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Molecular effects of lifestyle interventions are typically studied in a single tissue. Here, we perform a secondary analysis on the sex-specific effects of the Growing Old TOgether trial (GOTO, trial registration number GOT NL3301 (https://onderzoekmetmensen.nl/nl/trial/27183), NL-OMON27183, primary outcomes have been previously reported in ref. 1), a moderate 13-week combined lifestyle intervention on the transcriptomes of postprandial blood, subcutaneous adipose tissue (SAT) and muscle tissue in healthy older adults, the overlap in effect between tissues and their relation to whole-body parameters of metabolic health. The GOTO intervention has virtually no effect on the postprandial blood transcriptome, while the SAT and muscle transcriptomes respond significantly. In SAT, pathways involved in HDL remodeling, O_2/CO_2 exchange and signaling are overrepresented, while in muscle, collagen and extracellular matrix pathways are significantly overexpressed. Additionally, we find that the effects of the SAT transcriptome closest associates with gains in metabolic health. Lastly, in males, we identify a shared variation between the transcriptomes of the three tissues. We conclude that the GOTO intervention has a significant effect on metabolic and muscle fibre pathways in the SAT and muscle transcriptome, respectively. Aligning the response in the three tissues revealed a blood transcriptome component which may act as an integrated health marker for metabolic intervention effects across tissues.

Worldwide, the proportion of older people in our society is increasing, and with it the burden of age-related diseases on the quality of life and health economics[2,3]. Improving metabolic health is considered key to improving vitality at older ages[4,5]. Dietary, activity-based, or combined lifestyle interventions are known to improve culprit factors in human

ageing such as metabolic health and immunity. Although effects are usually heterogeneous, the metabolic health can significantly be improved, even in middle and older age[1,6–10].

The effect of lifestyle interventions can be studied by traditional blood-based markers of metabolic health (including: BMI, fasting

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insulin, systolic blood pressure (SBP), HDL cholesterol, diastolic blood pressure (DBP), dual-energy X-ray absorptiometry (DEXA)-based whole-body fat%, trunk fat%)[1,11] and a range of omics based assays such as the metabolome[1,12–14], proteome[15,16], epigenome[8] and the transcriptome[17–27], of blood[1,12–17,19,28,29], gut[15], adipose tissue[18,20,25,30,31], and muscle tissue[21,26,27]. Human intervention studies are usually small ($n = 120–250$)[1,29,32–34] and focus on one tissue only, mainly blood. Mostly, a strong effect is observed on the fasting blood proteome[16] and metabolome[1], but typically not on the fasting transcriptome, where the effects are milder and more variable[17,23,35], except for rigorous lifestyle interventions[29]. The transcriptomes of adipose tissue and muscle do react respectively on separate dietary[24] and physical activity[21,22,26,27] lifestyle interventions. In mice, combined lifestyle interventions were shown to affect the transcriptome of brown adipose tissue and skeletal muscle[36].

In humans, however, the simultaneous effect of combined lifestyle interventions on the transcriptome of blood, subcutaneous adipose tissue (SAT), and muscle was not investigated as yet. To understand how differences in omics profiles relate to changes over the years in health parameters, one often relies on population-based omics studies. However, such studies mainly rely on blood samples[37,38]. We speculate that some of the transcriptomic changes in different tissues in response to interventions align due to the systemic nature of lifestyle interventions and that a part of changes in the blood transcriptome reflect responses in SAT and muscle and related health gains. Hereto, an integrative analysis of the SAT, muscle and blood transcriptomic response to such intervention is necessary.

Integrative data analysis methods pool data from different datasets to jointly analyze them[39]. Joint and Independent Variation Explained (JIVE)[40] is one such integrative data analysis method. JIVE decomposes the variation across the different datasets in three components: (1) the joint variation between the datasets, (2) dataset-specific variations, and (3) the residual noise for each dataset separately. The blood transcriptome response in mild interventions is usually minimal, JIVE however, may reveal a part that represents the joint response variation across tissues. We are interested whether the joint response captures the intervention effects on metabolic health stronger than the blood transcriptome alone.

We applied the tissue-specific as well as the integrative data analysis approach on a 13-week combined physical activity and dietary lifestyle intervention trial in older adults; the Growing Old TOgether (GOTO) trial. The primary outcome of the GOTO trial was a reduction in fasting insulin along with general improvement in metabolic health, through a mild combined lifestyle intervention[1]. Within this trial the transcriptome of postprandial blood, SAT and muscle tissue before and after intervention has been measured. We report on genes whose expressions within each of the different tissues associated with whole-body health improvements across the intervention, and report on the observed joint transcriptional effect. We conclude that especially the SAT and muscle transcriptome respond to the intervention by HDL remodeling, cell signaling and CO_2/O_2 exchange for SAT, and collagen/extracellular matrix pathways for muscle. Especially the SAT transcriptome in both sexes associates to beneficial changes in whole-body composition. The blood transcriptome does not generally respond to intervention or reflect health gain. Our integrative analysis of blood, however, revealed that the joint effect of the three tissues associated with the parameters of whole-body composition parameters. This blood transcriptome component may act as an integrated health marker for metabolic intervention effects across tissues.

Results
GOTO lifestyle intervention trial
The Growing Old TOgether (GOTO) trial, was a 13-week combined lifestyle intervention with 164 participants, of whom 163 completed the trial. One participant withrew from the trial due to knee surgery.

The primary outcome of the trial was a significant reduction in fasting insulin, these effects have been reported in ref. 1. The GOTO trial is completed.

Baseline characteristics of the GOTO participants were similar among subsets
At baseline, the participants of the GOTO trial were non-diabetic overall healthy older adults, with a mean age of 63 for males and 62 for females (Table 1). Some of the participants were overweight and/or had elevated blood pressure. Transcriptome data are available at baseline and post intervention for different sets of participants of the complete GOTO trial group, i.e.: (1) 57 participants (33 males, 24 females) with transcriptome data of postprandial blood, SAT, and muscle samples, (2) 88 blood postprandial samples, containing 45 males and 43 females, (3) 78 SAT postprandial samples, which contains 38 males and 40 females, and 4) 82 muscle postprandial samples, 48 males and 34 females (Supplementary Fig. 1).

Between the participants in the different subsets, there was only a slight variation in baseline measurements of the metabolic health markers (Table 1). There were differences between the baseline characteristics of males and females, especially in whole-body fat%, trunk fat%, HDL cholesterol level, which measurements were all higher in females than in males: 38.16%, 37.4% & 1.64 mmol/L versus 26.5%, 27.17% & 1.36 mmol/L, respectively. Fasting insulin, the primary outcome of the GOTO trial, was higher at baseline in males than in females: 9.42 mU/L versus 8.35 mU/L. These differences in baseline metabolic health between the two sexes, along with earlier published results on different activity patterns and differences in the intervention effect on metabolic health between the sexes[1,41], prompted us to perform the analysis in a sex-stratified manner.

