey formula, eHF-W=extensively hydrolysed whey formula, eHF-C=extensively hydrolysed casein formula, None=no formula given. For details on formulas, selection and randomization see [2] and [3]
P-value from unequal-variance T-test for normally distributed variables (birthweight); Kruskal-Wallis test for categorical variables (nutritional intervention, breastfeeding duration global null hypothesis); Wilcoxon's two-tailed rank-sum test for binary and otherwise non-normal variables (all others.)
--if p>0.05.
** if pairwise comparison inappropriate (see global null), -- if p>0.05

Accelerometric protocol

This study followed the same protocol as Smith and Schulz [14] and Pfitzner et al. [15] each of which profiled subsets of GINIplus. Triaxial accelerometers (ActiGraph GT3X, Pensacola, Florida) were worn on the dominant hip for up to 7 days, after which device and diary were returned by mail. After consenting to accelerometry, subjects were mailed a package which contained the device, a pre-printed diary with instructions for filling it in and sample of proper methods, ([15] Figure S2) and a stamped, self-addressed envelope for returning monitors after one week of PA measurement.

Figure 1b: Accelerometry Response Rate



Activity Diary: Subjects documented each of the following events as close as possible to the time they occurred: time of waking up and going to bed; time and reason for removing one or both monitors (non-wear time) such as for showering or swimming; time and method of travel to and from school; time of starting and finishing school; time of starting and finishing school sport; and time and type of leisure-time sporting activity. Since school sport is mandatory in Germany, we considered only leisure-time sport as an indicator of active lifestyle in the current study.

Validation of days: Of a total 11,572 recorded days, 2740 (17.1%) were invalid. Most invalid days (1140, 58%) were the result of inconsistency between the diary and the NHANES weartime criteria, reflecting our high standard of data cleaning and suggesting a relatively accurate allocation of activity on the days that passed quality control. Other reasons included non-wear time issues (526 days, 26.7%), and technical issues (145 days, 7.4%). Many days were invalid for more than one reason. In addition, 271 days were excluded because the subject did not have at least 3 valid weekdays and one valid weekend day.

Validation of subjects: Subjects were required to have at least one valid weekend day of recording in addition to at least three valid weekdays. Days were required to have at least 10 hours of valid recording time to be considered valid, or as little as 7 hours if subjects were awake for less than 10 hours. (Figure 1b above)

Data Management and Quality Control: Sampling rate was set to 30 Hz and the measured accelerations stored at 1 Hz after conversion into activity counts. Activity counts of the vertical axis were assigned to the four intensity levels—sedentary, light, moderate, and vigorous physical activity—using Freedson's commonly-used cutpoints. [11, 12] Diary information was digitized using a 7-day template and reviewed by a second study assistant to avoid transcription errors.

Spirometric protocol

Spirometry was performed at the 15-year followup physical examinations for GINIplus and LISAplus. Measurements were performed in line with ATS/ERS recommendations (Miller et al, 2005[16]) using a pneumotachograph-type spirometer (EasyOne Worldspirometer, ndd, Zurich, Switzerland), calibrated daily before spirometry with a 3-L calibration pump supplied by the manufacturer.

Subjects were seated while wearing nose clips. They performed at least three but not more than eight trials per test under the guidance of trained and experienced examiners in order to obtain optimal flow-volume curves. Both flow-volume and volume-time curves were monitored by the examiner and visible to the participant to enable guided support of the participant.

Appendix 1: Population Characteristics – Accelerometry - Spirometry

Based on ATS/ERS acceptability criteria[16] and as recommended by [17] all tests were visually inspected by physicians to exclude manoeuvres performed incorrectly or with artefacts. Spirometric indices were taken from the best manoeuvre with the largest sum of FEV1 and FVC. Further parameters evaluated were the ratio of FEV1 and FVC (FEV1/FVC), peak expiratory flow (PEF), forced expiratory flow rates at 25, 50 and 75% of exhaled FVC (FEF25, FEF50 and FEF75) and the mean flow rate between 25 and 75% of FVC (FEF2575). In total, 2878 subjects from GINIplus and LISAplus underwent spirometry, of whom 2757 (96%) passed quality control

Comparison of Spirometric Z-scores: GLI vs. LUNOKID

Z-scores were calculated for our study population from two different reference equations: those published by the Global Lung Initiative [13] and those published by the LUNOKID study of Germans.[18] Although the GLI values were consistently lower than those for LUNOKID, the correlation between the two was very strong (over 99% of variance explained; see Table 1d below.)

Table 1d: Comparison of Z-Scores

Mean (SD): expected value is 0(1)

Index	LUNOKID[18]	GLI [13]	Pearson correlation between LUNOKID and GLI
FEV1	0.109 (1.2)	-0.510 (0.94)	0.996
FVC	-0.020 (1.2)	-0.489 (0.93)	0.997
FEV1/FVC	0.244 (1.2)	-0.069 (0.95)	0.997

GLI z-scores from [13]; LUNOKID z-scores from [18]

Final study population: 1196 subjects had complete accelerometry and spirometry (Figure 1 main text), of which 1102 (92%) confirmed no asthma. Of these, 1011 confirmed abstinence from tobacco. Only complete cases were analysed, but missing data were uncommon. Of the 1011, 895 (89%) were missing no confounder and thus were included in the models.

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