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RESEARCH ARTICLE

PLIS: A metabolomic response monitor to a lifestyle intervention study in older adults

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Abstract

The response to lifestyle intervention studies is often heterogeneous, especially in older adults. Subtle responses that may represent a health gain for individuals are not always detected by classical health variables, stressing the need for novel biomarkers that detect intermediate changes in metabolic, inflammatory, and immunity-related health. Here, our aim was to develop and validate a molecular multivariate biomarker maximally sensitive to the individual effect of a lifestyle intervention; the Personalized Lifestyle Intervention Status (PLIS). We used ^{1}H-NMR fasting blood metabolite measurements from before and after the 13-week combined physical and nutritional Growing Old TOgether (GOTO) lifestyle intervention study in combination with a fivefold cross-validation and a bootstrapping method to train a separate PLIS score for men and women. The PLIS scores consisted of 14 and four metabolites for females and males, respectively. Performance of the PLIS score in tracking health gain was illustrated by

Abbreviations: 1H-NMR, hydrogyen-1 nuclear magnetic resonance; AGO study, Active and Healthy Old study; Ala, alanine; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; BMI, body mass index; bOHBut, 3-Hydroxybutyrate; CHM, classical metabolic health marker; Cit, citrate; Crea, creatinine; CRP, C-reactive protein; DBP, diastolic blood pressure; DHA, docosahexaenoic acid; FAw3-FA, ratio of omega-3 fatty acids to total fatty acids; FAw6, omega-6 fatty acids; FAw6-FA, ratio of omega-6 fatty acids to total fatty acids; FRS, Framingham risk score; Glc, glucose; Gln, glutamine; GOTO study, Growing Old TOgether study; Gp, glycoprotein acetyls; HDL2-C, total cholesterol in HDL2; HDL3-C, total cholesterol in HDL3; HDL-C, total cholesterol in HDL; HDL-D, mean diameter for HDL particles; His, histidine; HOMA2-IR, Homeostasis Model Assessment of Insulin Resistance; IDL-C, total cholesterol in IDL; IDL-L, total lipids in IDL; LA, linoleic acid; Lac, lactate; LDL-C, total cholesterol in LDL; Leu, leucine; L-HDL-L, total lipids in large HDL; L-LDL-L, total lipids in large LDL; MHDLL, total lipids in medium HDL; M-HDL-L, total lipids in medium HDL; M-LDL-L, total lipids in medium LDL; MUFA, monounsaturated fatty acids; MUFA-FA, ratio of monounsaturated fatty acids to total fatty acids; M-VLDL-L, total lipids in medium VLDL; PC, phosphatidylcholine and other cholines; Phe, phenylalanine; PLIS, personalized Lifestyle Intervention Status; PUFA, polyunsaturated fatty acids; PUFA-FA, ratio of polyunsaturated fatty acids to total fatty acids; SBP, systolic blood pressure; SerumC, serum total cholesterol; SerumTG, serum triglycerides; SFA, saturated fatty acids; SFA-FA, ratio of saturated fatty acids to total fatty acids; S-LDL-L, total lipids in small LDL; SM, sphingomyelins; S-VLDL-L, total lipids in small VLDL; TotCho, total cholines; TotFA, total fatty acids; TotPG, total phosphoglycerides; Tyr, tyrosine; UnsatDeg, estimated degree of unsaturation; Val, valine; VLDL-C, total cholesterol in VLDL; VLDL-D, mean diameter for VLDL particles; WC, waist circumference; WHR, waist-to-hip ratio; XL-HDL-L, total lipids in very large HDL; XS-VLDL-L, total lipids in very small VLDL; XXL-VLDL-L, total lipids in chylomicrons and extremely large VLDL; ΔMetaboAge, delta MetaboAge.

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association of the sex-specific PLIS scores with several classical metabolic health markers, such as BMI, trunk fat%, fasting HDL cholesterol, and fasting insulin, the primary outcome of the GOTO study. We also showed that the baseline PLIS score indicated which participants respond positively to the intervention. Finally, we explored PLIS in an independent physical activity lifestyle intervention study, showing similar, albeit remarkably weaker, associations of PLIS with classical metabolic health markers. To conclude, we found that the sex-specific PLIS score was able to track the individual short-term metabolic health gain of the GOTO lifestyle intervention study. The methodology used to train the PLIS score potentially provides a useful instrument to track personal responses and predict the participant's health benefit in lifestyle interventions similar to the GOTO study.

KEYWORDS
bioinformatics, healthy ageing, lifestyle intervention, machine learning, metabolomics, response monitor

1 | INTRODUCTION

Worldwide, the proportion of older people in the human population is increasing and with it, the burden of late age diseases on quality of life and society.[1,2] Improving metabolic and immune-related health parameters is generally considered to be an important component of improving vitality in older age.[3,4] Various lifestyle interventions may beneficially influence such parameters and especially those based on combinations of physical activity and dietary intake.[5–10] However, the effects of short-term lifestyle intervention studies are often modest and do not immediately indicate changes in disease risk, they are often also ambiguous due to individual differences in response which mask beneficial effects for subgroups.[11,12]

To maximize the result of lifestyle interventions one needs to establish what benefit is gained by which intervention at a personal basis, expressing the need for sensitive molecular biomarkers. There are novel molecular biomarkers which have been trained to predict disease and metabolic health outcomes using machine learning algorithms in combination with different omics measures, including metabolomics.[13] Metabolomics is the term for studies into the small molecules within cells, biofluids, tissues, or organisms that represent the intermediate or end products of metabolism (metabolites).[13] The metabolome comprises the quantitative and qualitative measurements of these metabolites. Novel metabolomics-based biomarkers have been shown to be a valuable tool in precision nutrition and they indicate the risk of metabolic diseases.[14,15] Multivariate metabolomic markers have also been trained (based on chronological age and on mortality) to indicate biological age (MetaboAge and MetaboHealth).[16,17] Such biomarkers tend to predict disease outcomes and mortality equally or even better than classical clinical markers, potentially contributing to personalized medicine.[14–19] However, these biomarkers are trained on large sets of cohort data and do not detect the subtle changes in health caused by short-term interventions, further stressing the need for novel biomarkers specifically designed to record such changes caused by short lifestyle interventions, to follow the trajectory of the participants.

We set out to train and validate a novel response marker that can predict the effect of a short-term intervention based on metabolomics data. The data used to train this novel response predictor, which we named the Personalized Lifestyle Intervention Status (PLIS) score, were part of the short-term Growing Old TOgether (GOTO) lifestyle intervention study.[6] The aim of the GOTO intervention study was to improve the metabolic health in older adults through an increased energy expenditure by 25% for 13 weeks in part by a 12.5% reduction in caloric intake and a 12.5% increase in physical activity, following one of the criteria of the hallmark CALERIE intervention study.[20]

The aim of the current methodological study is to develop and validate a molecular multifaceted biomarker maximally sensitive to the individual effect of a mild lifestyle intervention, such as GOTO. We tested whether the PLIS score is sufficiently sensitive to detect heterogeneous responses to the GOTO intervention, whether it is significantly associated with more traditional metabolic health markers (such as BMI, trunk fat%, fasting HDL cholesterol, and fasting insulin), how well it would be able to select participants for a novel lifestyle intervention study and how it compared to a score trained on the investigated metabolic health markers and/or established metabolite health scores. Lastly, we investigated how the PLIS score

performed in an independent lifestyle intervention study called the Actief en Gezond Oud (Active and Healthy Old) (AGO) study.[11]

2 | MATERIALS AND METHODS

2.1 | Compliance calculation and selection of participants

The compliance in the GOTO study was self-reported. During each week of the intervention study, the participants filled in the number of days they were compliant to the dietary arm and the physical activity arm of the intervention. The mean compliance over 13 weeks was calculated separately for diet and physical activity. Participants with a mean dietary or a mean physical activity compliance above 3.5, were considered high compliant. Participants with a mean dietary compliance and a mean physical activity compliance below 3.5, were considered non-compliers and were removed from the dataset.