Health markers improve but improvement varies among subsets
Participants compliant to the intervention overall improved their metabolic health, with for some parameters slight sex differences in magnitude of effect (Table 2). The direction of effect on the health parameters in the subsets of participants for which data in different tissues is available was the same, though there were some differences in significance level. For example, in males, the intervention only had a significant effect on HDL cholesterol in the group with muscle transcriptome measurements. While in females, HDL cholesterol size was significantly different in only the participants with SAT transcriptome measurements, and the Framingham Risk Score (FRS) was significantly different in the complete set of measurements. These differences in effect could be partially explained by slight baseline classical metabolic health marker level differences between the groups (Table 1). To circumvent the slight differences in intervention effect on the classical metabolic health markers between the different groups, the associations between the transcriptome and the classical metabolic health markers will only be calculated on the participants with transcriptome data available in all three tissues (Supplementary Fig. 1).

Strong sex-dependent transcriptional response in subcutaneous adipose tissue and muscle
We investigated the effect of the GOTO intervention by comparing the transcriptome levels post intervention to the baseline transcriptome levels matched for each participant. We studied the transcriptional differences in blood, SAT, and muscle (Fig. 1, differential gene expression analysis, see "Methods"). In males, the number of significantly differentially expressed genes (DEGs) across the intervention varied considerably between blood (1), SAT (89), and muscle (251) (Fig. 2, Table 3, and Supplementary Datasets 1–3). The ratio between up and downregulated DEGs also differed between the affected tissues: a slightly larger number of downregulated genes in SAT whereas over 80% of the DEGs were upregulated in muscle. Noteworthy, the

Table 1 | Baseline measurements of metabolic health marker measurements per subset selection

| Health marker, baseline mean (SD) [limits]	Sex	Complete GOTO dataset 153 (75 male, 78 female)	Overlapping blood, SAT and muscle samples 57 (33 male, 24 female)	Blood postprandial samples 88 (45 male, 43 female)	SAT postprandial samples 78 (38 male, 40 female)	Muscle postprandial samples 82 (48 male, 34 female)
Age, years	Male	63.95 (5.36) [50.03–74.33]	65.08 (5.65) [50.03–74.33]	64.21 (5.68) [50.03–74.33]	64.86 (5.51) [50.03–74.33]	64.4 (5.5) [50.03–74.33]
BMI, kg/m²	Male	26.53 (1.95) [21.94–33.04]	26.58 (2.04) [21.94–32.11]	26.59 (2) [22.03–32.11]	26.78 (2.19) [21.94–32.11]	26.6 (2.05) [22.03–32.11]
Waist circumference	Male	98.2 (6.47) [75.00–117.00]	99.27 (6.03) [75.00–112.00]	98.6 (6.39) [81.00–112.00]	99.58 (6.09) [75.00–112.00]	98.9 (6.56) [82.00–112.00]
Systolic blood pressure, mm Hg	Male	86.22 (7.52) [66.50–107.50]	87.66 (7.99) [66.50–107.00]	86.87 (7.58) [66.50–107.00]	87.74 (7.91) [66.50–107.00]	86.79 (7.64) [66.50–107.00]
Diastolic blood pressure, mm Hg	Male	141.02 (14.84) [114.00–182.50]	142.95 (14.67) [114.00–182.50]	140.46 (15.13) [114.00–182.50]	142.36 (14.44) [114.00–182.50]	140.95 (14.76) [114.00–182.50]
Fasting insulin, mU/L	Male	9.42 (4.06) [2.00–22.60]	9.39 (3.23) [2.91–20.00]	9.07 (3.24) [2.00–20.00]	9.69 (3.57) [2.00–20.00]	9.36 (3.59) [3.43–20.00]
Whole-body fat, %	Male	26.5 (4.37) [14.83–38.06]	26.76 (4.64) [14.83–38.06]	26.25 (4.7) [16.14–38.06]	27.02 (4.7) [14.83–38.06]	26.5 (4.77) [16.14–38.06]
Trunk fat, %	Male	28.17 (5.38) [11.97–39.91]	28.09 (5.33) [11.97–39.91]	27.55 (5.68) [15.44–39.91]	28.42 (5.56) [11.97–39.91]	27.85 (5.84) [15.44–39.91]
Fasting HDL cholesterol, mmol/L	Male	1.36 (0.23) [0.81–1.92]	1.38 (0.24) [0.81–1.92]	1.36 (0.24) [0.81–1.92]	1.38 (0.23) [0.81–1.92]	1.36 (0.24) [0.81–1.92]
Fasting HDL cholesterol size, nm	Male	9.49 (0.14) [9.24–9.98]	9.49 (0.15) [9.29–9.98]	9.49 (0.14) [9.29–9.98]	9.5 (0.14) [9.29–9.98]	9.49 (0.14) [9.29–9.98]
FRS	Male	12.96 (1.54) [9.00–17.00]	13.21 (1.29) [9.00–17.00]	12.98 (1.44) [9.00–17.00]	13.11 (1.35) [9.00–17.00]	13.04 (1.35) [9.00–17.00]
Age, years	Female	62.02 (6) [46.70–75.11]	61.83 (4.84) [48.41–75.11]	62.74 (5.4) [48.41–69.25]	61.86 (5.27) [49.11–69.25]	61.99 (4.77) [49.11–69.25]
BMI, kg/m²	Female	27.04 (2.73) [21.04–33.73]	26.86 (2.68) [21.04–32.57]	26.67 (2.34) [21.04–33.55]	27.21 (2.68) [21.04–32.57]	26.85 (2.66) [21.04–32.57]
Waist circumference	Female	93.36 (7.83) [72.00–112.00]	93.33 (7.2) [73.00–112.00]	93.14 (7.48) [74.00–112.00]	95.1 (7.28) [73.00–112.00]	93.68 (7.35) [76.00–112.00]
Systolic blood pressure, mm Hg	Female	84.23 (8.6) [62.50–107.75]	85.18 (9.14) [62.50–107.75]	84.07 (8.46) [71.00–107.75]	85.89 (8.47) [71.00–107.75]	84.66 (9.23) [71.50–107.75]
Diastolic blood pressure, mm Hg	Female	137.72 (17.27) [101.25–177.50]	138.16 (17.06) [101.25–175.00]	136.05 (16.37) [107.50–175.00]	137.59 (15.84) [107.50–175.00]	136.48 (15.96) [107.50–175.00]
Fasting insulin, mU/L	Female	8.35 (3.99) [2.00–22.50]	7.78 (3.2) [2.00–16.90]	7.57 (3.59) [2.00–22.50]	8.24 (4.12) [2.00–18.70]	7.69 (3.25) [2.00–16.70]
Whole-body fat, %	Female	38.16 (4.39) [24.25–47.36]	37.34 (4.81) [24.25–45.51]	37.7 (4.24) [24.25–45.51]	37.85 (4.46) [24.25–45.51]	37.19 (4.92) [24.25–45.51]
Trunk fat, %	Female	37.4 (5.55) [18.22–49.03]	36.93 (5.82) [20.17–49.03]	36.82 (5.18) [18.22–49.03]	37.3 (5.79) [18.22–49.03]	36.72 (6.2) [20.17–49.03]
Fasting HDL cholesterol, mmol/L	Female	1.64 (0.28) [1.08–2.32]	1.64 (0.27) [1.08–2.32]	1.68 (0.29) [1.08–2.32]	1.66 (0.26) [1.13–2.17]	1.64 (0.26) [1.13–2.12]
Fasting HDL cholesterol size, nm	Female	9.62 (0.17) [9.31–10.06]	9.59 (0.16) [9.33–10.06]	9.62 (0.17) [9.33–10.06]	9.61 (0.16) [9.34–10.06]	9.62 (0.17) [9.34–10.02]
FRS	Female	14.88 (2.72) [7.00–21.00]	14.88 (2.58) [9.00–20.00]	14.91 (2.56) [9.00–20.00]	14.8 (2.33) [9.00–20.00]	14.71 (2.3) [9.00–20.00]

SD standard deviation, SAT subcutaneous adipose tissue, BMI body mass index, FRS Framingham Risk Score.

