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A novel healthy metabolic phenotype developed among a cohort of families enriched for longevity

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Abstract

Background: Long-lived individuals and their offspring have healthier metabolic characteristics than expected, such as more favorable levels of fasting glucose, insulin, and lipids than controls without longevity. Dysregulation in metabolic pathways has also shown to predict accelerated aging. Using information from the Long Life Family Study (LLFS), a multi-center study of two-

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Megan Marron completed the analysis and interpretation of the data, and wrote the manuscript. Dr. Iva Miljkovic contributed to the healthy metabolic phenotype development by determining the optimal markers to use, the interpretation of data, and edited the manuscript. Dr. Robert Boudreau helped with analysis and edited the manuscript. Dr. Kaare Christensen contributed to the conception and design of the LLFS and edited the manuscript. Drs. Mary Feitosa and Joseph Lee helped with the interpretation of results and edited the manuscript. Dr. Paola Sebastiani contributed to the design of the LLFS and edited the manuscript. Dr. Bharat Thyagarajan contributed to the acquisition of all blood-based biomarker data and edited the manuscript. Dr. Mary Wojczynski ensured data quality and edited the manuscript. Dr. Joseph Zmuda contributed to the interpretation of results and edited the manuscript. Dr. Anne B. Newman contributed to the conception and design of the study, guided the aims of the manuscript, helped with interpretation of the data, and edited the manuscript. All authors approved the manuscript prior to submission.

Disclosures

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generation families selected for exceptional longevity, we developed an indicator of healthy metabolism to determine whether metabolic health was more prevalent in a subset of LLFS families and whether it was heritable and associated with other metrics of healthy aging.

Methods: A Latent Profile Analysis was applied to age- and gender-adjusted z-scores of fasting levels of glucose, insulin, triglycerides, and high-density lipoprotein cholesterol, body mass index, waist circumference, interleukin-6, and C-reactive protein. Families were defined as meeting the healthy metabolic phenotype if ≥2 and ≥50% of their offspring were classified into a latent subgroup with a profile of healthier metabolic markers than expected given age and gender relative to all LLFS offspring.

Results: The log odds of being classified into the latent subgroup with a healthy profile of metabolic markers was heritable ($h^2 = 0.40$, $p < 0.001$). Among 388 families, 39 (10%) met the healthy metabolic phenotype. Participants from these families had somewhat better cognition than those from remaining families. Proband-generation participants from families who met the healthy metabolic phenotype also had better pulmonary functioning and physical performance.

Conclusions: The better cognition, pulmonary function, and physical performance among probands from families with the healthy metabolic phenotype may indicate that this subset of LLFS families have a more extreme longevity phenotype than other LLFS families since cognitive, physical, and pulmonary function are top mortality predictors for older adults. Future work is needed to determine if rare or protective alleles confer a healthy metabolic phenotype in this subset of LLFS families with exceptional metabolism.

Keywords

Longevity; Metabolism; Healthy aging; Cognition

1. Introduction

Genetic variants in metabolic pathways have been documented to promote longevity in model organisms [1]. For example, mutations in the pathway of insulin and insulin-like growth factor-1 (IGF-1) signaling have shown to increase lifespan across multiple species [2]. Since decreased insulin and IGF-1 signaling can increase longevity, a healthy metabolic profile of insulin sensitivity and healthy lipids could be an important intermediate phenotype for exceptional longevity in humans. In accordance, long-lived individuals and their offspring have healthier metabolic characteristics than expected for their age, such as more favorable levels of fasting glucose, insulin, and lipids than controls without longevity [1,3–5].

A variety of mechanisms contribute to insulin signaling pathway abnormalities and insulin resistance [6], as well as to metabolism as a whole. For example, inflammation and abdominal obesity also characterize age-related changes that impact metabolism [6]. Inflammation, in particular, is one of the few common risk factors for multiple major causes of death among community-dwelling older adults [7]. Inflammation negatively impacts insulin signaling [6], where the control of systemic inflammation has been proposed as a way to promote longevity [8]. Identifying individuals who are relatively robust to metabolic

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alterations occurring with aging may provide insights into novel factors influencing healthy aging and longevity.

The Long Life Family Study (LLFS) is a multicenter cohort of two-generation families who were selected because they had a clustering of family members with exceptional longevity. Similar to other longevity-related cohorts, participants in the LLFS offspring generation had healthier metabolic characteristics than offspring from the Framingham Heart Study [9]. In this report, we determined whether a subset of participants in the LLFS offspring generation had a healthy metabolic profile based on fasting glucose and insulin, lipids, body composition, and inflammation, with the goal of determining whether a subset of LLFS families had a clustering of individuals with a healthy metabolic profile and whether this was associated with other metrics of healthy aging, including chronic conditions and physical and cognitive function.

2. Methods

2.1 The Long Life Family Study (LLFS)

The LLFS is an international multicenter cohort of two-generation families with a clustering of longevity, designed to examine genetic, environmental, and behavioral determinants of exceptional survival. Families were recruited during 2006–2009 from Boston, Massachusetts; New York, New York; Pittsburgh, Pennsylvania; and Denmark. Families were primarily white and met the following eligibility criteria: 1) enrolled one long-lived participant (proband) aged ≥90, 2) enrolled ≥1 sibling of the proband, 3) enrolled ≥1 offspring of either the proband or the proband's sibling, and 4) the proband generation had a clustering of members with exceptional survival based on a family longevity selection score [10]. The two generations in the LLFS were labeled as the proband generation (long-lived individual and their enrolled siblings) and the offspring generation (all enrolled offspring of individuals in the proband generation). The LLFS also recruited as many spouses as possible. The LLFS protocol was approved by the Human Research Protection office of the coordinating center at Washington University, the Regional Scientific Ethical Committees for Southern Denmark, and the Institutional Review Boards at Boston University, Columbia University, and the University of Pittsburgh.

Fig. 1 illustrates the process of coming to the final analytic sample used to develop the healthy metabolic phenotype. Among the participants in the offspring generation, 303 were excluded from the current analysis because they were missing more than three of the eight markers used to develop the healthy metabolic phenotype. Among the remaining 2132 offspring, 77 (4%) were taking medication for diabetes. The 2055 offspring not taking medication for diabetes served as the study sub-sample for developing the individual-level healthy metabolic phenotype. Of these, 1763 (86%) had complete information on all eight metabolic markers and 292 (14%) were missing one to three metabolic markers. Available information on the eight metabolic markers, age, and gender were used to replace missing values using Monte Carlo Markov Chain multiple imputation [11]. Families included in the analysis had to have at least two offspring with information on the individual-level healthy metabolic phenotype. Thus, our final analytic sample size was 388 families that comprised 1093 probands and 1987 offspring.

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2.2 Healthy metabolic phenotype

We developed the healthy metabolic phenotype using fasting levels of glucose, insulin, triglycerides, and high-density lipoprotein cholesterol, body mass index, waist circumference, interleukin-6, and high-sensitivity C-reactive protein. We considered markers that characterized metabolic changes that occur with aging [6]. In addition, favorable levels of these metabolic markers have been associated with longevity [1,3–5,7,9], and have the potential to identify a unique subset of individuals who are resistant to developing metabolic abnormalities. We also considered systolic blood pressure and adiponectin as potential components of the phenotype, but they did not help differentiate latent subgroups.

To account for the wide age range (30 to 88 years old) in the offspring generation and gender differences in metabolic traits, we calculated age- and gender-adjusted z-scores for the eight metabolic traits relative to the whole LLFS offspring generation. Participants who were taking medication for diabetes were excluded when calculating z-scores of fasting glucose and insulin, but were still included when calculating z-scores for the other markers. For each of the eight metabolic markers, z-scores were calculated using information from linear regression models of the respective marker on age, while stratifying by gender, which provided standardized values describing how each participant's metabolic measurements deviated from what was expected for their age and gender. Triglycerides, insulin, interleukin-6, and high-sensitivity C-reactive protein were log-transformed prior to analysis.