2.2 | Diagnostic measurements

All measurements were performed in fasting serum collected through venipuncture. Measurements of cholesterol and C-reactive protein (CRP) were performed on the Roche/Hitachi Modular P800 analyzer (Roche Diagnostics, Almere, The Netherlands). Insulin was measured using an Immulite 2000 XPi (Siemens, Eschborn, Germany). The Homeostasis Model Assessment of Insulin Resistance (HOMA2-IR) was calculated using the publicly available HOMA calculator (https://www.dtu.ox.ac.uk/homacalculator/).[21] Complete methods of diagnostic measurements are described in van de Rest et al.[6]

2.3 | Hydrogen-1 nuclear magnetic resonance

Hydrogen-1 nuclear magnetic resonance (^{1}H-NMR) was used to analyze the metabolite levels in fasting serum using a previously described platform,[22] consisting of 58 underived measurements and 175 ratios derived from the 58 underived measurements. Sixty-three measurements were selected, the 58 underived measurements, and five fatty acid ratios (FAw3-FA, FAw6-FA, PUFA-FA, MUFA-FA, and SFA-FA). These measurements included: amino acids, ketone bodies, total lipid concentration, mean lipid size, fatty acid amounts, and metabolites involved in glycolysis, fluid balance, and immunity (Table 1; Table S1).

2.4 | Univariate analysis of the effect of GOTO intervention study on metabolic health markers

The intervention effect of each metabolic health marker was calculated using a linear mixed model, adjusted for age at baseline (fixed effects) and individual (random effects). The intervention effects of males and females were calculated separately.

2.5 | Training the PLIS score predictor

We used elastic net regression with both L1 and L2 penalization to train a sex-specific Personalized Lifestyle Intervention Status (PLIS) score using metabolomic measurements from baseline (output 0) and postintervention (output 1) (Table S1). Consequently, a PLIS score closer to 0 represents someone before the GOTO intervention and a PLIS score closer to 1 represents someone who has already been through the GOTO intervention. We trained our PLIS score using elastic net regression ($\alpha = 0.5$, λ was optimized through a bootstrapping method) using the function *glmnet* of the package *glmnet*.[23] We used a combination of fivefold cross-validation and bootstrapping ($N = 1000$) to evaluate the PLIS score. Based on outer-fold samples (data not used for training the models), the association between the PLIS scores and 11 metabolic health marker measurements are assessed using linear mixed models.

The metabolite measurements were normalized per outer training set, using natural log transformation, followed by rank-based inverse normal transformation (Figure S1, Supplementary Methods). For each outer training set: a bootstrap method ($N = 1000$) was used to grab 80% of the training data at random. The bootstrap sample selection was used for an inner fivefold cross-validation to train inner PLIS models across the whole shrinkage (λ) range. For each λ value, the inner PLIS score was calculated. The correlation between the inner PLIS scores and 11 metabolic health markers were calculated. For each λ value, the average of the correlations was calculated, the λ value with the strongest absolute mean correlation between the inner PLIS scores and all 11 metabolic health markers, was selected to train the outer model (Figures S2 and S3).

Males had the highest mean absolute correlations across the five outer folds: 0.172, 0.170, 0.173, 0.191, and 0.195 (with accompanying λ values: 1.16e-01, 1.07e-01, 1.26e-01, 1.07e-01, and 1.26e-01, respectively, Table S2). Although there is some minor variation between the different folds, the shrinkage levels for the male model are in the same order of magnitude. Females had lower mean absolute correlations: 0.162, 0.155, 0.120, 0.125, and 0.124

TABLE 1 Effect of the Intervention on body composition, health and functioning, and diagnostic measurements of the participants used to train the PLIS score

	Male			Female		
Characteristic, mean (SE)	***n***	**Difference**	***p*-value**	***n***	**Difference**	***p*-value**
Body composition						
Weight, kg	75	−3.48 (0.277)	5.47E-20	78	−3.35 (0.249)	6.99E-22
BMI, kg/m^2	75	−1.09 (0.09)	2.38E-19	78	−1.23 (0.093)	1.41E-21
Waist circumference, cm	75	−4.49 (0.608)	1.79E-10	78	−4.33 (0.63)	1.44E-09
Waist-to-hip ratio	75	−0.02 (0.005)	1.57E-04	78	−0.01 (0.006)	7.62E-02
Whole-body fat, %	63	−1.6 (0.209)	1.22E-10	62	−1.57 (0.217)	9.79E-10
Trunk fat, %	63	−2.47 (0.27)	3.17E-13	62	−2.22 (0.275)	3.13E-11
Health and functioning						
Systolic blood pressure, mm Hg[a]	53	−4.46 (1.516)	4.89E-03	52	−4.89 (1.362)	7.42E-04
Diastolic blood pressure, mm Hg[a]	53	−1.53 (0.888)	9.10E-02	52	−2.16 (0.913)	2.16E-02
FRS, %	75	−0.75 (0.447)	9.93E-02	78	−0.38 (0.151)	1.27E-02
Diagnostic measures						
Fasting glucose, mmol/L	75	−0.07 (0.047)	1	78	−0.13 (0.045)	6.23E-03
Fasting insulin, mU/L	75	−0.07 (0.038)	1	78	−0.05 (0.037)	3.34E-01
HOMA2-IR	75	−0.05 (0.049)	1	78	−0.05 (0.046)	2.92E-01
Fasting total cholesterol, mmol/L[b]	59	−0.28 (0.072)	.00527	66	−0.28 (0.072)	2.13E-04
Fasting HDL cholesterol, mmol/L[b]	59	0.04 (0.019)	.516	66	−0.05 (0.019)	1.08E-02
Fasting LDL cholesterol, mmol/L[b]	59	−0.15 (0.042)	.0118	66	−0.1 (0.039)	1.67E-02
Fasting serum triglycerides, mmol/L	75	−0.14 (0.056)	.302	78	−0.08 (0.047)	1.01E-01
Fasting CRP, mmol/L	75	−0.45 (0.4)	1	78	−0.35 (0.35)	3.27E-01

Note: The effects of the intervention were determined using a linear model adjusted for, age, status (longevity family member or control) (fixed effects), household, and individual (random effects).

[a]Individuals using antihypertensive agents were removed before analysis.

[b]Individuals using lipid-lowering medication were removed before analysis.

(with accompanying λ values: 6.61e-02, 5.19e-02, 9.87e-02, 8.89e-03, and 6.61e-02, respectively). Also, female scores varied more between the folds, and penalization weights varied more than in males.

Lastly, the optimized outer models were used to predict the PLIS scores in the independent validation sets.

2.6 | Association of PLIS scores to classical metabolic health marker measurements

The association between the PLIS score and each classical metabolic health marker separately was calculated using the function *lmer* of package *lmerTest*,[24] adjusting for age at baseline as a fixed effect and person ID as a random effect. To combine the *p*-values from the five independent test sets into one *p*-value, we used Fisher's method for combining *p*-values.[25] We adjusted the combined *p*-values for multiple testing, using the Bonferroni correction method.

2.7 | Training the classical metabolic health marker (CHM) score

The classical metabolic health marker (CHM) score was trained based on the 11 classical metabolic health marker measurements: BMI, WC, WHR, SBP, DBP, whole-body fat%, trunk fat%, fasting insulin, fasting HDL cholesterol,

fasting SerumTG, and fasting CRP. A similar procedure as for the PLIS score is used. Natural log and rank-based inverse normal transformation were performed with in the fivefold cross-validation, using the same folds as in the PLIS score training (Figure 1). The same Y labels as in the PLIS score were used. We used logistic regression to train the sex-specific models, using the function *glm* of the package *glmnet*. After training the CHM score was predicted in the independent validation sets.

2.8 | Actief en Gezond Oud (Active and Healthy Old) (AGO) study

The AGO study was a 12-week physical activity lifestyle intervention study.[11] The aim of the intervention was to increase the activity by 10%, compared to the participant's baseline activity. Two hundred and thirty-five participants were included in the study, 119 in the intervention group and 116 in the control group. The baseline age of the AGO participants was between 60 and 70 years old. Some participants of the AGO study had a higher baseline BMI than the maximum baseline BMI of the participants in the GOTO study. To select a group with more similar characteristics, AGO participants with a higher BMI at baseline than the maximum baseline BMI of the GOTO participants were removed. We selected 100 participants (62 males, 38 females) out of the intervention group of AGO to validate the PLIS score. These participants had a similar age at baseline as the GOTO participants (Tables S3 and S4). The effect of the AGO study on the majority of the investigated classical metabolic health markers was in the same direction as the effect of the GOTO study, however, the effects of the AGO study were weaker (Table 1; Table S5).