Table 2 | Effect of the 13-week lifestyle intervention on metabolic health marker measurements differs per subset selection

Health marker, mean (SD)	Sex	Complete GOTO dataset 153 (75 male, 78 female)		Overlapping blood, SAT and muscle samples 57 (33 male, 24 female)		Blood postprandial samples 88 (45 male, 43 female)		SAT postprandial samples 78 (38 male, 40 female)		Muscle postprandial samples 82 (48 male, 34 female)	
		Delta (SD)	P adjust	Delta (SD)	P adjust	Delta (SD)	P adjust	Delta (SD)	P adjust	Delta (SD)	P adjust
BMI, kg/m^2	Male	−1.09 (0.78)	2.4e-18*	−1.02 (0.78)	1.8e-07*	−1.15 (0.79)	1.4e-11*	−1.10 (0.77)	1.3e-09*	−1.18 (0.78)	6.9e-13*
Waist circumference	Male	−4.49 (5.26)	1.8e-09*	−3.82 (4.97)	1.1e-03*	−4.47 (5.29)	1.1e-05*	−4.42 (5.10)	4.8e-05*	−4.94 (5.41)	8.7e-07*
Systolic blood pressure, mm Hg	Male	−3.25 (12.54)	2.8e-01	−3.80 (14.36)	1.0e+00	−2.52 (13.42)	1.0e+00	−3.91 (14.14)	9.7e-01	−3.05 (13.53)	1.0e+00
Diastolic blood pressure, mm Hg	Male	−0.75 (6.98)	1.0e+00	−3.04 (7.09)	1.9e-01	−2.27 (6.67)	2.7e-01	−3.09 (7.10)	1.1e-01	−2.32 (6.86)	2.3e-01
Fasting insulin, mU/L	Male	−0.31 (3.30)	1.0e+00	0.14 (3.63)	1.0e+00	0.04 (3.62)	1.0e+00	−0.11 (3.71)	1.0e+00	−0.23 (3.72)	1.0e+00
Whole-body fat, %	Male	−1.60 (1.65)	1.2e-09*	−1.17 (1.55)	1.5e-03*	−1.43 (1.71)	8.2e-06*	−0.92 (1.52)	9.3e-05*	−1.77 (1.66)	4.8e-07*
Trunk fat, %	Male	−2.47 (2.13)	3.2e-12*	−1.96 (1.81)	3.3e-05*	−2.26 (2.13)	2.3e-07*	−1.48 (1.74)	1.1e-06*	−2.59 (2.04)	8.7e-09*
Fasting HDL cholesterol, mmol/L	Male	0.04 (0.14)	8.2e-02	0.04 (0.13)	1.0e+00	0.06 (0.14)	7.7e-02	0.05 (0.13)	3.4e-01	0.07 (0.14)	1.7e-02*
Fasting HDL cholesterol size, nm	Male	0.06 (0.09)	2.0e-06*	0.06 (0.08)	9.9e-04*	0.08 (0.08)	2.0e-06*	0.06 (0.09)	4.6e-04*	0.08 (0.09)	1.3e-06*
FRS	Male	−0.40 (1.22)	5.8e-02	−0.61 (1.37)	1.6e-01	−0.53 (1.25)	6.6e-02	−0.58 (1.31)	9.7e-02	−0.54 (1.24)	3.9e-02*
BMI, kg/m^2	Female	−1.23 (0.82)	1.4e-20*	−1.26 (0.72)	1.4e-07*	−1.27 (0.64)	2.7e-15*	−1.35 (0.73)	3.0e-13*	−1.36 (0.74)	2.5e-11*
Waist circumference	Female	−4.33 (5.57)	1.4e-08*	−4.71 (5.74)	5.4e-03*	−4.70 (5.30)	7.5e-06*	−4.58 (5.31)	3.0e-05*	−5.21 (5.32)	2.3e-05*
Systolic blood pressure, mm Hg	Female	−3.01 (11.69)	2.6e-01	−1.67 (11.69)	1.0e+00	−2.85 (11.30)	1.0e+00	−2.46 (11.73)	1.0e+00	−1.35 (12.07)	1.0e+00
Diastolic blood pressure, mm Hg	Female	−1.25 (6.52)	9.5e-01	−1.19 (5.75)	1.0e+00	−1.66 (5.64)	6.1e-01	−2.29 (6.27)	2.6e-01	−1.43 (6.54)	1.0e+00
Fasting insulin, mU/L	Female	−0.36 (3.25)	1.0e+00	−0.30 (2.35)	1.0e+00	−0.02 (3.01)	1.0e+00	−0.33 (3.63)	1.0e+00	−0.90 (2.84)	7.3e-01
Whole-body fat, %	Female	−1.57 (1.70)	9.8e-09*	−1.33 (2.22)	1.1e-02*	−1.64 (1.85)	5.2e-05*	−1.58 (1.98)	7.2e-05*	−1.98 (2.07)	2.5e-04*
Trunk fat, %	Female	−2.22 (2.15)	3.1e-10*	−1.76 (2.78)	7.1e-03*	−2.32 (2.36)	1.2e-05*	−2.31 (2.48)	2.6e-05*	−2.60 (2.57)	1.1e-04*
Fasting HDL cholesterol, mmol/L	Female	−0.04 (0.15)	2.7e-01	−0.03 (0.14)	1.0e+00	−0.05 (0.16)	4.7e-01	−0.05 (0.15)	5.9e-01	0.00 (0.14)	1.0e+00
Fasting HDL cholesterol size, nm	Female	0.03 (0.08)	1.7e-02*	0.05 (0.09)	1.7e-01	0.02 (0.08)	6.0e-01	0.04 (0.08)	2.6e-02*	0.04 (0.09)	9.1e-02
FRS	Female	−0.65 (1.60)	5.5e-03*	−0.46 (1.59)	1.0e+00	−0.42 (1.47)	6.8e-01	−0.62 (1.76)	3.1e-01	−0.68 (1.79)	3.4e-01