2.2.1 Latent Profile Analysis—We applied a Latent Profile Analysis to identify a subset of participants in the offspring generation who had a healthy profile of metabolic characteristics. Latent Profile Analysis is a clustering technique that classifies participants into subgroups based on similar patterns of multiple continuous measurements. We used Mclust [12] to apply the Latent Profile Analysis to the eight metabolic z-scores among offspring who were not taking medication for diabetes. Model selection was performed to determine the optimal number of subgroups using the Bayesian Information Criterion [13] and the following a priori criteria: 1) at least 0.80 mean posterior probability of correctly classifying participants into subgroups and 2) at least 5% of participants classified into each subgroup. We did not solely use the Bayesian Information Criterion because it is known to be problematic in mixture modeling since it can continue to improve as the number of latent subgroups increase, suggesting an unreasonable number of groups [14,15]. Others have suggested using subjective criteria in addition to the Bayesian Information Criterion and recommend balancing parsimony with distinctness so that there are no more subgroups than what is necessary [14]. Models with more than 4 groups had at least one subgroup with <5% of participants and/or at least one subgroup with an average subgroup posterior probability <0.80. Among the two-, three-, and four-group models, the four-group model was most optimal according to the Bayesian Information Criterion.

The Latent Profile Analysis methodology does not accommodate multiple imputations for missing values. For this initial subgroup classification phase, we opted to use the average of five Monte Carlo Markov Chain imputations to replace a missing measurement for the respective metabolic marker among the 292 (14%) participants who were missing one to

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three of the eight metabolic markers. This average estimates the mean of the posterior distribution [11].

2.2.2 Individual-level healthy metabolic phenotype—We classified offspring as meeting the individual-level healthy metabolic phenotype if the Latent Profile Analysis classified them into a subgroup with a healthy profile of metabolic characteristics, represented by higher than expected high-density lipoprotein cholesterol and lower than expected values for the other seven metabolic markers than participants of the same age and gender. Offspring taking medication for diabetes were not included in the Latent Profile Analysis and, instead, automatically classified as not meeting the healthy metabolic phenotype.

2.2.3 Family-level healthy metabolic phenotype—Similar to a previously developed healthy blood pressure phenotype in the LLFS [16], we classified families as meeting the healthy metabolic phenotype if ≥2 and ≥50% of their offspring met the individual-level healthy metabolic phenotype. That is, families were classified as metabolically healthy if the majority of their offspring had a profile of healthy metabolic characteristics.

2.3 Examination

Sociodemographic factors, including date of birth, gender, race, and education, smoking status, difficulty with activities of daily living, health status, and chronic conditions were determined by interview, as well as a blood sample was collected, in the participant's home near the time of enrollment (2006–2009). History or presence of heart disease, stroke, cancer, emphysema, and chronic obstructive pulmonary disease was based on self-report of a physician's diagnosis. Hypertension was defined as systolic and diastolic blood pressure ≥140/90 mmHg or taking anti-hypertensive medication. Diabetes was defined as fasting glucose ≥126 mg/dL or taking diabetes-related medication. All prescription and non-prescription medications were examined in their original containers for a medication inventory.

Information on weight, waist circumference, systolic and diastolic blood pressure, and performance measures was collected. Lung function was measured by forced expiratory volume in one second using EasyOne spirometers. Grip strength was the average of two measurements using the Jamar Hydraulic Hand Dynamometer on the stronger hand. Gait speed was averaged over 4 m, or 3 m if a 4 m space was not available. The short physical performance battery was based on gait speed, three balance tests, and repeated chair stands [17]. Overall cognitive performance was assessed using the digit symbol substitution task [18], the mini-mental state examination [19], and a cognitive endophenotype based on semantic fluency, digit forward and backward, and immediate and delayed recall [20]. Semantic fluency was the sum of animals and vegetables fluency, measuring the time it took to name as many animals or vegetables, respectively, as possible in 60 s [21]. Overall memory was the sum of how well participants could recall a short passage both immediately and 30 min after hearing it [22]. Attention/working memory was the sum of digit span

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forward and backward, which tested participants' ability to repeat a sequence of numbers, increasing in difficulty, both forward and backward, respectively [22].

Blood-based biomarkers were measured by a central laboratory at the University of Minnesota. Participants were asked to fast for at least eight hours prior to the blood draw, though phlebotomy was performed regardless of fasting time. For the current analysis, measurements of glucose, insulin, triglycerides, and cholesterol were only used if participants fasted for ≥8 h. There were 273 offspring who fasted for <8 h. Among participants without diabetes who were missing fasting glucose but had information on glycated hemoglobin, fasting glucose was estimated using the following equation [23]: 28.7*HbA1c - 46.7, and then multiplied by the mean observed fasting glucose levels divided by the mean estimated fasting glucose. Interleukin-6, high-sensitivity C-reactive protein, creatinine, and insulin-like growth factor-1 were also measured in the blood.

The metabolic syndrome was examined as a way to validate our healthy metabolic phenotype, since it was expected that participants who met the healthy metabolic phenotype would have a much lower likelihood of the metabolic syndrome. The metabolic syndrome was defined based on the 2009 Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity [24], as meeting at least three of the following five criteria: elevated waist circumference (≥102 cm for men, ≥88 cm for women), elevated triglycerides (≥150 mg/dL), low high-density lipoprotein cholesterol (<40 mg/dL for men, <50 mg/dL for women), elevated blood pressure (systolic ≥ 130 mmHg, diastolic ≥ 85 mmHg, or taking anti-hypertensive medication), and elevated fasting glucose (≥100 mg/dL or taking medication for diabetes). Insulin resistance was quantified using the homeostatic model assessment [25]: $(\text{glucose}_{\text{mg/dL}}*\text{insulin}_{\text{mg/dL}})/405$.

2.4 Statistical analysis

Mean (standard deviation) or frequency (percent) was used to describe differences among offspring who met the individual-level healthy metabolic phenotype versus offspring who did not. Effect sizes were examined using Cohen's d for continuous measures and phi coefficient for categorical measures. Differences were tested using generalized estimating equations in SAS 9.4, adjusting for relatedness of individuals. Differences were also tested while additionally adjusting for age and gender for all measures except cognitive performance, which additionally adjusted for age and education. A Benjamini-Hochberg correction was used to account for multiple comparisons [26] with a 10% false discovery rate. Distributions were assessed for normality and transformations were applied as needed. For comparison, we examined the percentage who met the individual-level healthy metabolic phenotype among the offspring versus the offspring-generation spousal controls. The same descriptive statistics and tests were performed when comparing offspring and probands from families who met the healthy metabolic phenotype versus offspring and probands, respectively, from remaining families.

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The log odds of being classified into the latent subgroup with a healthy metabolic profile was calculated for both generations, separately, using logistic regression models of an indicator for the latent subgroup with a healthy metabolic profile on the age- and gender-adjusted z-scores of the eight metabolic markers. Heritability of the log odds of being classified into the latent subgroup with a healthy metabolic profile was determined using a variance component-based family analysis adjusting for field center and significant population principal components in SOLAR.