3 | RESULTS

3.1 | A 13-week combined lifestyle intervention study improved the metabolic health of its participants

Participants of the GOTO intervention study increased their physical activity by 12.5% and decreased their caloric intake by 12.5% for a duration of 13 weeks. Classical metabolic health markers and ^{1}H-NMR metabolites were measured at baseline and after the intervention. In previous work, we showed that the GOTO intervention study significantly improved health indicators of its participants,[6] including BMI, whole-body fat%, trunk fat%, total cholesterol, and several metabolites. Out of the 164 GOTO participants, we selected 153 participants (75 males and 78 females) that both had a high compliance (see Section 2) and fasting metabolite measurements at baseline as well as after the intervention. This subset of participants responded similarly or slightly stronger in terms of the relevant health indicator as compared to the entire study, that is, the effects were stronger in weight, BMI, waist circumference (WC), systolic blood pressure (SBP), diastolic

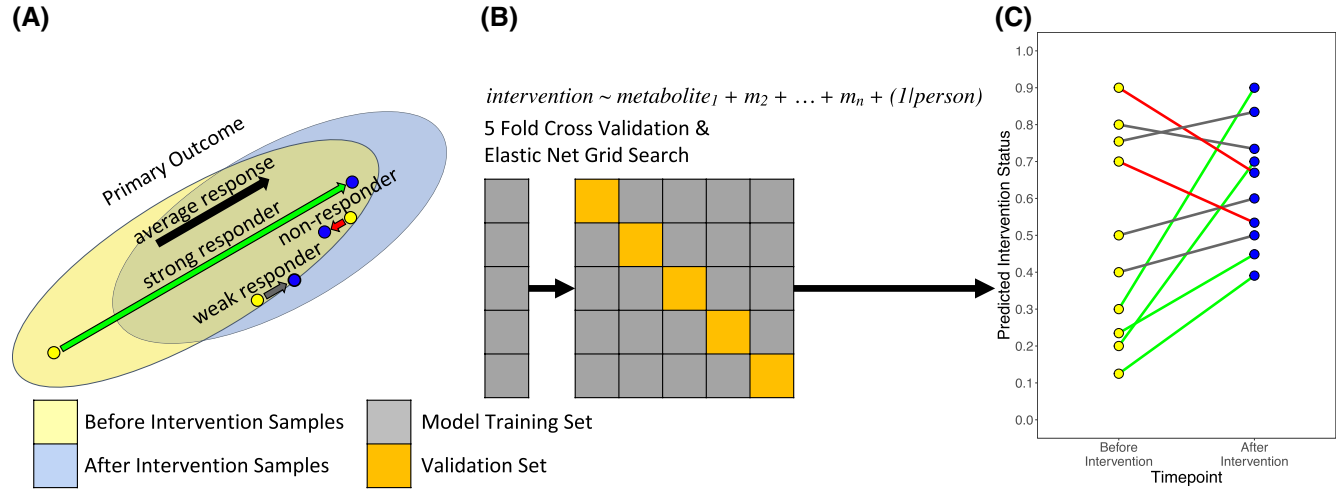

FIGURE 1 Overview of concept and training of the PLIS score. (A) Heterogeneity of the response to an intervention study if generally ignored when only looking at the average response. Yellow area represents the before intervention measurements, blue area represents postintervention measurements. Yellow dots represent baseline samples, blue dots represent postintervention samples. Arrows represent participants' responses to an intervention. (B) Training of the PLIS score. X = fasted ^{1}H-NMR metabolite measurements, Y = intervention time point. PLIS score was trained using fivefold cross-validation in combination with elastic net regression. (C) Predictions of the PLIS score. Green lines represent a positive response to the intervention, gray lines represent a weak response to the intervention, red lines represent a negative response to the intervention.

blood pressure (DBP) (females only), Framingham risk score 10-year risk (FRS), fasting glucose (Glc), fasting insulin (men only), HOMA2-IR, serum triglycerides (SerumTG), and fasting CRP (Table 1; Table S6).

3.2 | The GOTO intervention had a similar effect on most of the metabolic health markers in males and females

As health parameters usually differ between males and females especially at older age,[26] we stratified for testing sex difference in intervention responses. Here we report on the GOTO intervention effects in the 153 participants we selected. Out of 17 selected metabolic health markers, six were significantly influenced by the intervention in both males and females, after adjusting for multiple testing (Table 1). However, waist-to-hip ratio (WHR) and LDL cholesterol concentration were only significantly influenced in males. SBP was only significantly decreased in females. The direction of the intervention effect on the metabolic health markers was the same in males and females, except for fasting HDL cholesterol, which was increased in males and decreased in females. From this we conclude that the GOTO intervention study had a largely similar effect on the metabolic health markers in males and females.

3.3 | The GOTO intervention had a different effect on the ^{1}H-NMR metabolites in males and females

In addition to the metabolic health markers, the ^{1}H-NMR-based fasted blood metabolome was measured before and after the intervention. The metabolite profiles consist of 63 measurements including: amino acids, glycolysis-related metabolites, lipoprotein subclasses, and fatty acid ratios (see Section 2). In the 153 participants we selected, the GOTO intervention significantly influenced 23 of the 63 metabolites, in both males and females (His, M-VLDL-L, S-VLDL-L, XS-VLDL-L, IDL-L, L-LDL-L, M-LDL-L, S-LDL-L, IDL-C, SerumC, VLDL-C, LDL-C, HDL-C, HDL2-C, TotPG, SM, ApoB, TotFA, LA, FAw6, PUFA, MUFA, and SFA-FA) (Table S1). Seven metabolites were significantly different only in males (Cit, Gp, L-HDL-L, VLDL-D, HDL-D, SerumTG, and MUFA-FA). Eight metabolites were only significantly different in females (Gln, Tyr, Leu, Glc, M-HDL-L, PC, TotCho, and ApoA1). Forty-eight metabolites had an effect in the same direction in males and females. Thirteen metabolites had an opposite effect in males and females (Ala, Phe, Val, Lac, XL-HDL-L,M-HDL-L, HDL-C, HDL2-C, HDL3-C, ApoA1, DHA, Faw6-FA, and PUFA-FA), and two metabolites had an effect in males but not in females (SFA and UnsatDeg). These results indicated that the metabolomic response to the GOTO intervention was sex specific.

3.4 | An intervention status predictor to reconstruct variation in individual intervention responses

The effect of the GOTO intervention on metabolites showed a big variation between the different participants (Table S1). The individual variation of metabolite levels after the intervention is based on differences at baseline and their change from pre- to postintervention status. To capture heterogeneity in the response to the intervention (Figure 1), we trained a model based on all pre- and post-metabolite levels and generated a score per metabolic profile of an individual that indicates to what extend someone resembles the metabolome status before or after the intervention. This Personalized Lifestyle Intervention Status (PLIS) score ranges from 0 to 1 and was calculated for all individuals, both, before and after the intervention. The closer the PLIS score for an individual approaches 0, the more that individual resembles the average score before the intervention and, vice versa, a PLIS score closer to 1 indicates the person resembles the average level after the intervention (see Section 2). The model that generates these PLIS scores was trained on 63 ^{1}H-NMR metabolite markers measured at baseline and postintervention. To train the model, we used penalized logistic regression in combination with a double fivefold cross-validation and a bootstrapping method ($n = 1000$) (Supplementary Methods) (Figure 1). Since the metabolome of males and females responded differently to the GOTO intervention two separate models were trained.

3.5 | Characteristics of the optimized PLIS score models

The differences in metabolomic response to the GOTO intervention between males and females resulted in two sex-specific PLIS models with a low overlap of betas for the different metabolites. The metabolites selected by the penalized regression highlight the differences and similarities in the metabolic response to the GOTO intervention in males and females. The male PLIS score was influenced by four metabolites: a higher level of citrate (Cit), and ratio of saturated fatty acids to total fatty acids (SFA-FA) positively influenced the male PLIS score, resembling the (supposedly healthier) profile after the intervention, while higher concentrations of histidine (His) and total

lipids in small VLDL (S-VLDL-L) were associated with a lower PLIS score in males, resembling the profile before the intervention. The female PLIS score was influenced by 14 metabolites. A higher level of glutamine (Gln), phenylalanine (Phe), Cit, docosahexaenoic acid (DHA), and SFA-FA concentration positively influenced the female PLIS score, while higher concentrations of His, tyrosine (Tyr), leucine (Leu), glucose (Glc), 3-Hydroxybutyrate (bOHBut), creatinine (Crea), total lipids in chylomicrons and extremely large VLDL (XXL-VLDL-L), sphingomyelins (SM), and apolipoprotein A1 (ApoA1) resulted in a lower female PLIS score (Table 2, Table S7).