SD standard deviation, SAT subcutaneous adipose tissue, BMI body mass index, FRS Framingham Risk Score.
Significance levels were calculated using a two-sided linear mixed model. P values were adjusted for multiple tests using the Bonferroni correction method. An adjusted P value below 0.05 was considered significant. Significant effects are indicated by an asterisk.
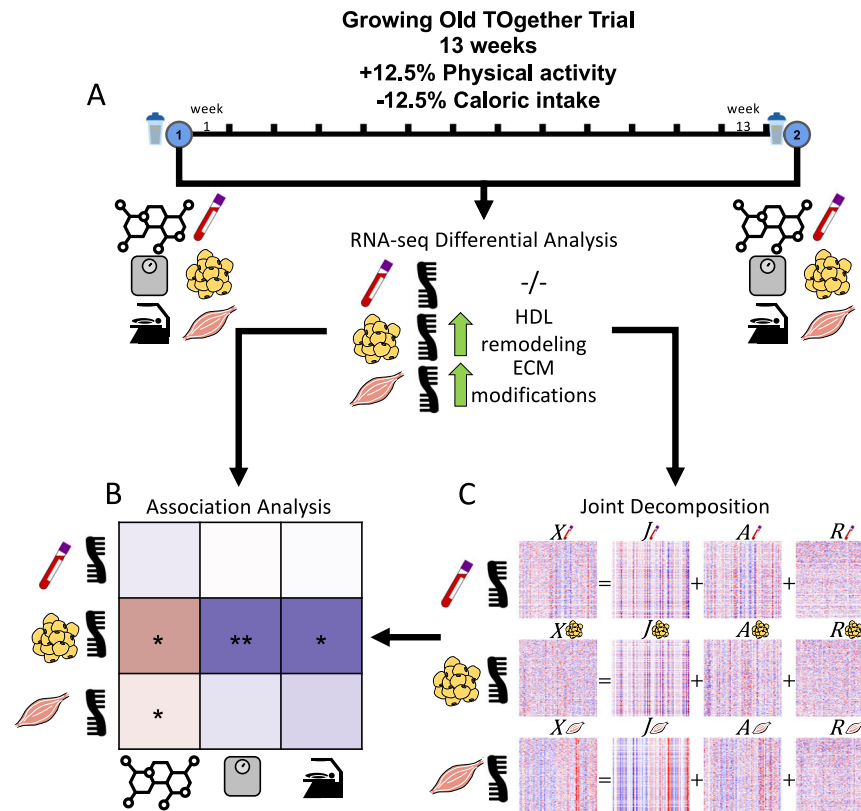
Fig. 1 | Outline of the transcriptomic analysis of the Growing Old TOgether trial. A The participants of the Growing Old TOgether (GOTO) trial changed their lifestyle for a duration of 13 weeks. Both before and after the intervention measurements were performed, including anthropometrics, Dual-energy X-ray absorptiometry and blood metabolite levels. In addition, following a standardized nutritional challenge, postprandial blood, subcutaneous adipose tissue (SAT) and muscle tissue were sampled. Blood, SAT and muscle RNA-seq levels were studied and a differential analysis was performed to study the effect of the intervention on these three tissues. Subsequently, a pathway overrepresentation analysis was performed. **B** To study which tissue best represented the metabolic health changes, the association between the RNA-seq levels of the overrepresented pathways of the three tissues and metabolic health marker levels was studied. **C** Joint and Individual Variation Explained (JIVE) was applied to investigate whether there was a joint variation between the expression levels of the three tissues. JIVE decomposed the expression levels of each tissue (X) into the joint (J), individual (A), and the residual (R) effect, with the joint effect representing the shared variation across the expression levels of the three tissues. Subsequently, the drivers of the joint effect in blood were identified. Lastly, the association between the joint effect drivers in blood and the metabolic health markers was studied and compared to the postprandial blood individual effect expression levels (A).

muscle DEGs contained 14 collagen genes, all of which were upregulated (Supplementary Dataset 3).

For females, the number of DEGs across the different tissues were somewhat similar as in males, but the effect sizes were smaller (Fig. 2 and Table 3): zero DEGs in blood, 168 in SAT, and 168 in muscle (Supplementary Datasets 4 and 5). Like the male samples, more than half of the DEGs in SAT were downregulated, while in muscle over two-thirds of significant DEGs were upregulated. The female muscle DEGs contained 7 collagen genes, all of them upregulated (Supplementary Dataset 5).

Overall, in both males and females, the intervention had hardly any effect on the blood transcriptome, while in SAT both up- and downregulated genes were observed, whereas in muscle the majority of the DEGs were upregulated.

Lipid and collagen-related pathways enriched in SAT and muscle response, respectively

We then investigated which pathways were associated with the found DEGs (pathway overrepresentation analysis, see Methods section). In male SAT, five pathways were significantly overrepresented (Fig. 3 and Supplementary Dataset 6). The three upregulated pathways were involved with HDL remodeling, nuclear receptor signaling and cholesterol transport, while the downregulated pathway was involved in fertilization, the remaining pathway (equally down -and upregulated) was involved in ligand clearance. In male muscle, all 18 enriched pathways were upregulated. The majority of these were involved in collagen formation or extracellular matrix formation/degradation (Fig. 3 and Supplementary Dataset 6). The remaining pathways were involved in myogenesis (NCAM1 pathways), mesenchymal stem cell differentiation (PDGF pathway), scavenger receptors (binding and uptake, class A receptors), and protein O-glycosylation.

In female SAT, seven pathways were significantly overrepresented (Fig. 3 and Supplementary Dataset 6). The three upregulated pathways were involved in signaling pathways and HDL remodeling. The four downregulated pathways were related to MAPK signaling and CO_2/O_2 exchange. However, the three CO_2/O_2 pathways were only overrepresented due to the same three genes (Supplementary Dataset 6). In female muscle, all eight overrepresented pathways were upregulated (Fig. 3 and Supplementary Dataset 6), six of which were involved in collagen formation and extracellular matrix organization/degradation. The remaining pathways were involved in in netrin-1 signaling and osteoblast differentiation.