3. Results

Fig. 2 illustrates the average age- and gender-adjusted z-scores for the eight metabolic markers by the four latent subgroups, where each group was characterized by a different metabolic profile. Group four (20% of offspring) had the healthiest profile of metabolic characteristics. All metabolic markers were at least 0.4 standard deviations better, on average, than the overall age- and gender-specific sample means. Fasting insulin and high-density lipoprotein cholesterol were the most extreme; both were more than one standard deviation better, on average, than the overall age- and gender-specific sample mean. Group three was the largest subgroup and had a metabolic profile that was closest to the overall age- and gender-specific sample averages. The remaining two groups had unhealthy profiles with similar average adjusted z-scores for body mass index, waist circumference, and fasting glucose, but group one was characterized by high inflammation-related biomarkers and group two was characterized by worse insulin, triglycerides, and high-density lipoprotein cholesterol.

3.1 Healthy metabolic phenotype

Among 1987 offspring, 388 (20%) met the individual-level healthy metabolic phenotype because they were classified into the latent subgroup with a healthy profile of metabolic characteristics and 72 (4%) offspring were automatically classified as not meeting the individual-level healthy metabolic phenotype because they were taking medication for diabetes. The log odds of being classified into the latent subgroup with a healthy metabolic profile was heritable ($h^2 = 0.40$, $p < 0.001$). Only 11% of offspring-generation spousal controls met the individual-level healthy metabolic phenotype. Among the 388 families, 39 (10%) met the family-level healthy metabolic phenotype because ≥2 and ≥50% of their offspring met the individual-level healthy metabolic phenotype.

3.2 Individual-level healthy metabolic phenotype comparison

Table 1a–1d compares offspring who met the individual-level healthy metabolic phenotype versus remaining offspring. More offspring were from New York, whereas fewer were from Pittsburgh among those who met the healthy metabolic phenotype (Table 1a). Fewer offspring who met the healthy metabolic phenotype were current smokers and fewer reported heart disease, hypertension, and difficulty with ≥1 activity of daily living than remaining offspring (Table 1b). Offspring with the healthy metabolic phenotype also had a lower average systolic and diastolic blood pressure and low-density lipoprotein cholesterol, and a slightly higher average forced expiratory volume in 1 s and slightly lower average creatinine and insulin-like growth factor-1 than remaining offspring (Table 1b). Offspring

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who met the healthy metabolic phenotype also had a slightly better average gait speed and short physical performance battery score, as well as a better average cognitive endophenotype (Table 1d).

3.3 Family-level healthy metabolic phenotype comparison

Table 2a–2d compares offspring and probands from families who met the family-level healthy metabolic phenotype versus offspring and probands, respectively, from remaining families. Fewer offspring from families with healthy metabolism were from Denmark and Pittsburgh and more were from Boston and New York, as well as more had greater than a high school education than offspring from remaining families (Table 2a). Offspring from families with healthy metabolism also had lower average systolic and diastolic blood pressure, and low-density lipoprotein cholesterol (Table 2b). Fewer offspring from families with healthy metabolism had heart disease and hypertension and fewer were taking lipid-lowering medication and anti-hypertensive medication, though, more had a history or presence of cancer than offspring from remaining families (Table 2b). Offspring from families with healthy metabolism also performed better on the digit symbol substitution task, the cognitive endophenotype, attention/working memory, and the short physical performance battery than offspring from remaining families (Table 2d).

Similar to the offspring generation, fewer probands from families with healthy metabolism were from Denmark and more were from New York, as well as more had greater than a high school education than probands from remaining families (Table 2a). Probands from families with healthy metabolism had a better average forced expiratory volume in one second and fewer had chronic obstructive pulmonary disease or emphysema (Table 2b). Consistent with the definition of our phenotype, probands from families with healthy metabolism also had a better average fasting insulin, triglycerides, high-density lipoprotein cholesterol, and high sensitivity C-reactive protein (Table 2c). Also, fewer probands had the metabolic syndrome among families with healthy metabolism. Probands from families with healthy metabolism had a faster average gait speed and better average short physical performance battery score, as well as performed better, on average, on the digit symbol substitution task than probands from remaining families (Table 2d). As a sensitivity analysis, we included individuals who had non-fasting measures for high-density lipoprotein cholesterol and triglycerides and results did not differ substantively from the primary analysis.

4. Discussion

In the LLFS, we identified a subset of families (10%) who had a clustering of offspring with a healthier profile of metabolic characteristics than expected given age and gender relative to all offspring in the cohort. Among these families, the offspring had somewhat better cognitive performance than offspring from families who did not meet the healthy metabolic phenotype. When examining participants from the proband generation, those from families who met the healthy metabolic phenotype had better pulmonary functioning, physical performance, and cognitive performance than those from remaining families.

Similar to the healthy blood pressure phenotype and the healthy memory phenotype developed in LLFS [16,27], few families (10%) had a clustering of offspring with a healthy

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metabolic profile. This is partly because the healthy phenotypes were developed based on health relative to the whole LLFS offspring generation, a cohort of individuals selected for exceptional familial longevity. In addition, offspring taking medication for diabetes were not included in the Latent Profile Analysis and instead were automatically classified as not meeting the individual-level healthy metabolic phenotype. Because of this, the Latent Profile Analysis identified a subset of offspring who had a profile of healthy markers relative to a total group of offspring who were healthy enough to not be taking medication for diabetes or who had not yet been prescribed medication for diabetes. We deliberately chose to take this approach so that we could identify especially unusual families within the LLFS cohort for deeper molecular characterization. There is likely a genetic component contributing to metabolic health among families enriched with both longevity and healthy metabolism since all nine of the hallmarks of aging have been linked to metabolic perturbations [28]. In addition, current interventions (e.g., caloric restriction) that extend lifespan across a variety of species do so by enhancing metabolic fitness. Healthy metabolism was heritable in the LLFS cohort, with a heritability of 0.40 for the log odds of being classified as having a profile of healthy metabolic characteristics. It remains to be seen whether there are rare variants segregating with metabolic fitness in this extreme subset of LLFS families.

Our approach to defining a healthy metabolic phenotype identified a subset of LLFS families with a clustering of offspring members who were metabolically healthy. By defining families using the offspring generation, we validated the phenotype with the finding that it was also expressed in the proband generation. Probands from families who met the healthy metabolic phenotype had lower averages of fasting insulin, triglycerides, and high-sensitivity C-reactive protein and higher average high-density lipoprotein cholesterol than probands from remaining families. Among families defined as having a healthy metabolic phenotype, 25% of probands and 64% of offspring were classified as having a healthy metabolic profile, whereas among families who did not meet the healthy metabolic phenotype, 18% of probands and 16% of offspring had a healthy metabolic profile. This illustrates the concordance of healthy metabolism across generations, but also the low prevalence of healthy metabolism at advanced old age. In addition to the markers used to develop the healthy metabolic phenotype, we found more optimal values for cardio-metabolic risk factors, such as lower systolic and diastolic blood pressure and low-density lipoprotein cholesterol, as well as a lower proportion with heart disease, hypertension, and taking lipid-lowering or anti-hypertensive medications among offspring from families with the healthy metabolic phenotype versus offspring from remaining families.

Overall, the prevalence of metabolic syndrome among all LLFS probands (age range: 71–110) was 26%, which was much lower than the U.S. prevalence. Among Americans aged ≥70, 62% and 58% of non-Hispanic white women and men, respectively, have the metabolic syndrome [29]. This further supports that the LLFS participants were healthier than the U.S. population. In addition, when examining the subset of probands from families who met the healthy metabolic phenotype, the prevalence of metabolic syndrome was only 12%, providing more evidence that our healthy metabolic phenotype successfully identified a subset of LLFS families with a clustering of probands, in addition to offspring, with metabolic health.

