Appendix 2: Statistical Methods

Statistical Methods

All analyses were done using SAS 9.2. Population characteristics (Table 1, main text; Tables 1b and 1c, Appendix 1) are given as mean (standard deviation) for centrally distributed variables, percent of total for categorical variables, and as mean (median); 5th percentile, 95th percentile for skewed variables. P-values for group comparisons were calculated with Wilcoxon's two-tailed rank-sum test for binary or otherwise non-normal variables such as VPA and weight; t- test for centrally-distributed variables such as spirometric indices and height; and Kruskal-Wallis for global null hypothesis if there were more than two categories (nutritional intervention, breastfeeding duration.)

Statistical models were fit using generalized linear modelling. Spirometric indices (FEV1, FVC, FEV1/FVC, and FEF2575) and GLI Z-scores for each were treated as normally-distributed outcomes. Inspection of Q-Q plots confirmed normality. To give larger parameter estimates, all measurements in litres were converted to millilitres before modelling, FEV1/FVC is modelled as percentage rather than decimal, and relationships with z-scores are multiplied by 1,000.

Each spirometric index or Z-score was modelled as a statistical function of a subset of confounders and also one PA measure at a time. No model contained either more than one PA measure or more than one spirometric index. To check for effect modification or confounding we created three models with different subsets of confounders; in increasing order of complexity these were the crude, basic, and main models. Confounders were chosen a priori and left in the models regardless of statistical significance.

The crude model corrected only for sex, age and height. The basic model contained these three and also weight, BMI, study centre Munich compared to Wesel, average daily accelerometer weartime, nutritional intervention, and parental education (socioeconomic status.) Lastly, the main model and all sensitivity analyses contained all of these predictors and also birthweight, breastfeeding, and pre- and postnatal tobacco-smoke exposure. For details on definitions and choice of confounders, see below.

To confirm effect homogeneity, we conducted sensitivity analyses. First, to reduce the effect of outliers we reanalysed the subset of our population (N= 743/895, 83%) without extreme values for spirometry or PA. Second, we modelled flow indices (PEF, FEF25, FEF50, and

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FEF75) in addition to the more typical FEV1, FVC, FEV1/FVC, and FEF2575. Third, we assessed potential confounding from air pollution (annual average PM2.5 and NOx at the subject's home address at age 15) when data were available. Lastly, we re-ran the models that were significant at p=0.05 using only data-driven confounders; for both FEV1 and FVC these were only gender, height, study center, and BMI. This last sensitivity analysis is not shown but results did not change.

At $p \leq 0.05$ our sample has 80% power to detect a difference as small as approximately 100 mL FEV1 or FVC between the top and bottom quintiles of MVPA. This is comparable to the effect size estimated in the literature [1],[2-5] so we choose the traditional $p \leq 0.05$ to avoid missing an effect. Strict Bonferroni correction is $p \leq 0.0003$ (three models, four spirometric indices and 12 PA measures, counting each MVPA quintile).

Choice of Confounders

Crude Model

Sex: Males are more active and have larger lungs than females at all ages, so all models were corrected for sex.

Age: Age predicts both lung function and PA, with PA declining throughout life and lung function reaching a maximum around age 18-20. While we did not expect to find a strong effect of age in this sample (age 15.2 ± 0.3 years at the time of the physical exam) we nevertheless corrected for it for consistency with other studies.

Height: Height strongly predicts lung size at all ages, and was measured objectively at the time of spirometry.

Basic Model

The basic model contained all the predictors in the crude model as well as:

Body mass index (BMI): In the basic model we further corrected for body size by considering body mass index as a predictor. BMI was calculated from height and weight (measured objectively at the physical exam) as kg/m^2, and BMI cutoffs between *underweight, normal weight, overweight,* and *obese* were chosen from the 10^{th}, 90^{th}, and 97^{th} age- and sex-specific percentiles from a large German population.[6]

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Study centre: All subjects were from either the urban environment of Munich, or the rural/suburban environment of Wesel. This difference may affect lung function, PA, or both. Study centre Munich was considered as a binary predictor in the basic and main models.

Accelerometer weartime: Since all activity takes place during accelerometer weartime, it is possible that longer wear is associated with higher apparent activity. As a result we corrected for weartime in the basic and main models.

Nutritional intervention: The GINIplus cohort was originally founded to track the effect of hydrolysed milk-protein baby formulas on later allergy development in children with at least one parent or biological sibling with a history of allergic disease, and who were thus at elevated risk of allergy. These subjects (the intervention arm) were randomized at birth in nearly equal numbers (see Appendix 1) to one of four nutritional interventions (three hydrolysed formulas, one with cows' milk) and the process of allergy development monitored over time. The observational arm of the study, consisting of a random population sample, was followed up but given no formula.

The four formulas were: partially or extensively hydrolysed whey (pHF-W, eHF-W); extensively hydrolysed casein (eHF-C) or cows' milk formula (CMF). Intervention was equally distributed among the groups with approximately 550 subjects each. For further details see [7, 8] and Appendix 1. We did not differentiate in the model between the different formulas, but instead used 2 categories, "treated" for children from the GINIplus intervention arm and "untreated" for those from the GINIplus observational arm and from LISAplus, since no intervention was used for LISAplus.

Parental education: Parental education was included as a proxy for high socioeconomic status, measuring whether the higher-educated birth parent entered college. Roughly half of subjects' families achieved this cutoff.

Main Model

The main model contained all the predictors in the basic model, and in addition some that are known to predict health in general, lung function in particular, and /or are further proxies for socioeconomic status.

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*Birthweight**:*** Both cohorts were limited to full-term births and LISAplus specifically excluded subjects with low birthweight; however, some small effect may remain and was corrected for.

Exclusive breastfeeding: Exclusive breastfeeding was modelled as a three-level categorical predictor: never, between ages 1 and 4 months only, and to the fifth month or later, as reported by the mother.

Prepartum smoking: We defined prenatal tobacco-smoke exposure as whether the mother reported smoking any cigarettes during pregnancy.

Childhood secondhand-smoke exposure: We defined childhood exposure to secondhand smoke as whether anyone in the household smoked up to the child's age of 6.

Sensitivity Analyses

Unless otherwise stated, all models used the full set of confounders (the main model described above.) This includes the sensitivity analyses for the effect of air pollution; the models of spirometric flow parameters (PEF and FEFs); and the model which excluded extreme-valued subjects (potential outliers).

Air Pollution: Most subjects (n=858 / 895) were also enrolled in the ESCAPE project, a multicentre study of air pollution exposure and childhood asthma prevalence. For project details see [9-13]. For these subjects, we conducted a sensitivity analysis of a possible mediating effect of air pollution on any relationship between PA and lung function. In the current study, air pollution was quantified as the annual average exposure to PM2.5 and NOx at the subject's home address at age 15. Baseline concentrations (mean (median); 5^{th}, 95^{th} percentile) were 15.0 (14.1); 12, 18 μg/m^3 for PM2.5, and 33.6 (32.7); 24, 46 μg/m^3 for NOx. For further details on data collection and definitions, see [9-13].

Exclusion of Extreme Values: To assess the possible effect of extreme-valued individuals, we re-ran models without them and compared effect estimates. For each sex we calculated the mean and standard deviation for FEV1, FVC, FEV1/FVC, moderate PA, vigorous PA, and MVPA, and included only those 743 subjects (83%) whose values were all within two standard

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deviations of the mean (Table 3.) 68 subjects had extreme values for PA, 91 for spirometry, and 7 for both.

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