Taken together, both in males and females, the overrepresented pathways in muscle were largely involved in collagen and extracellular matrix remodeling. In SAT, there were fewer enriched pathways which were less consistent in terms of direction (both -up and downregulated). The overrepresented pathways in SAT were involved in signaling, O_2/CO_2 exchange, HDL remodeling, and signaling.
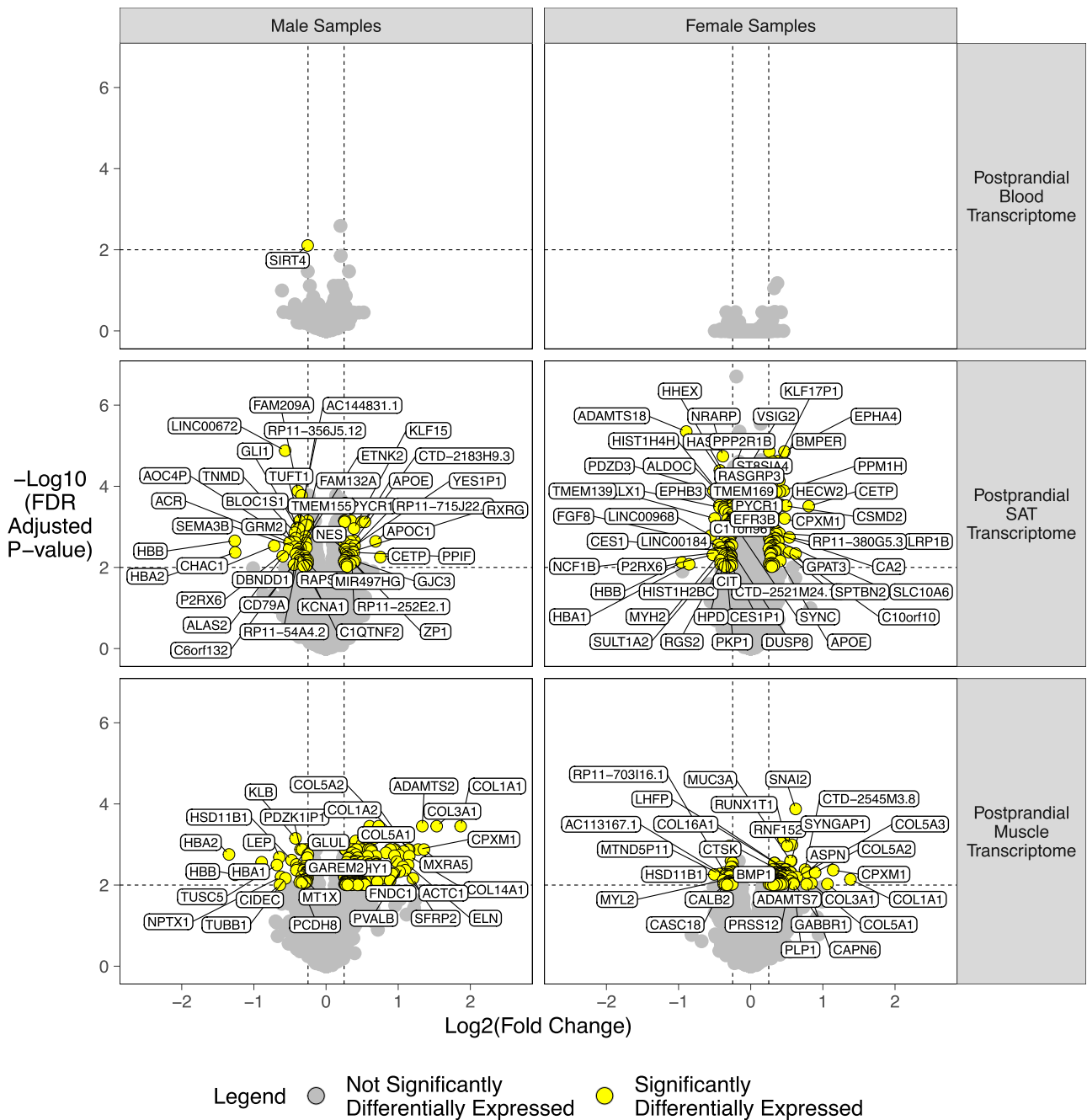
Fig. 2 | Intervention effect on postprandial blood, SAT and muscle gene expression levels in men and women. The effect of the intervention was studied using two-sided linear mixed model analysis. The significance levels were adjusted for multiple testing using the false discovery rate (FDR). The log2 fold change is plotted on the x axis, the -log10 FDR-adjusted *P* value is plotted on the y axis. The dashed horizontal line represents the significance threshold of FDR 0.01, the two vertical lines represent the log2 fold change thresholds of −0.25 and 0.25. Genes with an absolute log2 fold change ≥0.25 and an FDR-adjusted *P* value < 0.01 were considered significantly differentially expressed. Each dot represents a gene. Yellow dots represent the significantly differentially expressed genes (DEGs). Gray dots represent genes with an absolute log2 fold change < 0.25 and/or FDR ≥ 0.01. Significantly differentially expressed genes with the strongest log2 fold changes were labeled with their gene symbol. SAT subcutaneous adipose tissue.

Response of tissue-specific overrepresented pathways reproduced across SAT and muscle, not in blood

Next, we questioned whether there was a transcription response common to the three tissues. Hereto, we examined the response of the mean expression of the DEGs within each overrepresented pathway of SAT and muscle (Fig. 3). For example, the mean expression of the male-upregulated SAT DEGs involved in the HDL remodeling pathway was also significantly positively upregulated in muscle, whereas it was non-significantly upregulated in blood (second row in top panel of Fig. 4).

Similarly, in females, we noted correspondence between SAT and muscle, albeit weaker than in males. The HDL remodeling pathway was weakly upregulated in both SAT and muscle but had no response in blood (second row, third panel from the top, Fig. 4). Since there was only one significant DEG in postprandial blood (Fig. 2 and Table 3), no overrepresented blood down- or upregulated pathways were present.

In general, we noted a similar behavior of the overrepresented postprandial SAT pathways across the three tissues, both in males and females (Fig. 4). In addition, two overrepresented SAT pathways (HDL

Table 3 | The 13-week lifestyle intervention had the strongest effect on postprandial SAT and muscle transcriptomics

Sex	Tissue	State	Direction of effect	Expressed genes	Genes with Log2FC ≥ 0.25	Genes with FDR < 0.01	Genes with Log2FC ≥ 0.25 & FDR < 0.01
Male	Blood	Postprandial	Downregulated	8602	52	1	1
Male	Blood	Postprandial	Upregulated	10,093	45	1	0
Male	SAT	Postprandial	Downregulated	10,099	402	123	48
Male	SAT	Postprandial	Upregulated	9614	173	180	41
Male	Muscle	Postprandial	Downregulated	9413	228	139	46
Male	Muscle	Postprandial	Upregulated	7166	674	239	205
Female	Blood	Postprandial	Downregulated	9619	63	0	0
Female	Blood	Postprandial	Upregulated	9143	32	0	0
Female	SAT	Postprandial	Downregulated	9860	254	331	92
Female	SAT	Postprandial	Upregulated	9599	143	267	76
Female	Muscle	Postprandial	Downregulated	9464	292	127	46
Female	Muscle	Postprandial	Upregulated	7570	827	132	122

SAT subcutaneous adipose tissue.

remodeling, *NR1H3* & *NR1H2* gene regulation), were significantly positively associated with the intervention time point in postprandial muscle expression of males. In contrast to the overrepresented SAT pathways, the intervention had an opposite effect on the overrepresented muscle pathways in the SAT transcriptome, when compared to the muscle expression levels. In addition, the ECM proteoglycans pathway, which was significantly upregulated in muscle, had a significant negative effect in SAT (in males only). The intervention had a weak effect on the remaining overrepresented muscle pathways, in either sex. Lastly, the intervention had hardly an effect on the postprandial blood expression levels of any of the overrepresented pathways, showing that the genes that were differentially expressed in SAT and/or muscle were not changed by the intervention in blood.

In summary, when investigating the intervention effect of the genes strongest affected by the intervention, no overlap of transcriptional response was observed between blood and SAT or muscle. Some relationships did exist within the transcriptional response between SAT and muscle, although not always consistent in the direction of effect.

At baseline, the transcriptomes of postprandial blood, SAT, and muscle are weakly associated with the metabolic health status
To investigate the interplay between the transcriptome and metabolic health at baseline, we calculated the association between the baseline mean expression of the overrepresented pathways to the baseline measurements of ten clinical metabolic health markers: I) two DEXA scan body composition measurements: whole-body fat%, trunk fat%; II) seven traditional metabolic health markers: BMI, Waist Circumference (WC) fasting insulin, fasting HDL cholesterol, fasting HDL cholesterol size, SBP, DBP; and III) the Framingham Risk Score (FRS), which is a cardiovascular risk score (Supplementary Fig. 2).