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Both offspring and probands from families who met the healthy metabolic phenotype performed somewhat better on the digit symbol substitution test, a measure of information processing speed, working memory, and visuospatial scanning. Consistent with other studies, metabolism plays an important role in brain health, though does not necessarily explain dementia risk among older adults [30,31]. Two common pathological mechanisms potentially leading to both diabetes and cognitive dysfunction is insulin resistance, by disrupting insulin transport across the blood-brain barrier [32], and inflammation. Metabolic-related interventions shown to increase lifespan, such as administering rapamycin or intermittent fasting, improved cognitive performance and lowered inflammation in mice [28,33]. In community-dwelling older adults, the metabolic syndrome only predicted cognitive impairment in those with high inflammation [34]. Inflammation has also been linked to lung disease [35], where LLFS probands from families who met the healthy metabolic phenotype also had better pulmonary functioning.

Participants in the proband generation from families who met the healthy metabolic phenotype also had better cognitive, physical, and lung function. Probands from families with versus without the healthy metabolic phenotype had a clinically meaningful difference in gait speed and the short physical performance battery, a measure of lower extremity function [36]. Both physical and cognitive performance measures are among the best predictors of mortality for older adults [37] and can best illustrate older adults' overall health [38]. In addition, pulmonary dysfunction has shown to be a risk factor for multiple causes of death among older adults [7]. The better average cognitive and physical performance and lung function among probands from families who met the healthy metabolic phenotype may indicate that members of these families have a greater likelihood of surviving to later ages, or in other words these families have a more extreme familial longevity than probands from remaining families.

When examining offspring from families who met the healthy metabolic phenotype versus offspring from remaining families, there were minimal differences in physical performance measures, unlike what was observed in the proband generation. This is likely because of the younger average age among the offspring generation (mean age: 60) when compared to the proband generation (mean age: 90), where poor physical performance is more likely to manifest at later ages. For example, a significant decline in gait speed typically does not occur until around the sixth decade of life [39], thus, more striking differences in physical performance among offspring from families who met the healthy metabolic phenotype versus offspring from remaining families may not be apparent until later follow-up visits.

Though focused on distinguishing types of metabolic dysregulation, rather than health, a cluster analysis in the Cardiovascular Health Study (CHS) found varying degrees of insulin resistance and impaired insulin secretion [40]. Interestingly, their reference healthy group was similar to what we found with lower average values of fasting glucose and insulin, body mass index, and C-reactive protein than the remaining cohort. The prevalence of their healthy reference group was higher than what we found in the LLFS (33% vs. 20%, respectively); likely because the LLFS healthy group consisted of a more extreme set of individuals with values for metabolic markers that were even lower than the CHS healthy reference group. Prospectively, the CHS reference group had lower risks of incident

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diabetes, cardiovascular disease, disability, mobility limitation, and mortality than almost all other groups. The low rate of adverse health outcomes among their healthy reference group supports the importance of healthy metabolism as a healthy aging phenotype and the potential that our healthy metabolic phenotype can identify a subset of LLFS families with a greater likelihood of surviving to advanced age, which may be due to rare genetic variants in this pathway.

A larger percentage of offspring presented with a history of cancer among families with the healthy metabolic phenotype, though the absolute difference between the two groups was minimal. It should be noted a small number of offspring (1%) had very high high-density lipoprotein cholesterol, which has been paradoxically associated with high mortality [41], but excluding these individuals in sensitivity analyses did not influence results. A limitation of this report was its cross-sectional design, which does not allow for assessing temporality between the healthy metabolic phenotype and its correlates. However, future longitudinal analyses on these families will have the potential to overcome this. Other potential limitations were the mostly white cohort, which limits the generalizability of results, as well as families with one offspring in the study were excluded since we were unable to determine if the majority of their offspring were metabolically healthy when there is information on only one individual. Our study has several important strengths, including the well-characterized and novel cohort of families enriched for longevity allowing us to examine several correlates of metabolic health, as well as the home visits allowing data collection on as many participants from families as possible, including those who may have not been healthy enough to leave their home.

5. Conclusions

We have demonstrated that families with clustering of especially healthy metabolism can be identified within families enriched for longevity from the Long Life Family Study. Future research is needed to determine if rare or protective alleles contribute to a healthy metabolic phenotype in the LLFS families.

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Abbreviations:

LLFS	Long Life Family Study
CHS	Cardiovascular Health Study

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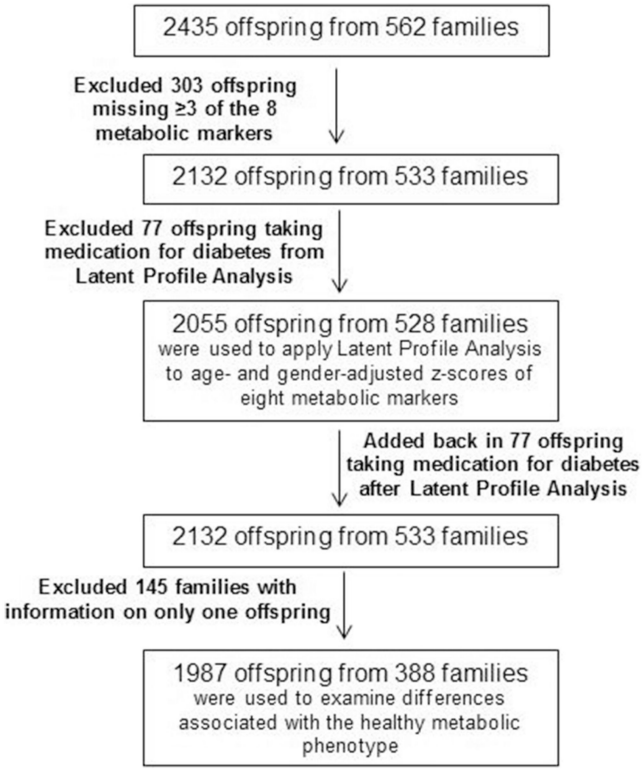

Fig. 1.

Flowchart of LLFS participants from the offspring generation involved in developing the healthy metabolic phenotype.