S-VLDL-L had a strong significant intervention effect in males (Table S1), but not in females, explaining why it is only present in the male model (Table 2). Of the 11 metabolites that are only present in the female PLIS score, three had an opposite effect in males and females (Phe, ApoA1, and DHA) and six metabolites were only significantly influenced by the intervention in female samples (Tyr, Leu, Glc, Crea, SM, and ApoA1), highlighting the difference in the male and female metabolomic response to the GOTO intervention. Only three metabolites influenced both the male and female PLIS models: His, Cit, and SFA-FA. The effect of the GOTO intervention on these three metabolites was similar in males and females.

The 15 metabolites that influence the two scores represent different aspects of metabolic health and include five amino acids, three lipids-related metabolites, two citric acid cycle-related metabolites, two fatty acids, and the others are ketone bodies, involved in fluid balance, or cholesterol related (Tables S1 and S7).

3.6 | The PLIS score associates significantly with the changes in metabolic health

The majority of the participants (74.7% of males and 75.6% of females) increased in their PLIS score as a result of the GOTO intervention (Figure 2). To evaluate how well the PLIS score captures the changes in metabolic health, we investigated to what extent the change in PLIS score for each participant associated with a change in 11 classical parameters of health gain: body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR), systolic and diastolic blood pressure (SBP and DBP), whole-body fat%, trunk fat%, fasting insulin, fasting HDL cholesterol, fasting serum triglycerides (SerumTG), and fasting CRP (Figure 3). These classical metabolic health markers were chosen because they represent three different aspects of metabolic health: glucose metabolism, fat metabolism, and inflammation, and have been linked to a wide range of metabolic diseases.[27–29]

The male PLIS score change was significantly negatively associated with the change in 6 (out of 11) investigated classical metabolic health markers: BMI, waist circumference, WHR, whole-body fat%, trunk fat%, and fasting SerumTG (Figure 3). The only significant positive

TABLE 2 PLIS Model betas for male and female models

Biomarker	Full name	Male model betas	Female model betas
Gln	Glutamine	0	0.15599
His	Histidine	−0.2084	−0.26048
Phe	Phenylalanine	0	0.14138
Tyr	Tyrosine	0	−0.20236
Leu	Leucine	0	−0.04071
Glc	Glucose	0	−0.13918
Cit	Citrate	0.01581	0.11538
bOHBut	3-Hydroxybutyrate	0	−0.10615
Crea	Creatinine	0	−0.08286
XXL-VLDL-L	Total lipids in chylomicrons and extremely large VLDL	0	−0.20585
S-VLDL-L	Total lipids in small VLDL	−0.15834	0
SM	Sphingomyelins	0	−0.03204
ApoA1	Apolipoprotein A1	0	−0.25456
DHA	Docosahexaenoic acid	0	0.14235
SFA-FA	Ratio of saturated fatty acids to total fatty acids	0.15861	0.28787

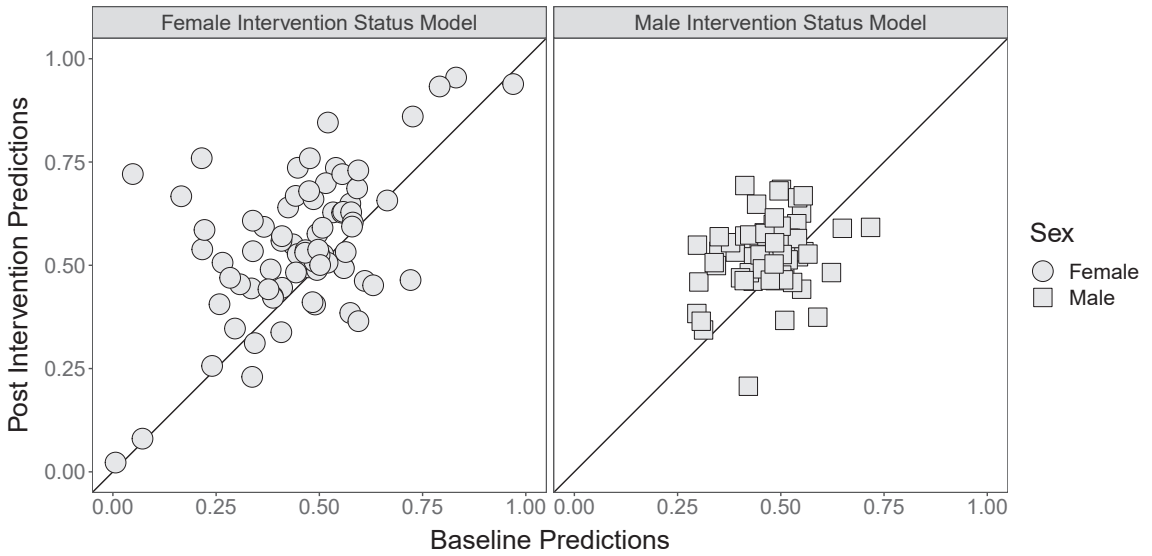

FIGURE 2 The PLIS score increases due to the GOTO Intervention study. The baseline PLIS score is plotted on the x-axis, the postintervention PLIS score is plotted on the y-axis. Each point on the plot represents a participant in the intervention study, the shape of the points represents the sex of the participant; circles indicate female samples, squares indicate male samples. Predictions were made using two different predictors, (A) Female predictor; 75.6% increased their PLIS score, (B) Male predictor; 74.7% increased their PLIS score.

association was with fasting HDL cholesterol changes. Furthermore, the male PLIS score had a weak negative interaction with SBP, DBP, and fasting insulin changes. There was no association between the male PLIS score and fasting CRP.

The female PLIS score change was significantly negatively associated with the change in nine classical metabolic health markers: BMI, WC, SBP, DBP, whole-body fat%, trunk fat%, fasting insulin, fasting HDL cholesterol, and fasting SerumTG (Figure 3). There was a nonsignificant negative association between the female PLIS score and WHR, and a nonsignificant positive interaction with fasting CRP.

3.7 | The PLIS score has stronger association with changes in health than before/after intervention labels

Next, we assessed whether the change in classical metabolic health markers due to the intervention associated stronger with traditional intervention status (0 for samples taken before intervention, 1 for samples taken after intervention) or by the PLIS score (at baseline and postintervention). For males, the traditional intervention status was significantly associated with five classical metabolic health marker measurements (BMI, waist circumference, WHR, whole-body fat%, and trunk fat%) (Figure S4).

The male PLIS score also associated significantly with these five classical metabolic health markers. There was, however, a difference in effect size. Overall, the effect sizes of the PLIS score associations were 10-fold higher than that of the traditional intervention status. We observed a stronger association for the male PLIS score than the traditional intervention status for the classical metabolic health markers with a large variation in intervention effect: fasting insulin, fasting HDL cholesterol, and fasting SerumTG (Table 1). Contrary, fasting CRP associations showed a stronger effect when using the intervention status label instead of the PLIS score. The association effects were all in the same direction, except for fasting CRP.

For females, traditional intervention status was significantly associated with four classical metabolic health marker measurements (BMI, WC, whole-body fat%, and trunk fat%) (Figure S4). The female PLIS scores also associated significantly with the four significant classical metabolic health markers. Additionally, the female PLIS score was significantly associated with five other classical metabolic health markers as well (SBP, DBP, fasting insulin, fasting HDL cholesterol, and fasting SerumTG). These classical metabolic health markers had a high variation in their intervention effect (Table 1). The effect sizes of the associations with WHR, SBP, and fasting HDL cholesterol were of similar strength when using the intervention status in comparison to when using the PLIS score. The PLIS score did show a larger effect size for fasting SerumTG and fasting insulin. Finally, fasting CRP associated weakly in both cases, however, there was a difference in the direction.

For both males and females, the PLIS score represented the classical metabolic health changes more accurately than the traditional intervention status.