To account for the differences in the baseline metabolic health marker levels between the different subsets (Table 1), we performed this analysis only on the participants with transcriptome measurements in all three tissues (57; 33 males, 24 females) (Supplementary Fig. 1).

At baseline, none of the associations between the expression levels of the different pathways in the three tissues and health marker levels were significant. However, some trends were present. In males, the SAT transcriptome was strongest associated to the metabolic health marker levels. In SAT, three of the overrepresented pathways (nuclear receptor signaling, HDL remodeling, cholesterol transport) had a negative association with whole-body and trunk fat%, BMI, WC, fasting insulin, the FRS and a positive association with the HDL cholesterol level and size. Interestingly, the pathway scavenger receptor ligand binding and all the pathways overrepresented in muscle,

showed an opposite trend with metabolic health, compared to the three aforementioned SAT pathways. The blood transcriptome was weakly associated to the metabolic health status. However, the association between the blood expression levels and metabolic health, was opposite to the SAT transcriptome associations reflecting that changes in blood cells poorly relate to those in the tissues. Lastly, the expression levels of muscle showed a weak association to the metabolic health levels, except for a positive association between signaling by the nuclear receptor pathway, the HDL measurements and DBP.

In females, the overrepresented SAT pathways were strongest positively associated with metabolic health markers when expressed in postprandial blood. The effect sizes were largest between blood expression of the signaling pathways, O2/CO2 exchange pathways, and whole-body fat%, trunk fat%, DBP and SBP. In postprandial SAT, only the pathway MAPK family signaling cascades showed a positive association with whole-body fat%, trunk fat%, BMI, and WC. In postprandial muscle, the overrepresented muscle pathways showed a weak negative association with the BMI, WC, fasting insulin and the body composition measurements.

Taken together, the baseline expression levels in none of the three tissues were significantly associated to the metabolic health status. However, when comparing the three tissues, the postprandial SAT transcriptome best captured the baseline metabolic health status in males, while in females the postprandial blood transcriptome was strongest associated with the baseline metabolic health status.

Especially SAT- and muscle-specific overrepresented pathways associate with health markers across the intervention
To better understand the differential expressed transcriptome, we investigated the associations between the mean expression of the overrepresented pathways and the metabolic health markers across the two time points of the intervention ("Methods", Fig. 4). Since the intervention effect on some of the health markers differed between the subgroups (Table 2), which can cause differences in effects due to differences in subsets, the relation between the expression data and metabolic health markers was performed on the overlapping blood, SAT and muscle samples (57; 33 males, 24 females) (Supplementary Fig. 1).

In general, the postprandial blood expression levels were weakly associated to the metabolic health markers (Fig. 4). However, in males, the postprandial blood expression levels of two pathways (HDL remodeling, cholesterol transport) were significantly negatively associated to trunk fat%, while two other pathways (ligand binding & collagen fibril crosslinking) were significantly positively associated to trunk fat% and the fasting HDL cholesterol size. In addition, in females,
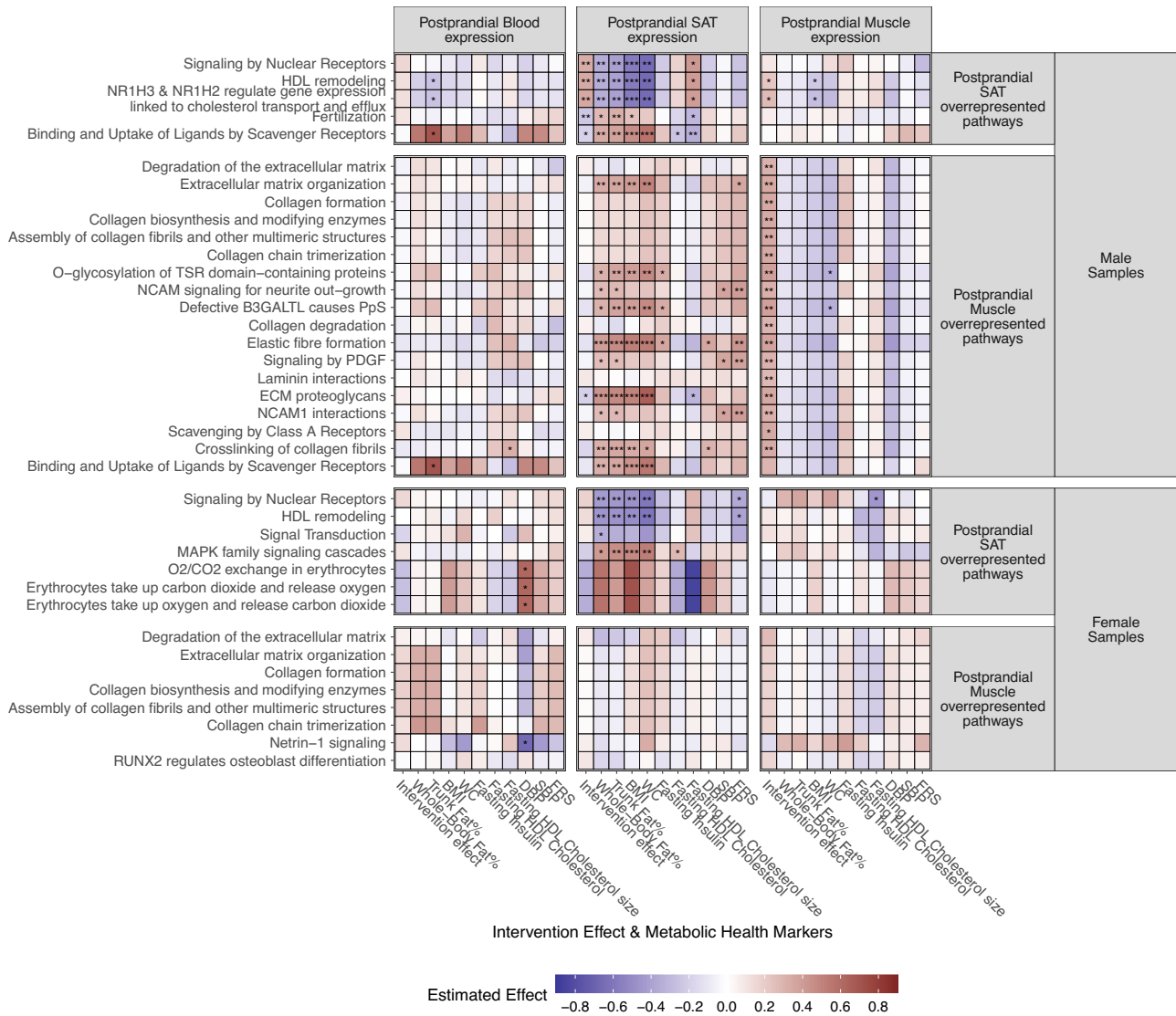
Fig. 3 | Significantly overrepresented pathways in postprandial SAT and muscle transcriptome. A one-sided functional enrichment test was performed to identify the overrepresented pathways. The overrepresented pathways are plotted on the *y* axis. Overrepresented pathways in the male samples are plotted on the left, overrepresented samples in female samples, on the right. The fill color represents the normalized direction of effect: dark blue (−1) all significantly differentially expressed genes in the pathway were underexpressed, dark red (1) all significantly differentially expressed genes in the pathway were overexpressed. *P* values were adjusted for multiple testing using the Bonferroni correction method. Significance is indicated by the asterisks (*P adjust <0.05, **P adjust <0.01, ***P adjust <0.001). SAT subcutaneous adipose tissue.

the pathways involved in O_2/CO_2 exchange were significantly positively associated to the changes in DBP, while DBP and netrin-1 signaling were significantly negatively associated.