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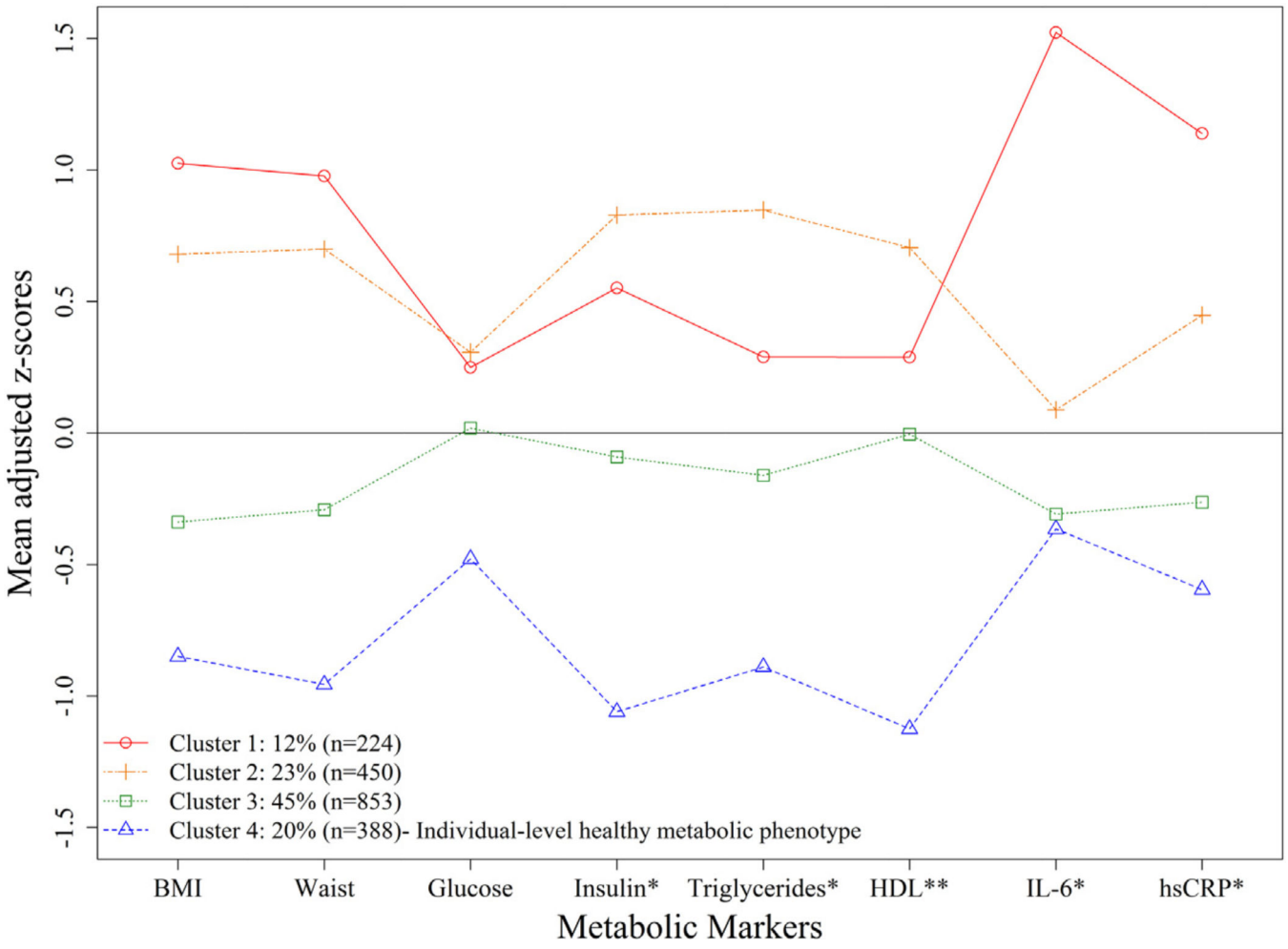

Fig. 2.

Mean age- and gender-adjusted z-scores of the eight metabolic markers by four latent subgroups among n = 1915 participants in the offspring generation who were not taking medication for diabetes (lower z-scores indicate healthier values) *Log transformation applied prior to computing z-scores **HDL cholesterol z-scores were multiplied by −1 Twenty percent of participants in the offspring generation were classified into a subgroup with a higher average value for high-density lipoprotein cholesterol and lower average values for body mass index, waist circumference, fasting glucose, fasting insulin, triglycerides, interleukin-6, and high-sensitivity C-reactive protein than the overall sample mean.

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Table 1a:

Individual-level healthy metabolic phenotype: Comparing demographics among offspring who met the healthy metabolic phenotype versus remaining offspring.

Mean (SD) or frequency (%)	Offspring who met the individual-level healthy metabolic phenotype (n = 388)	Offspring who did not meet the individual-level healthy metabolic phenotype (n = 1599)	Effect size**	Unadjusted *p*-value	Adjusted *p*-value*	False discovery rate
Age	60 (7.6)	60 (8.4)	−0.04	0.55	0.55	0.61
Male	170 (44%)	686 (43%)	0.007	0.75	0.74	0.76
White	388 (100%)	1597 (99.9%)	−0.02	–	–	–
Field center:						
Boston	112 (29%)	436 (27%)	0.01	0.001	0.001	0.002
Denmark	108 (28%)	479 (30%)	−0.02			
New York	82 (21%)	214 (13%)	0.09			
Pittsburgh	86 (22%)	470 (29%)	−0.06			
Education:						
Less than high school	18 (5%)	108 (7%)	−0.03	0.05	0.08	0.11
High school or equivalency	21 (5%)	138 (9%)	−0.05			
More than high school	349 (90%)	1350 (85%)	0.06			

*Adjusted for age and gender.

**Effect size: Cohen's d for continuous measures: $\frac{Mean_1 - Mean_2}{pooled\ standard\ deviation}$ and Phi Coefficient for categorical measures: $\sqrt{\frac{\chi^2}{n}}$

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Table 1b:

Individual-level healthy metabolic phenotype: Comparing disease prevalence and markers of disease among offspring who met the healthy metabolic phenotype versus remaining offspring.

Mean (SD) or frequency (%)	Offspring who met the individual-level healthy metabolic phenotype (n = 388)	Offspring who did not meet the individual-level healthy metabolic phenotype (n = 1599)	Effect Size**	Unadjusted *p*-value	Adjusted *p*-value*	False discovery rate
Disease prevalence:						
Heart disease	4 (1%)	73 (5%)	−0.07	0.003	0.005	0.01
Stroke	8 (2%)	42 (3%)	−0.01	0.49	0.59	0.64
Hypertension	98 (25%)	731 (46%)	−0.16	<0.0001	<0.0001	<0.0001
Cancer (excluding non-melanoma skin cancer)	33 (9%)	143 (9%)	−0.006	0.79	0.95	0.95
Emphysema or chronic obstructive pulmonary disease	3 (0.8%)	32 (2%)	−0.04	0.10	0.12	0.17
Lipid-lowering medication	24 (7%)	258 (18%)	−0.12	<0.0001	<0.0001	<0.0001
Anti-hypertensive medication	69 (21%)	463 (33%)	−0.10	0.0001	<0.0001	0.0002
Markers of disease: Smoking status:						
Never smoked	227 (59%)	857 (54%)	0.04	0.05	0.05	0.08
Former smoker	130 (34%)	565 (36%)	−0.02			
Current smoker	29 (8%)	168 (11%)	−0.04			
Weight (kg)	67 (11)	81 (16)	−1.0	<0.0001	<0.0001	<0.0001
Systolic blood pressure (mg/dL)	122 (20)	129 (19)	−0.34	<0.0001	<0.0001	<0.0001
Diastolic blood pressure (mg/dL)	76 (11)	80 (11)	−0.33	<0.0001	<0.0001	<0.0001
Cholesterol (mg/dL)	208 (37)	206 (41)	0.04	0.43	0.40	0.49
Low-density lipoprotein cholesterol (mg/dL)	116 (30)	125 (36)	−0.29	<0.0001	<0.0001	<0.0001
Creatinine (mg/dL)	0.96 (0.2) Median = 0.96	0.99 (0.3) Median = 0.96	−0.13	0.05	0.01	0.03
Insulin-like growth factor-1 (ng/mL)	136 (76) Median = 125	141 (52) Median = 132	−0.08	0.08	0.05	0.08
Forced expiratory volume in 1 s (L)	2.8 (0.8)	2.7 (0.8)	0.09	0.12	0.03	0.06
Difficulty with ≥1 activities of daily living	5 (1%)	59 (4%)	−0.06	0.02	0.03	0.05

* Adjusted for age and gender.

** Effect size: Cohen's d for continuous measures: $\frac{Mean_1 - Mean_2}{pooled\ standard\ deviation}$ and Phi Coefficient for categorical measures: $\sqrt{\frac{\chi^2}{n}}$

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Table 1c:

Individual-level healthy metabolic phenotype: Comparing markers of metabolic health among offspring who met the healthy metabolic phenotype versus remaining offspring.