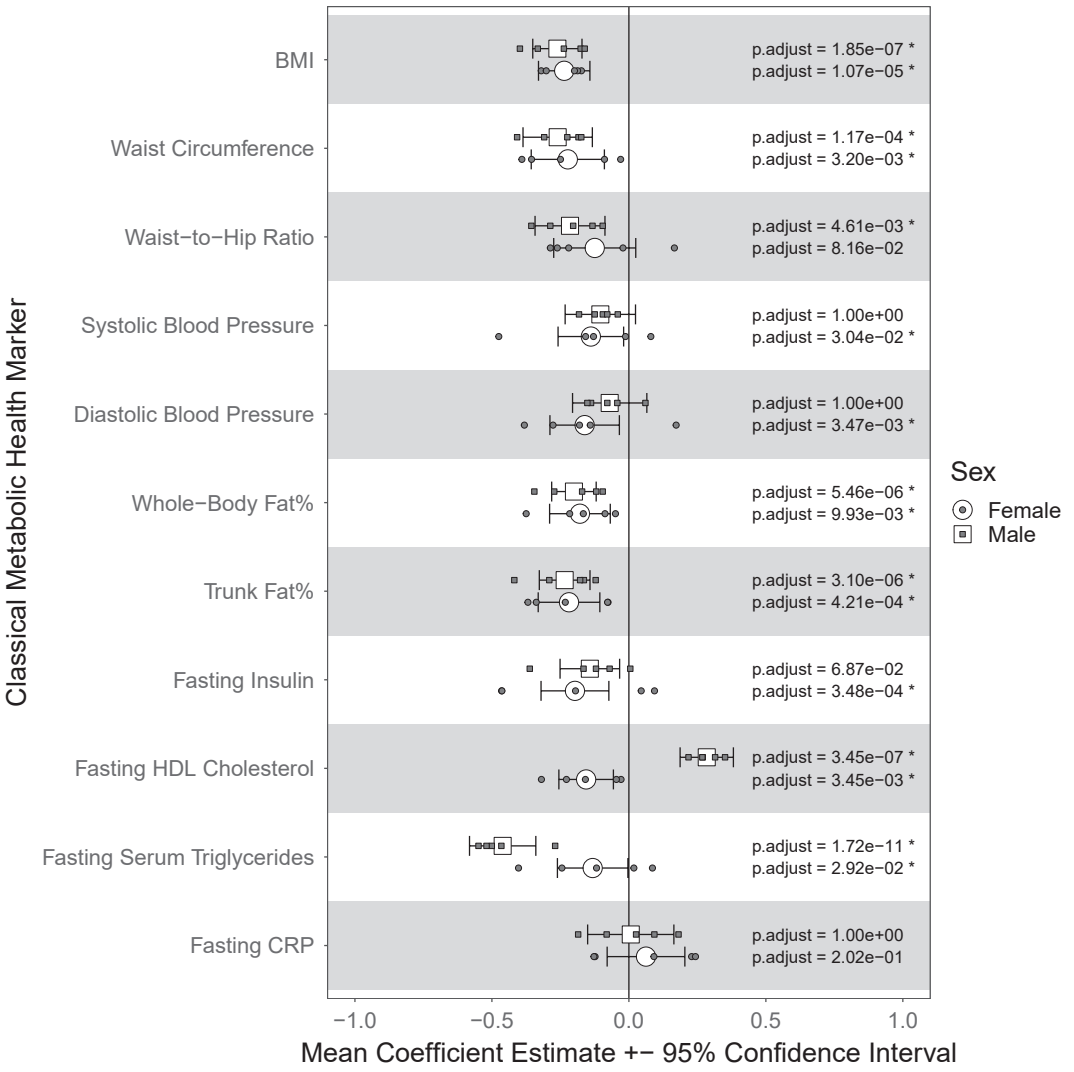

FIGURE 3 The PLIS score is significantly associated with 11 classical metabolic health markers. Association strength between the PLIS score and classical health markers are plotted on the x-axis, adjusted for age. Metabolic health markers are plotted on the y-axis. Interaction was calculated per outer fold test set for each of the sexes. Circles represent female samples; squares represent male samples. Gray dots represent the interaction between the individual fold test sets, white points represent the mean interaction over all five test sets. Error bars indicate the 95% confidence interval of the interaction. Combined *p*-values were calculated using Fisher's method and adjusted for multiple testing using the Bonferroni correction method. Asterisks indicate adjusted *p*-value <.05.

3.8 | The PLIS score has weak nonsignificant associations to Classical Metabolic health markers in an Independent Lifestyle Intervention Study AGO

To test the replicability of the PLIS score we applied it to an independent other intervention study: the Actief en Gezond Oud (Active and Healthy Old) AGO lifestyle intervention study.[11] The AGO study encompassed a 12-week physical activity lifestyle intervention study in sedentary older adults, with no dietary component (see Section 2). Ten of the 11 classical metabolic health markers discussed in this paper, were measured in the AGO study; only trunk fat% was not measured. In males the directions of effects between the classical metabolic health markers and the PLIS score were the same in the AGO study as in the GOTO study, except for WHR which unexpectedly had a positive association with the PLIS score in the AGO study (Figure 4). The associations in AGO were not as significant and the effect sizes were smaller, compared to GOTO. Fasting HDL cholesterol had a strong, but not significant, effect in AGO. Only fasting SerumTG was significantly associated with the male PLIS score.

In females of the AGO study, associations between the PLIS scores and health markers in the AGO study were in the same direction as in the GOTO study (Figure 4). Fasting HDL cholesterol showed a strong but nonsignificant effect. None of these associations were significant. Overall, the PLIS score had a similar but weaker

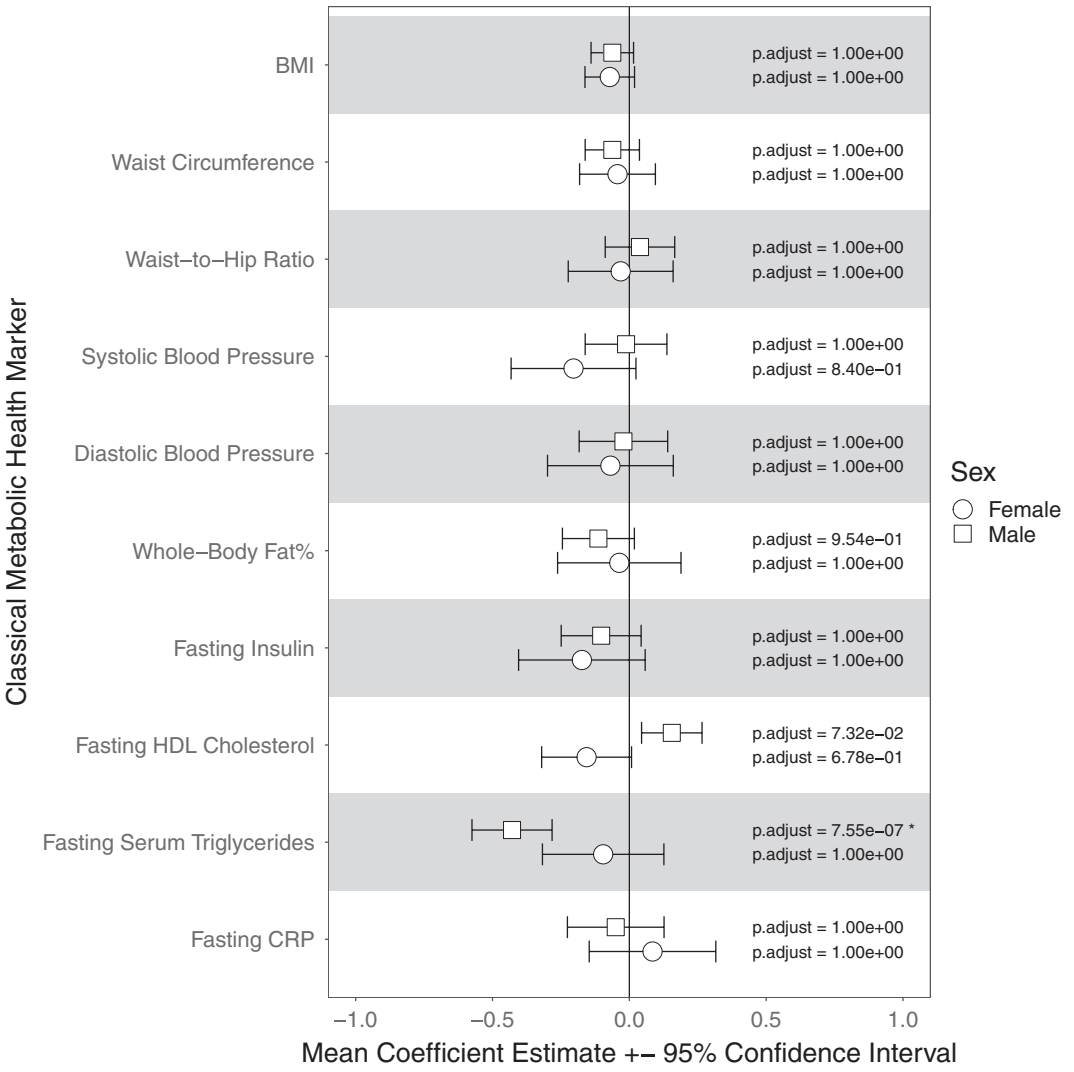

FIGURE 4 The PLIS score has weak nonsignificant associations to the metabolic health marker measurements in the AGO study. Interaction strength between the PLIS score and classical health markers are plotted on the x-axis. Metabolic health markers are plotted on the y-axis. Association was calculated for male and female samples separately. Circles represent female samples; squares represent male samples. Error bars indicate the 95% confidence interval of the interaction. *p*-values were adjusted for multiple testing using the Bonferroni correction method.

association with the classical metabolic health markers in the AGO study than in the GOTO study.