The postprandial SAT expression levels of the majority of both SAT and muscle-overrepresented pathways were significantly associated to the change in DEXA body composition parameters (whole-body fat%, trunk fat%), three of the seven traditional metabolic health markers (BMI, WC and fasting HDL cholesterol level) and the FRS, in both sexes, and with the fasting HDL cholesterol size, DBP and SBP only in men. The SAT pathways involved in nuclear receptor signaling, HDL remodeling

Fig. 4 | Significantly overrepresented pathway expression levels in SAT and muscle are significantly associated with the intervention time point and metabolic health markers. Associations between the overrepresented pathways and metabolic health markers were calculated using a two-sided linear mixed model. Overrepresented pathways are plotted on the *y* axis. Intervention time point and metabolic health markers are plotted on the *x* axis. Colors indicate the estimated effect between the mean expression in the pathways and the intervention effect/metabolic health markers: blue, a negative effect, white, no effect, red, a positive effect. *P* values were corrected for multiple testing using the false discovery rate. The significance level is indicated by the asterisks: *FDR <0.05, **FDR <0.01, ***<0.001.

cholesterol transport and signaling transduction were significantly negatively associated to whole-body fat%, trunk fat, BMI and WC, and positively associated to the HDL cholesterol measurements. Conversely, the remaining SAT pathways (fertilization, ligand binding and uptake, MAPK cascades) showed an opposite trend with similar strength of effect. The expression levels of the muscle-overrepresented pathways were positively associated to all metabolic health markers, excepting the fasting HDL level and size. Interestingly, only the overrepresented pathways of the muscle tissue were significantly positively associated to the two blood pressure measurements.

The transcriptome of postprandial muscle was weakly associated to the changes in the metabolic health markers. In males, pathways involved in HDL remodeling, *NR1H3 & NR1H2* gene regulation, and TSR O-glycosylation and PpS, were significantly negatively associated to BMI and WC, respectively. In females, there was one significant negative association between the pathway nuclear receptor signaling and fasting HDL cholesterol size.

Interestingly, in males, the expression levels of HDL remodeling and *NR1H3/NR1H2* gene regulation were significantly associated to weight-related metabolic health markers in all three tissues. Moreover, the direction of effect between the expression of these pathways and trunk fat%, whole-body fat%, WC, and BMI was negative in all three tissues.

Compared to the baseline associations between the pathway expression and metabolic health marker levels (Supplementary Fig. 2), the effects were stronger across the intervention for both the SAT and muscle transcriptomes. Contrarily, in postprandial blood, the expression of the overrepresented muscle pathways was strongly associated at baseline than across the intervention. The direction of effect remained the same for the overrepresented SAT pathways between the two analyses, while the associations between the muscle-overrepresented pathways and part of the health markers (whole-body fat%, trunk fat%, BMI, WC) were opposite at baseline and across the intervention, when expressed in blood and muscle.
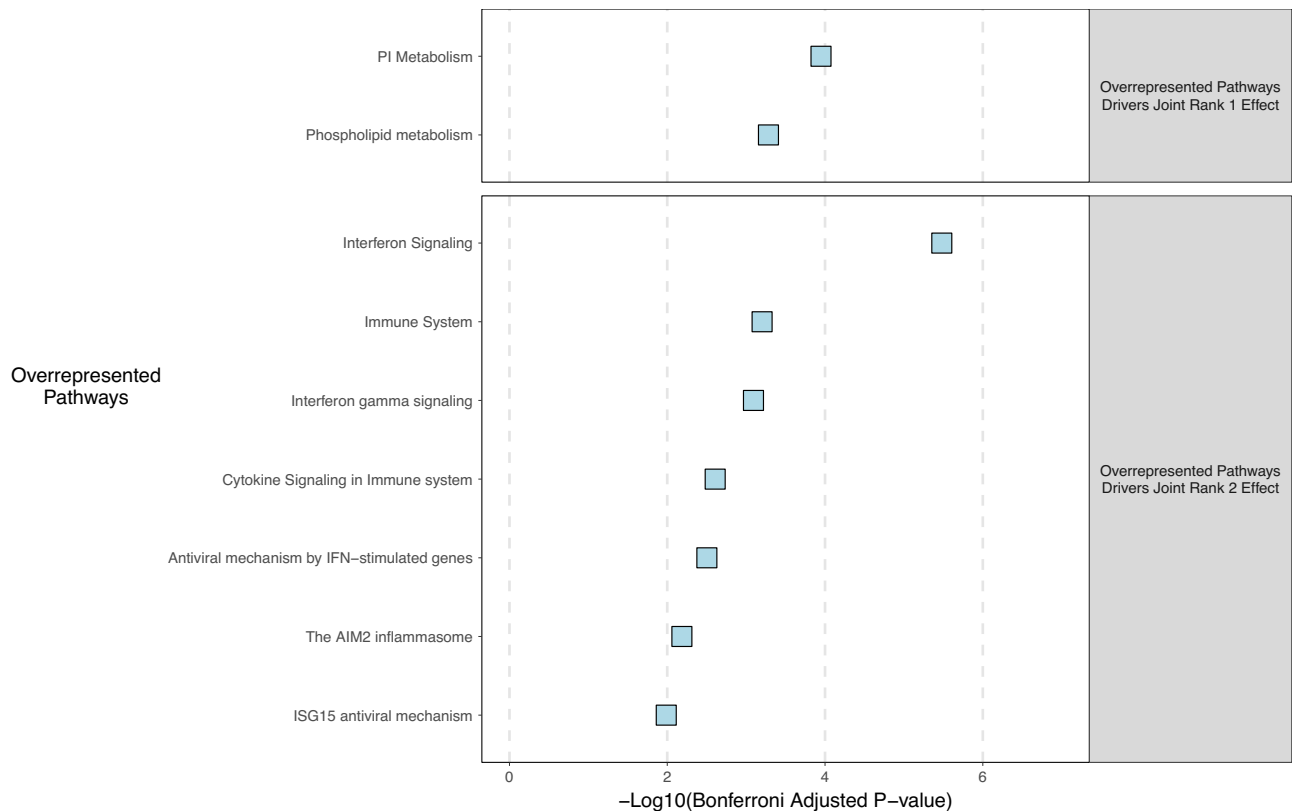
Fig. 5 | Significantly overrepresented pathways in the top 250 drivers of the postprandial blood joint effect. A one-sided statistical test was performed to identify the overrepresented pathways. *P* values were adjusted for multiple testing using the Bonferroni correction method. The overrepresented pathways are plotted on the *y* axis. *Y* axis is ordered by significance, the strongest significantly overrepresenting pathways first. The *x* axis represents -log10 of the Bonferroni adjusted *P* value.