Mean (SD) or frequency (%)	Offspring who met the individual-level healthy metabolic phenotype (n = 388)	Offspring who did not meet the individual-level healthy metabolic phenotype (n = 1599)	Effect size**	Unadjusted *p*-value	Adjusted *p*-value*	False discovery rate
Diabetes	0	72 (5%)	−0.11	–	–	–
Diabetes medication	0	72 (5%)	−0.10	–	–	–
Metabolic syndrome	2 (0.5%)	437 (30%)	−0.28	<0.0001	<0.0001	<0.0001
HOMA insulin resistance	0.85 (0.4) Median = 0.78	2.2 (1.6) Median = 1.8	−1.2	<0.0001	<0.0001	<0.0001
Glycated hemoglobin	5.38 (0.3)	5.90 (0.6)	−0.46	<0.0001	<0.0001	<0.0001
Metabolic markers used in latent profile analysis:						
Body mass index (kg/m^2)	23 (2.7) Median = 23 Range: 16, 33	29 (5.0) Median = 28 Range: 18, 57	−1.3	<0.0001	<0.0001	<0.0001
Waist circumference (cm)	82 (9.3) Median = 83 Range: 59, 108	98 (14) Median = 97 Range: 54, 158	−1.3	<0.0001	<0.0001	<0.0001
Fasting glucose (mg/dL)	86 (11) Median = 87 Range: 34, 119	92 (10) Median = 92 Range: 51, 125	−0.58	<0.0001	<0.0001	<0.0001
Fasting insulin (mU/L)	3.9 (1.7) Median = 3.7 Range: 0.33, 11	9.9 (6.6) Median = 8.0 Range: 0.33, 69	−1.2	<0.0001	<0.0001	<0.0001
Triglycerides (mg/dL)	65 (24) Median = 60 Range: 15, 169	126 (82) Median = 106 Range: 28, 1031	−1.0	<0.0001	<0.0001	<0.0001
High-density lipoprotein cholesterol (mg/dL)	79 (18) Median = 77 Range: 28, 150	56 (15) Median = 55 Range: 17, 130	1.4	<0.0001	<0.0001	<0.0001
Interleukin-6 (pg/mL)	0.76 (0.60) Median = 0.56 Range: 0.11, 4.0	1.6 (4.0) Median = 0.77 Range: 0.15, 73	−0.28	<0.0001	<0.0001	<0.0001
High-sensitivity C-reactive protein (mg/L)	1.1 (1.3) Median = 0.77 Range: 0.10, 12	3.0 (4.8) Median = 1.5 Range: 0.12, 56	−0.54	<0.0001	<0.0001	<0.0001

*Adjusted for age and gender.

**Effect size: Cohen's d for continuous measures: $\frac{Mean_1 - Mean_2}{pooled\ standard\ deviation}$ and Phi Coefficient for categorical measures: $\sqrt{\frac{\chi^2}{n}}$

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Table 1d:

Individual-level healthy metabolic phenotype: Comparing physical and cognitive performance among offspring who met the healthy metabolic phenotype versus remaining offspring.

Mean (SD) or frequency (%)	Offspring who met the individual-level healthy metabolic phenotype (n = 388)	Offspring who did not meet the individual-level healthy metabolic phenotype (n = 1599)	Effect size**	Unadjusted *p*-value	Adjusted *p*-value*	False discovery rate
Physical performance:						
Gait speed (m/s)	1.19 (0.2)	1.16 (0.2)	0.12	0.04	0.05	0.08
Average grip (kg)	32 (10)	32.3 (12)	−0.001	0.98	0.48	0.56
Short physical performance battery	11.6 (0.9) Median = 12	11.3 (1.3) Median = 12	0.28	<0.0001	<0.0001	<0.0001
Chair stand time (s)	10.1 (7.1) Median = 9.3	12.0 (12) Median = 10.2	−0.19	0.0001	0.0001	0.0003
Cognitive performance:						
Digit symbol substitution task	51 (12)	51 (13)	0.06	0.34	0.54	0.61
Attention/working memory:	16 (4.0)	15 (4.0)	0.09	0.16	0.27	0.37
Digit span forward total	8.6 (2.2)	8.5 (2.2)	0.04	0.56	0.72	0.75
Digit span backward total	7.0 (2.3)	6.7 (2.3)	0.12	0.05	0.10	0.15
Mini-mental state exam score	29 (2.0) Median = 30	29 (2.1) Median = 29	0.08	0.19	0.28	0.38
Overall episodic memory:	26 (7)	25 (8.0)	0.08	0.19	0.43	0.51
Logical memory 1A-immediate total	13 (3.5)	13 (4.0)	0.06	0.29	0.61	0.65
Logical memory IIA-delayed total	12 (3.8)	12 (4.4)	0.09	0.15	0.33	0.42
Semantic fluency:	38 (8.8)	37 (8.6)	0.13	0.03	0.08	0.11
Category fluency-animals total	23 (5.8)	22 (5.7)	0.09	0.13	0.31	0.40
Category fluency-vegetables total	16 (4.6)	15 (4.4)	0.14	0.02	0.04	0.08
Cognitive endophenotype ***	0.56 (2.4)	−0.13 (2.6)	0.27	<0.0001	0.0002	0.0004

* Adjusted for age and gender for physical measures and adjusted for age and education for cognitive measures;

** Effect size: Cohen's d for continuous measures: $\frac{Mean_1 - Mean_2}{pooled\ standard\ deviation}$ and Phi Coefficient for categorical measures: $\sqrt{\frac{\chi^2}{n}}$

*** Based on semantic fluency, digit forward and backward, and immediate and delayed recall [20].

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Table 2a:

Family-level healthy metabolic phenotype: Comparing demographics among participants from families who met the healthy metabolic phenotype versus participants from remaining families.

Mean (SD) or frequency (%)	Offspring from 39 families with the healthy metabolic phenotype (n = 163)	Offspring from 349 families without the healthy metabolic phenotype (n = 1824)	Offspring comparison:				Probands from 39 families with the healthy metabolic phenotype (n = 100)	Probands from 349 families without the healthy metabolic phenotype (n = 993)	Proband comparison:			
			Effect size**	Unadjusted *p*-value	Adjusted* *p*-value	FDR			Effect size**	Unadjusted *p*-value	Adjusted* *p*-value	FDR
Age	61 (7.8)	60 (8.3)	0.06	0.57	0.58	0.67	90 (6.2)	90 (6.2)	−0.03	0.81	0.99	0.99
Male	81 (50%)	775 (42%)	0.04	0.05	0.05	0.08	55 (55%)	436 (44%)	0.06	0.03	0.03	0.12
White	163 (100%)	1818 (99.7%)	−0.01	-	-	-	100 (100%)	985 (99.2%)	−0.02	-	-	-
Field center:												
Boston	66 (40%)	482 (26%)	0.09	0.001	0.001	0.003	32 (32%)	247 (25%)	0.05	0.007	0.008	0.06
Denmark	12 (7%)	575 (32%)	−0.15				4 (4%)	216 (22%)	−0.13			
New York	52 (32%)	244 (13%)	0.14				38 (38%)	196 (20%)	0.13			
Pittsburgh	33 (20%)	523 (29%)	−0.05				26 (26%)	334 (34%)	−0.05			
Education:												
Less than high school	4 (2%)	122 (7%)	−0.05	0.05	0.04	0.07	15 (15%)	294 (30%)	−0.09	0.03	0.06	0.20
High school or equivalency	8 (5%)	151 (8%)	−0.03				21 (21%)	224 (23%)	−0.01			
More than high school	151 (93%)	1548 (85%)	0.06				64 (64%)	473 (48%)	0.09			

FDR = False discovery rate.

*Adjusted for age and gender.

**Effect size: Cohen's d for continuous measures: $\frac{Mean_1 - Mean_2}{pooled\ standard\ deviation}$ and Phi Coefficient for categorical measures: $\sqrt{\frac{\chi^2}{\text{n.}}}$

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Table 2b:

Family-level healthy metabolic phenotype: Comparing disease prevalence and markers of disease among participants from families who met the healthy metabolic phenotype versus participants from remaining families.