3.9 | Participants with a low baseline PLIS score have a larger positive response to the GOTO intervention study

Selecting participants for a specific lifestyle intervention study that are expected to benefit the most can be difficult. We examined whether selecting participants on the basis of the PLIS score could be a useful inclusion criterium. To this end, we stratified the participants into tertiles based on their PLIS score at baseline. We reasoned that a low baseline PLIS score indicates a low metabolic health, and that these participants would then also benefit the most from the GOTO intervention. In males, fasting HDL cholesterol and fasting SerumTG showed a significant difference only for the tertile with the lowest baseline PLIS score (tertile 1). Whole-body fat% was only significantly different in tertile 1 and 2 and WHR was only significantly different in tertile 2. The remaining classical metabolic health markers had a similar significance in all three tertiles.

For females, SBP was significantly different in tertile 1 only. To our surprise, DBP was only significantly different in tertile 3. WC and whole-body fat% were significantly different in all tertiles, but had the strongest significance in tertile 1. Trunk fat% was also significantly

different in all three tertiles but had the strongest effect in tertile 1 and 2. The remaining classical metabolic health markers had a similar significance in all three tertiles.

When we focused on the median deltas in the tertiles and compared them with the median delta of the whole group (no selection), we found a striking difference between males and females (Figure 5). In the male samples, the median delta of tertile 1 was the biggest in only four classical metabolic health markers (fasting insulin, fasting HDL cholesterol, fasting SerumTG, and fasting CRP). For DBP the median delta was the same in tertile 1 and the entire group of male samples. BMI, SBP, whole-body fat%, trunk fat%, waist circumference, and WHR all had a bigger delta in the entire group of males.

For females there were slight differences in the median; tertile 1 had a bigger median delta, compared to the median delta of all females, in 9 of the 11 classical metabolic health markers (BMI, whole-body fat%, WC, SBP, DBP, fasting insulin, fasting SerumTG, WHR, and fasting CRP) (Figure 5). The median delta of trunk fat% was the same in the two groups, the median delta of fasting HDL cholesterol was bigger in the entire dataset than in tertile 1. Taken together, it seems we can use the PLIS score to select female participants that have a larger response to the GOTO intervention, while the PLIS score is less able to do this for males.

3.10 | A score comprising of metabolites is more predictive of the intervention response than a score comprising of classical metabolic health markers

In practice, individuals for an intervention would be selected on the basis of health criteria (high glucose, LDL cholesterol etc.). Such criteria based on classical metabolic health markers are based on cohort studies and life course risks, not on intervention studies. Therefore, we wondered how a selection of individuals that profit the most from the GOTO intervention based on PLIS score would compare to a selection based on classical metabolic health markers. To test that, we trained a multivariate logistic model, using the measured 11 classical metabolic health markers at baseline and postintervention, and called this score the Classical Health Marker (CHM) score (see Section 2). Next, we performed the same tertile approach for the CHM score as we did for the PLIS score to select participants expected to respond best to the intervention.

In males, tertile 1 of the CHM did not have a stronger significance than the entire group (Figure S5). When we focused on the median deltas, we found that three classical metabolic health markers had a bigger median for tertile 1 of the CHM score than in the entire male group (fasting SerumTG, WHR, and fasting CRP) (Figure S5). The median delta was the same in tertile 1 and all males for

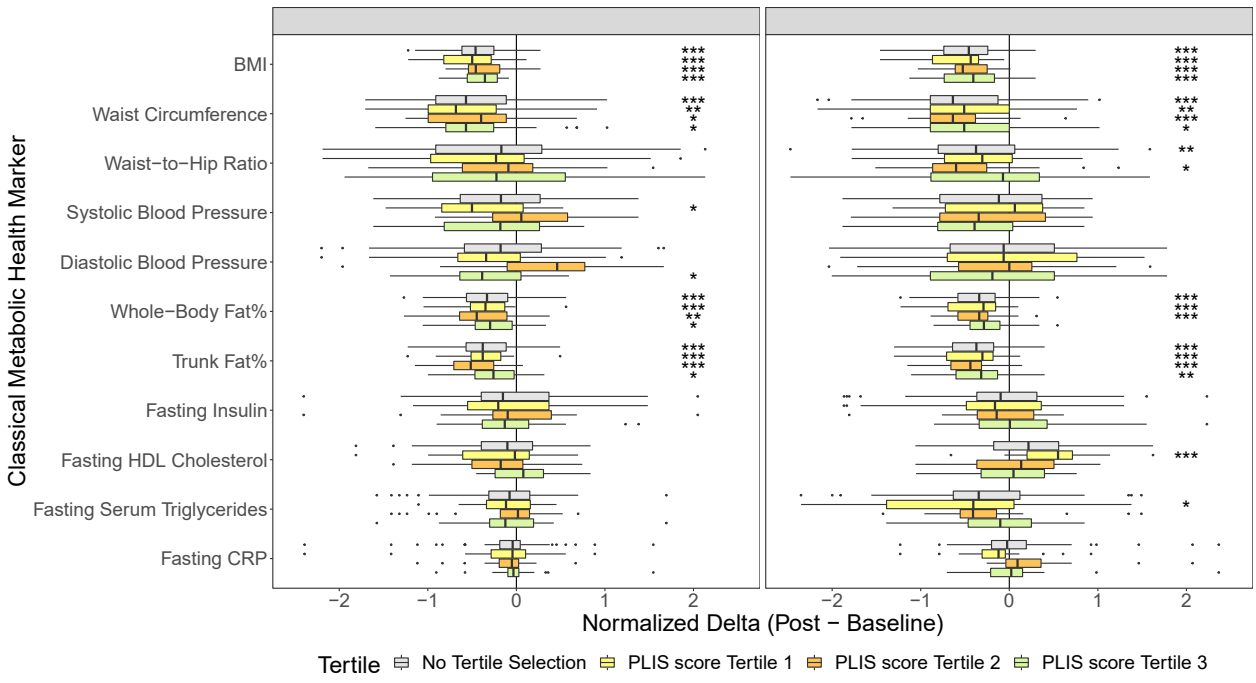

FIGURE 5 Samples in the lowest Baseline PLIS score tertile have bigger deltas for majority of metabolic health markers. Metabolic health markers are plotted on the x-axis, normalized deltas are plotted on the y-axis. Colors represent different PLIS score tertiles: gray; no tertile selection, yellow; tertile 1, orange; tertile 2, green; tertile 3. *p*-values were adjusted for multiple testing using the Bonferroni correction method. Significance is indicated by the asterisks (* = *p*-adjust <.05, ** = *p*-adjust <.01, *** = *p*- adjust <.001). The effects of the intervention were determined using a linear model adjusted for, age (fixed effect) and individual (random effect).

three classical metabolic health markers (BMI, DBP, and WC). Five classical metabolic health markers had a bigger median delta when considering all males than when considering only the males in tertile 1 (SBP, whole-body fat%, trunk fat%, fasting insulin, and fasting HDL cholesterol). For males, this score based on the classical metabolic health markers was not predictive of the response to the GOTO intervention study and was less predictive than the PLIS score.

For females, eight classical metabolic health markers had a bigger median delta in tertile 1 than for the whole group of females (BMI, SBP, DBP, whole-body fat%, trunk fat%, WC, fasting insulin, and fasting SerumTG) (Figure S5). Fasting HDL cholesterol, WHR, and fasting CRP had a higher median delta in the entire female group than in females in tertile 1. For females, the CHM score was more predictive of the response to the GOTO intervention study, than in males. However, also in females, the CHM score was less predictive of the response as the PLIS score.

3.11 | The PLIS score outperformed established metabolomic predictors of metabolic health

Since the PLIS score outperformed the CHM score, we wanted to investigate how the PLIS score would compare to other metabolic health scores based on metabolites. We selected two other scores that used the same metabolite platform. The MetaboHealth[17] and the MetaboAge[16] score. The MetaboHealth was trained on metabolomic data of over 44 000 participants and gives a score that is significantly positively associated with all-cause mortality. For males, the change in the MetaboHealth was significantly associated with the change in fasting insulin, fasting HDL cholesterol, and fasting CRP (Figure S6). Overall, the associations are a lot weaker than in the PLIS score, which had seven significant associations for males (Figure 3). When the tertile approach was used in combination with the MetaboHealth, we found that participants in the tertile with the highest MetaboHealth at baseline do not respond better to the intervention than participants in the other two tertiles (Figure S7). A low PLIS score was associated with a more positive response to the GOTO intervention study (Figure 5).

For females, we find similar results as for males. The change in MetaboHealth was significantly associated with the change in fasting HDL cholesterol and the change in fasting CRP (Figure S6). The PLIS score was significantly associated with 9 of the 11 classical metabolic health markers (Figure 3). A high MetaboHealth at baseline was not associated with a better response to the intervention (Figure S7). A PLIS score did show a stronger positive GOTO intervention effect for females (Figure 5).

The MetaboAge is trained on metabolomic data of over 25 000 participants. When a participant's MetaboAge is higher than their chronological age, they have a positive delta MetaboAge (ΔMetaboAge) and are metabolically unhealthy for their age, participants with a negative ΔMetaboAge are metabolically healthy for their age. The ΔMetaboAge is used as a biomarker. For males, the change in ΔMetaboAge was not significantly associated with any of the classical metabolic health marker changes (Figure S8). A higher ΔMetaboAge at baseline was not associated with a stronger intervention effect (Figure S9). On both these measures, the PLIS score easily outperformed the MetaboAge score (Figures 3 and 5).

For females, the change in ΔMetaboAge was significantly associated with the change in serum triglycerides (Figure S8). As for males, there was no association between a high MetaboAge at baseline and a stronger response to the intervention in females (Figure S9). For females, the PLIS score outperformed the MetaboAge as well (Figures 3 and 5).

In the independent lifestyle intervention study AGO, both the MetaboHealth and the MetaboAge were weakly associated with the classical metabolic health marker levels.

In males, the change in the MetaboHealth was significantly associated with fasting HDL cholesterol and fasting CRP (Figure S10). In females, the MetaboHealth was only significantly associated with fasting HDL cholesterol.

The ΔMetaboAge only had one significant association in males, a significant negative association with fasting serum triglycerides (Figure S11). In females, the ΔMetaboAge was not significantly associated with any of the classical metabolic health markers.

4 | DISCUSSION

By performing metabolic biomarker profiling in 153 participants of the 13-week GOTO combined lifestyle intervention study, we identified 38 metabolomic biomarkers associating with the intervention, 15 of which with sex-specific effects. The identified metabolomics biomarkers represent immuno-metabolic health for ages ranging 50 to 75 years. The metabolomics biomarkers were then used to calculate sex-specific PLIS scores that indicate intervention effects. For both sexes, these scores were significantly associated with several classical metabolic health markers. We observed that the PLIS score represents the intervention induced change in classical metabolic health markers better than the traditional intervention status (labeled 0 for samples taken before intervention,

1 for samples taken after intervention). In GOTO the PLIS score also outperformed a predictor trained on the 11 classical metabolic health markers in selecting participants who are expected to respond beneficially to future lifestyle interventions with similar characteristics to the GOTO study. Furthermore, we have shown that the PLIS score associates with changes in an independent lifestyle intervention study.

The 15 metabolites that form the sex-specific PLIS scores, are involved in different biological processes, including fatty acid and lipoprotein metabolism, renal function, energy metabolism, protein synthesis, and the immune system (Figure 6).[30–37] Several of these metabolites have previously been found to associate with health and were included in predictors of metabolic age and mortality.[16,17] This is the first time they were included in a predictor to study the effectiveness of a lifestyle intervention.

Out of the 15 metabolites, Histidine, Citrate, and SFA-FA are both used to determine the PLIS score in males and females. S-VLDL-L is only used in males. Gln, Phe, Tyr, Leu, Glc, bOHBut, Crea, XXL-VLDL-L, SM, ApoA1, and DHA are used to calculate the PLIS score only in females (Figure 6).

SFA-FA, bOHBut, and DHA are involved in fatty acid metabolism,[38,39] which has been shown to change upon a combined dietary and physical activity lifestyle intervention study in glucose intolerant participants of older age.[40] XXL-VLDL-L, S-VLDL-L, SM, and ApoA1 play a role in lipid metabolism,[41] which is related to change in multiple lifestyle intervention studies.[42,43] Gln is a nonessential amino acid that plays a role in the immune system,[30] the decline of which is closely linked to older age,[44] but can be improved again upon an increase in activity and a change in diet.[45] His, Phe, and Leu are essential amino acids that play a role in protein synthesis as well as in energy metabolism,[31,36,37] which are both affected by aging,[46,47] both have also shown to improve as a result of a lifestyle intervention.[48–50] Tyr is

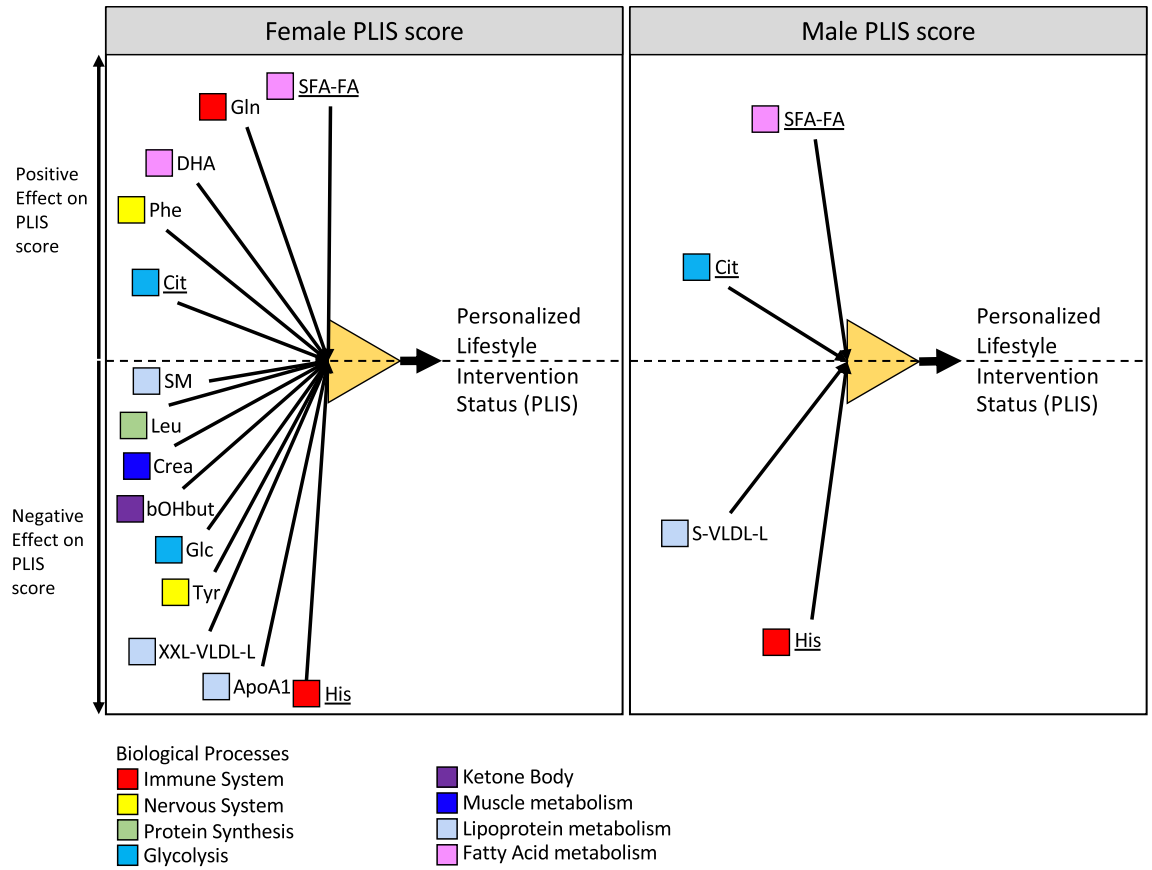

FIGURE 6 The PLIS score is influenced by metabolites that play a role in different biological processes. Schematic representation of the metabolites that influence the PLIS score. Y-axis represents the strength of the effect, metabolites above the dashed horizontal line have a positive effect on the PLIS score, metabolites below the dashed horizontal line have a negative effect on the PLIS score. The further away the metabolite from the horizontal line is, the stronger the effect. (A) The female PLIS score is influenced by 14 metabolites which are involved in eight biological processes. (B) The male PLIS score is influenced by four metabolites that are involved in at four biological processes. Metabolites can be involved in more than one biological process. Three metabolites influence both the female and the male PLIS score, these metabolites are underlined.