Mean (SD)or frequency (%)	Offspring from 39 families with the healthy metabolic phenotype (n = 163)	Offspring from 349 families without the healthy metabolic phenotype (n = 1824)	Offspring comparison:				Probands from 39 families with the healthy metabolic phenotype (n = 100)	Probands from 349 families without the healthy metabolic phenotype (n = 993)	Proband comparison:			
			Effect size**	Unadjusted *p*-value	Adjusted* *p*-value	FDR			Effect size**	Unadjusted *p*-value	Adjusted* *p*-value	FDR
Disease prevalence:												
Heart disease	1 (0.6%)	76 (4%)	−0.05	0.05	0.05	0.07	16 (16%)	161 (16%)	−0.002	0.94	0.76	0.86
Stroke	6 (4%)	44 (2%)	0.02	0.33	0.36	0.48	16 (16%)	158 (16%)	0.0006	0.99	0.96	0.99
Hypertension	49(30%)	780 (43%)	−0.07	0.0008	<0.0001	<0.0001	61 (61%)	654 (66%)	−0.03	0.37	0.54	0.71
Cancer (excluding non-melanoma skin cancer)	25 (l5%)	151(8%)	0.07	0.002	0.001	0.003	14 (14%)	177 (18%)	−0.03	0.39	0.32	0.52
Emphysema or chronic obstructive pulmonary disease	1 (0.6%)	34 (2%)	−0.03	0.27	0.26	0.38	0	46 (5%)	−0.07			
Lipid-lowering medication	13 (8%)	269 (17%)	−0.07	0.001	0.0006	0.002	25 (26%)	164(17%)	0.06	0.04	0.07	0.20
Anti-hypertensive medication	36 (23%)	496(32%)	−0.05	0.04	0.03	0.06	69 (70%)	645 (68%)	0.02	0.59	0.57	0.71
Markers of disease:												
Smoking status:												
Never smoked	97(60%)	987(54%)	0.03	0.54	0.47	0.59	63 (63%)	635 (64%)	−0.008	0.73	0.73	0.85
Former	53 (33%)	642(35%)	−0.02				36 (36%)	332 (34%)	0.01			
smoker												
Current smoker	13 (8%)	184(10%)	−0.02				1 (1%)	21 (2%)	−0.02			
Weight (kg)	73 (15)	79(17)	−0.36	<0.0001	<0.0001	<0.0001	67 (14)	66 (14)	0.02	0.85	0.38	0.55
Systolic blood pressure (mg/dL)	123 (17)	128 (20)	−0.27	0.002	<0.0001	<0.0001	136 (26)	139 (25)	−0.11	0.33	0.51	0.69
Diastolic blood pressure (mg/dL)	76 (11)	79(11)	−0.27	0.006	0.002	0.005	71 (11)	74(12)	−0.20	0.08	0.07	0.21

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Mean (SD)or frequency (%)	Offspring from 39 families with the healthy metabolic phenotype (n = 163)	Offspring from 349 families without the healthy metabolic phenotype (n = 1824)	Offspring comparison:				Probands from 39 families with the healthy metabolic phenotype (n = 100)	Probands from 349 families without the healthy metabolic phenotype (n = 993)	Proband comparison:			
			Effect size**	Unadjusted *p*-value	Adjusted* *p*-value	FDR			Effect size**	Unadjusted *p*-value	Adjusted* *p*-value	FDR
Cholesterol (mg/dL)	2 02 (36)	207(41)	−0.13	0.21	0.33	0.46	190 (40)	189(44)	0.03	0.85	0.39	0.55
Low-density lipoprotein cholesterol (mg/dL)	113 (31)	124(35)	−0.34	0.0007	0.0009	0.003	110(32)	111 (36)	−0.05	0.66	0.99	0.99
Creatinine (mg/dL)	0.98 (0.2) Median = 0.98	0.98 (0.3) Median = 0.96	0.002	0.50	0.57	0.67	1.2 (0.4) Median = 1.2	1.2 (0.4) Median = 1.1	0.12	0.15	0.33	0.52
Insulin-like growth factor-1 (ng/mL)	137 (47) Median = 132	140 (58) Median = 130	−0.06	0.70	0.58	0.67	103 (45) Median = 95	102(48) Median = 96	0.02	0.74	0.91	0.96
Forced expiratory volume in 1 s (L)	2.8 (0.8)	2.8 (0.8)	0.05	0.58	0.69	0.75	1.8 (0.7)	1.6 (0.6)	0.34	0.008	0.01	0.06
Difficulty with ≥1 activity of daily living	4(3%)	60 (4%)	−0.02	0.47	0.43	0.56	28 (29%)	391 (43%)	−0.08	0.05	0.04	0.13

FDR = False discovery rate.

*Adjusted for age and gender.

**Effect size: Cohen's d for continuous measures: $\frac{Mean_1 - Mean_2}{pooled\ standard\ deviation}$ and Phi Coefficient for categorical measures: $\sqrt{\frac{\chi^2}{n}}$

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Table 2c:

Family-level healthy metabolic phenotype: Comparing markers of metabolic health among participants from families with the healthy metabolic phenotype versus participants from remaining families

Mean (SD)or frequency (%)	Offspring from 39 families with the healthy metabolic phenotype (n = 163)	Offspring from 349 families without the healthy metabolic phenotype (n = 1824)	Offspring comparison:				Probands from 39 families with the healthy metabolic phenotype (n = 100)	Probands from 349 families without the healthy metabolic phenotype (n = 993)	Proband comparison:			
			Effect size**	Unadjusted *p*-value	Adjusted* *p*-value	FDR			Effect size	Unadjusted *p*-value	Adjusted* *p*-value	FDR
Diabetes	1 (0.7%)	71 (5%)	−0.06	0.04	0.04	0.07	2 (3%)	59 (7%)	−0.05	0.13	0.11	0.25
Diabetes medication	1 (0.7%)	71 (5%)	−0.06	0.05	0.04	0.07	2 (2%)	59 (6%)	−0.05	0.11	0.09	0.22
Metabolic syndrome	7 (5%)	432 (26%)	−0.14	<0.0001	<0.0001	<0.0001	9 (12%)	210 (27%)	−0.10	0.007	0.01	0.06
HOMA insulin resistance	1.2(0.9) Median = 0.88	2.0 (1.5) Median = 1.6	−0.63	<0.0001	<0.0001	<0.0001	1.4 (1.0) Median = 1.0	1.6 (1.1) Median = 1.4	−0.25	0.01	0.008	0.06
Glycated hemoglobin Metabolic markers used in latent profile analysis:	5.47 (0.3)	5.56 (0.58)	−0.20	0.004	0.0007	0.002	5.69 (0.4)	5.74 (0.5)	−0.10	0.29	0.27	0.46
Metabolic markers used in latent profile analysis:												
Body mass index (kg/m^2)	25 (3.9) Median = 25 Range: 18, 40	28 (5.1) Median = 27 Range: 16, 57	−0.54	<0.0001	<0.0001	<0.0001	26 (3.3) Median = 26 Range: 19, 37	26 (4.3) Median = 26 Range: 13, 58	−0.07	0.54	0.45	0.63
Waist circumference (cm)	88 (12) Median = 88 Range: 64, 119	95 (14) Median = 94 Range: 54, 158	−0.55	<0.0001	<0.0001	<0.0001	94 (11) Median = 94 Range: 62, 124	94 (12) Median = 94 Range: 42, 149	0.02	0.87	0.77	0.86
Fasting Glucose (mg/dL)	87 (9.4) Median = 87 Range: 60, 119	92 (11) Median = 91 Range: 34,125	−0.40	<0.0001	<0.0001	<0.0001	90 (9.5) Median = 90 Range: 72,120	92 (11) Median = 92 Range: 54,125	−0.19	0.12	0.09	0.22
Fasting Insulin (mU/L)	5.5 (4.3) Median = 4.3 Range: 0.33,27	9.0 (6.5) Median = 7.2 Range: 0.33, 69	−0.62	<0.0001	<0.0001	<0.0001	6.0 (4.2) Median = 4.8 Range: 0.67, 23	7.1 (4.7) Median = 6.0 Range: 0.67, 50	−0.25	0.005	0.004	0.06
Triglycerides (mg/dL)	85 (61) Median = 68 Range: 23,494	117 (79) Median = 96 Range: 15,1031	−0.45	<0.0001	<0.0001	<0.0001	91 (43) Median = 80 Range: 33, 304	109 (59) Median = 94 Range: 24, 500	−0.34	0.003	0.006	0.06
High-density lipoprotein	72 (19) Median = 70	60 (18) Median = 58	0.66	<0.0001	<0.0001	<0.0001	62 (17) Median = 59	56 (16) Median = 54	0.37	0.02	0.006	0.06

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Mean (SD)or frequency (%)	Offspring from 39 families with the healthy metabolic phenotype (n = 163)	Offspring from 349 families without the healthy metabolic phenotype (n = 1824)	Offspring comparison:				Probands from 39 families with the healthy metabolic phenotype (n = 100)	Probands from 349 families without the healthy metabolic phenotype (n = 993)	Proband comparison:			
			Effect size**	Unadjusted *p*-value	Adjusted* *p*-value	FDR			Effect size	Unadjusted *p*-value	Adjusted* *p*-value	FDR
cholesterol (mg/dL)	Range: 31,128	Range: 17,150					Range: 34, 112	Range: 21,126				
Interleukin-6 (pg/mL)	1.0 (1.6) Median = 0.60 Range: 0.14, 15	1.4 (3.7) Median = 0.74 Range: 0.11, 73	−0.14	0.02	0.01	0.02	3.3 (5.7) Median = 1.6 Range: 0.11, 35	4.5 (11) Median = 2.0 Range: 0.20, 116	−0.14	0.07	0.05	0.16
High-sensitivity C-reactive protein (mg/L)	1.6 (2.8) Median = 0.82 Range: 0.10, 26	2.7 (4.5) Median = 1.4 Range: 0.12, 56	−0.31	<0.0001	<0.0001	<0.0001	3.5 (7.5) Median = 1.2 Range: 0.17, 61	5.6 (13) Median = 2.1 Range: 0.14, 203	−0.20	0.005	0.005	0.06
Prevalence of individual-level healthy metabolic phenotype	104(64%)	284 (16%)	0.33	<0.0001	<0.0001	<0.0001	20 (25%)	147 (18%)	0.05	0.16	0.16	0.30

FDR = False discovery rate.

*Adjusted for age and gender

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Table 2d:

Family-level healthy metabolic phenotype: Comparing physical and cognitive performance among participants from families with the healthy metabolic phenotype versus participants from remaining families.

Mean (SD) or frequency (%)	Offspring from 39 families with the healthy metabolic phenotype (n = 163)	Offspring from 349 families without the healthy metabolic phenotype (n = 1824)	Offspring comparison:				Probands from 39 families with the healthy metabolic phenotype (n = 100)	Probands from 349 families without the healthy metabolic phenotype (n = 993)	Proband comparison:			
			Effect size**	Unadjusted *p*-value	Adjusted* *p*-value	FDR			Effect size	Unadjusted *p*-value	Adjusted* *p*-value	FDR
Physical performance:												
Gait speed (m/s)	1.19 (0.2)	1.17 (0.2)	0.12	0.17	0.12	0.18	0.79 (0.2)	0.71 (0.3)	0.31	0.009	0.009	0.06
Average grip (kg)	33 (11)	32 (11)	0.08	0.40	0.98	0.98	21 (8.7)	19 (7.6)	0.22	0.11	0.35	0.53
Short physical performance battery	11.5 (1.1) Median = 12	11.3 (1.3) Median = 12	0.15	0.06	0.03	0.06	7.8 (3.1) Median = 7	6.7 (3.4) Median = 8	0.33	0.009	0.01	0.06
Chair stand time (s)	11.3 (13) Median = 9.3	11.7 (11) Median = 10.1	−0.03	0.69	0.62	0.70	32.6 (37) Median = 14.1	45.0 (41) Median = 16.7	−0.32	0.008	0.03	0.12
Cognitive performance:												
Digit symbol substitution task	53 (12)	50 (12)	0.20	0.10	0.04	0.07	32 (9.5)	28 (13)	0.39	0.002	0.0003	0.02
Attention/working memory:	17 (4.0)	15 (4.0)	0.43	<0.0001	0.0001	0.0004	14 (3.4)	13 (4.0)	0.31	0.01	0.15	0.29
Digit span forward total	9.3 (2.1)	8.5 (2.2)	0.40	0.0001	0.0001	0.0005	7.9 (2.0)	7.3 (2.3)	0.32	0.01	0.14	0.29
Digit span backward total	7.6 (2.4)	6.7 (2.3)	0.38	0.0003	0.0005	0.002	5.7 (2.0)	5.2 (2.2)	0.24	0.04	0.24	0.41
Mini-mental state exam score	29 (1.1) Median = 30	29 (2.1) Median = 29	0.15	0.04	0.05	0.07	26 (2.8) Median = 27	26 (3.8) Median = 26	0.20	0.04	0.15	0.29
Overall episodic memory:	25 (6.9)	25 (7.9)	0.05	0.60	0.81	0.84	14 (7.6)	14 (8.9)	0.0003	0.99	0.67	0.80
Logical memory 1A-immediate	13 (3.4)	13 (3.9)	0.06	0.49	0.74	0.78	8.2 (4.0)	8.1 (4.6)	0.03	0.83	0.85	0.93
Logical memory IIA-delayed	12 (3.8)	12 (4.3)	0.04	0.71	0.86	0.88	6.0 (3.9)	6.1 (4.6)	−0.01	0.91	0.60	0.74
Semantic fluency:	38 (8.8)	37 (8.6)	0.10	0.40	0.45	0.57	26 (7.6)	24 (7.9)	0.24	0.07	0.09	0.22
Category fluency-animals	23 (5.2)	22 (5.8)	0.06	0.58	0.68	0.75	15 (5.2)	14(5.1)	0.24	0.09	0.12	0.25
Category fluency-vegetables	16 (4.9)	15 (4.4)	0.12	0.32	0.34	0.47	11 (3.9)	10 (4.0)	0.18	0.15	0.20	0.37

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Mean (SD) or frequency (%)	Offspring from 39 families with the healthy metabolic phenotype (n = 163)	Offspring from 349 families without the healthy metabolic phenotype (n = 1824)	Offspring comparison:				Probands from 39 families with the healthy metabolic phenotype (n = 100)	Probands from 349 families without the healthy metabolic phenotype (n = 993)	Proband comparison:			
			Effect size**	Unadjusted *p*-value	Adjusted* *p*-value	FDR			Effect size	Unadjusted *p*-value	Adjusted* *p*-value	FDR
Cognitive endophenotype***	0.74 (2.5)	−0.05 (2.6)	0.31	0.01	0.005	0.01	0.16 (2.3)	−0.21 (2.9)	0.14	0.32	0.57	0.71

FDR = False discovery rate.

*Adjusted for age and gender for physical measures and adjusted for age and education for cognitive measures;

**Effect size: Cohen's d for continuous measures: $\frac{Mean_1 - Mean_2}{pooled\ standard\ deviation}$ and Phi Coefficient for categorical measures: $\sqrt{\frac{\chi^2}{n}}$

***Based on semantic fluency, digit forward and backward, and immediate and delayed recall [20].