a nonessential amino acid that also plays a role in protein synthesis, as well as in cognition,[51,52] the latter of which has been positively associated with physical activity.[53] Cit and Glc are an intermediate and end product of the citric acid cycle and play a role in energy metabolism.[54,55] Crea is in involved in muscle metabolism, improves upon increased physical activity and is a marker for kidney health.[56,57] In summary, the 15 metabolites that made up the algorithm of the PLIS score are all either involved in the aging process or have previously been found to change upon a lifestyle intervention, highlighting the strong associations between the PLIS score, healthy aging, and intervention effect.

The male and female PLIS models have three overlapping metabolites. This small overlap of metabolites could be explained by a number of factors. First, the difference in baseline metabolite levels of males and females (Table S3),[58] due to metabolic differences in the sexes, which in turn could explain the sex-specific response to the intervention study (Table S1). Second, the GOTO intervention had a diet and an activity component, and it is possible that males and females were prone to focus on different aspects of the intervention, through their own choice or as an advice from the physical activity/dietary supervisors. Third, males and females could have performed the physical activity aspect of the intervention differently. Males are overall more physically active,[59] and could also be more prone to higher intensive activities than females during the intervention. Fourth, the difference in adherence to the GOTO intervention. A study that compared the adherence to a lifestyle intervention in males and females, found that males had a higher adherence than females,[60] which could also explain why males overall had a larger intervention effect than females (Table 1), which could in turn have led to stronger associations between the PLIS score and the majority of the classical metabolic health markers. There are multiple possibilities for these differences However, this is not clear from our compliance data. Finally, it could also be that males have a stronger relationship between their fasting metabolite levels and classical metabolic health markers than females.

Metabolic health has different aspects, including BMI, lipid profile, blood pressure, fasting glucose, and inflammation.[61,62] These aspects are reflected in the 11 classical metabolic health markers we have used to determine metabolic health. The change in PLIS score in GOTO was significantly associated with several of these classical metabolic health markers in both males and females. Six classical metabolic health markers were significantly associated with the PLIS score in both males and females (BMI, WC, whole-body fat%, trunk fat%, fasting HDL cholesterol, and fasting SerumTG). WHR was only significantly associated with the PLIS score in males. Contrarily, SBP, DBP, and fasting insulin were only significantly associated with the PLIS score in females.

The significant associations between the PLIS score and 11 classical metabolic health markers, showed that the change in PLIS score is indicative of the metabolic health change. Moreover, participants with minor changes in some of the classical metabolic health markers still showed a big difference in the PLIS score. This may indicate that the PLIS score potentially records a broader spectrum of metabolic changes than the classical markers investigated here and/or smaller changes in metabolic health that may have gone undetected by the classical metabolic health markers.

In both males and females, the PLIS score showed a stronger association with the classical metabolic health markers than categorization by traditional intervention status (0 for samples taken before intervention, 1 for samples taken after intervention) (Figure 2; Figure S4). These results suggest that the PLIS score: (a) better captures the metabolic health state of the GOTO participants than the traditional intervention status and (b) may provide a better indicator of metabolic change due to the intervention.

In addition, we found that a low PLIS score was stronger associated with a positive response to the GOTO intervention study than a low score composed of classical metabolic health markers (CHM score, Figure 5; Figure S5), in both males and females. This result may indicate that the 15 metabolites capture the metabolic health gain better than the 11 classical metabolic health markers used here, something which has been hinted at in other studies.[63]

We also found a stronger association between the change in PLIS score and changes in classical metabolic health markers than was found for the change in two recently developed metabolomic health scores namely MetaboAge and MetaboHealth (Figure 3; Figure S6 and S8). The PLIS score was also better at selecting participants who would respond positively to the intervention than both the MetaboAge and MetaboHealth, especially for females (Figure 5; Figure S7 and S9). This illustrates the higher sensitivity of the PLIS score as compared to the metabolomics health risk estimators.

Taken together our results indicate that the benefit in metabolic health gained by a mild intervention study like GOTO is best monitored by a score trained for this purpose and sex-specific PLIS generated from fasting metabolites seem a suitable first step. When one would want to select older persons inclined to respond positively to GOTO like interventions baseline PLIS screening would clearly be preferred over the individual classical metabolic health markers tested here as well as over a score based on these and finally over metabolomics health risk estimators such as MetaboAge and the MetaboHealth.

Overall, the associations of the PLIS scores and health markers in the independent replication AGO study were in the same direction as in the GOTO study, indicating that the PLIS score can capture the effect of the AGO study (Figure 5). The effect sizes and the significances in the AGO study were lower than in the GOTO study. This reduction in effect sizes and significance could be explained by the lower number of the participants in AGO (62 males, 38 females) than in GOTO (75 males, 78 females), especially in females (Table 1; Table S4). In addition, the fact that the AGO study was only a physical activity intervention while the GOTO intervention combined diet and physical activity, resulted in weaker intervention effects on metabolic health markers in the AGO study, compared to the GOTO study (Table S5; Table 1).[11] Consequently, the MetaboHealth and the PLIS scores were weaker associated with the classical metabolic health markers in AGO than in GOTO (Figures 3 and 4; Figures S6 and S10). The associations between MetaboAge and classical metabolic health markers were weak and nonsignificant in both studies (Figures S8 and S11). These results indicate that a score trained, using the same methodology as the PLIS score, on results from a lifestyle intervention study with only a physical activity component might estimate the intervention status in AGO more accurately than our current PLIS score. However, we do see the same direction of effect between the PLIS score and the classical metabolic health markers in AGO and GOTO, which shows that the PLIS score was able to pick up an intervention effect in an independent lifestyle intervention study.

5 | CONCLUSION

The PLIS score showed that an omics-based biomarker specifically trained on capturing individual lifestyle intervention effects, was able to monitor minor metabolic health changes, which more traditional metabolic health markers and omics-based health risk biomarkers were not able to. Furthermore, these results highlighted that intervention-specific biomarkers could be applied to select participants at baseline most likely to profit most from a novel lifestyle intervention than the classical metabolic health markers investigated here and could be used as inclusion criterium for personalized lifestyle intervention studies. The PLIS score methodology may potentially provide a useful instrument to indicate for similar types of lifestyle interventions which participants are expected to respond positively.

AUTHOR CONTRIBUTIONS

Fatih A. Bogaards, Thies Gehrmann, Erik Ben van den Akker, Marcel J. T. Reinders, and P. Eline Slagboom designed the study. Ondine van de Rest, Marian Beekman, Raymond Noordam, and Simon P. Mooijaart acquired the data. Fatih A. Bogaards, Thies Gehrmann, Marcel J. T. Reinders, Lisette C. P. G. M. de Groot, and P. Eline Slagboom performed the research and interpreted the data. All authors were involved in drafting and revising the manuscript.

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DISCLOSURES

The authors have stated explicitly that there is no conflict of interest in connection with this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are accessible upon request to the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

ETHICS STATEMENT

The Medical Ethical Committee of the Leiden University Medical Center approved the study (P11.187) and all participants signed a written informed consent. All experiments were performed in accordance with relevant and approved guidelines and regulations. This trial was registered at the Dutch Trial Register (http://www.trialregister.nl) as NTR3499.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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