Taken together, especially in the SAT transcriptome, the SAT and muscle-overrepresented pathways reflected changes in DEXA body composition markers (whole-body and trunk fat%), classical metabolic health markers (BMI, WC, fasting HDL cholesterol level and size, DBP, SBP) and the FRS. Many effects were similar in males and females, although more pronounced in males.

Integrated analysis reveals male postprandial blood, SAT, and muscle transcriptome share a joint variation
To explore the tissue dependency in a more intricate way, we captured the joint transcriptional reaction to the intervention across the three different tissues using Joint and Individual Variation Explained (JIVE) analysis ("Methods", Supplementary Fig. 3). The joint representation is a linear subspace spanned by the genes, analogous to a principal component analysis (PCA), but that now also explains the joint variation in expression in all three tissues across the intervention ("Methods"). Hence, although informed by all three tissues, the blood expression of a participant (before and after the intervention) can be mapped to this joint subspace without needing information about the muscle or SAT transcriptome. In other words, the blood transcriptome of a participant is decomposed in three (independent) transcriptome terms: (1) a term that reflects the joint transcriptomic variation in blood, SAT, and muscle, (2) a term reflecting the remaining structured variation in blood only (also referred to as the individual variation), and (3) a term that represents the residual noise in the blood transcriptome, i.e., the signal that is not explained by the joint and individual terms. Subsequently, we can measure the differential expression across the intervention in either the joint subspace (of blood, SAT, and muscle) or the individual subspaces (for each tissue

type). The number of joint and individual components are determined through permutation testing ("Methods").

In the male samples, JIVE identified 2 joint components for the shared transcriptomic variation in blood, SAT and muscle, and 11, 15, and 15 individual components for the blood, SAT, and muscle, respectively (Supplementary Dataset 7). In postprandial blood and SAT, the joint components together explained 8% and 7.2% of the total variation in those tissues, respectively. In the postprandial muscle transcriptome, both joint components explained 31.3% of the variation. In female samples, JIVE did not identify a joint term that was independent of the individual terms (Supplementary Fig. 4).

In male samples, the drivers of the joint components were involved in phospholipid metabolism and the immune system
To investigate what genes expressed in postprandial blood were the drivers of the joint effect, the 250 genes that had the largest loading factors for each of the two joint components were selected, respectively ("Methods"). Both sets of genes were subsequently used in a pathway enrichment analysis. In postprandial blood, the genes driving the first joint component (JC1) that explains most of the joint variance across the tissues were involved in phospholipid metabolism, while the genes driving the second joint component (JC2) were involved in interferon signaling, inflammation, and other aspects of the immune system (Fig. 5).

Phospholipid metabolism genes that drive the joint effect also capture the intervention effect individually
Next, we wondered whether the genes driving the significant effects of JC1 and JC2 also showed an intervention effect by themselves. Within both sets of 250 genes that had the highest loading factors for each of

the joint components, we selected those genes that overlapped with the found enriched pathways. Interestingly, we found a remarkable difference between the intervention effect of the genes involved in phospholipid metabolism and those involved in immunity (Fig. 6): the joint effect of the genes involved in phospholipid metabolism was more strongly influenced by the intervention than the individual effect, however, neither effect crossed the significant threshold after adjustment for multiple testing (Fig. 6). Compared to the phospholipid metabolism genes, the effect sizes in both the joint nor individual effect of genes involved in the immune system were considerably weaker. These effects were also non-significantly associated with the intervention effect.

Immune genes that relate to the joint effect capture metabolic health effects of the intervention

Next, we investigated whether changes in the genes involved in phospholipid metabolism and immunity associated with changes in the metabolic health markers. Here we found that neither the joint nor individual effect of the phospholipid genes associated to these health marker effects (Fig. 6). In contrast, the joint effect of the genes involved in the immune system did strongly associate with markers of body composition (whole-body and trunk fat%) while, except for *RAB44* and *RAPGEF3* (which was associated to the fasting HDL cholesterol level), the individual effect did not. Hence, it is possible to detect the effects of the intervention in the postprandial blood transcriptome, but only when the individual blood effect has been removed.

Variance in the muscle and SAT transcriptome explain the selection of contributing genes to the joint components of blood differently

To investigate which of the two tissues, SAT or muscle, correlated most with the joint components in blood, we calculated the correlations of the top 250 genes with the highest loading factors for each of the joint components (JC1 and JC2) in blood with those in muscle and SAT (Supplementary Fig. 5). The JC1 blood genes showed increasingly higher absolute correlations with the JC1 and JC2 muscle genes, whereas the JC2 blood genes showed an opposite behavior and were merely not correlated with the JC1 or JC2 muscle genes. On the other hand, the JC1 and JC2 SAT genes showed a stronger absolute correlation with the JC2 blood genes as opposed to the JC1 blood genes. These results indicate that the JC1 blood genes (dominated by genes involved in phospholipid metabolism) are largely correlated with the variance in the muscle transcriptome, the JC2 blood genes (dominated by genes involved in the immune system) are more correlated with the variances in the SAT transcriptome.

Discussion

We investigated the effects in men and women of a mild combined lifestyle intervention (Growing Old TOgether trial, trial registration number GOT NL3301 (https://onderzoekmetmensen.nl/nl/trial/27183), NL-OMON27183 in older adults on the postprandial transcriptome of blood, subcutaneous adipose tissue (SAT) and muscle tissue, and related the response to changes in parameters of metabolic health. Furthermore, using JIVE, we explored the shared variation between the three transcriptomes. In both sexes, the 13-week combined lifestyle intervention had virtually no effect on the postprandial blood transcriptome, while the postprandial SAT and muscle transcriptomes responded significantly. In postprandial SAT, pathways involved in HDL remodeling, cell signaling, and CO_2/O_2 exchange (both sexes), ligand binding by scavenger receptors, NR1H3/NR1H2 cholesterol-related gene expression and fertilization (male only), and signal transduction and MAPK signaling cascades (female only) were overrepresented, while in the postprandial muscle, pathways involved in collagen, extracellular matrix remodeling, protein O-glycolysation

(male only) and osteoblast differentiation (female only) were significantly overexpressed.

The response of the SAT transcriptome in both sexes associated most strongly with changes in the Framing Risk Score (FRS), and metabolic health, including HDL cholesterol level and size, fasting insulin, and specifically in body composition markers (whole-body and trunk fat%, BMI and Waist Circumference (WC)), and so did a part of the changes in the muscle transcriptome (whole-body and trunk fat%, BMI, fasting HDL cholesterol, WC and the FRS). In postprandial blood, the expression of a few pathways was associated with trunk fat%, fasting HDL cholesterol size, and diastolic blood pressure (DBP). Interestingly, in males, the expression levels in all three tissues of pathways involved in HDL remodeling and *NR1H3* & *NR1H2* regulated gene expression were significantly negatively associated to one or more body composition makers, which might indicate a shared biological process between the three tissues. We did observe sex differences in the association effect sizes. Lastly, we identified shared variation between the three transcriptomes, but only in the tissues of men. We identified in the blood transcriptome a joint intervention effect dominated by genes involved in phospholipid metabolism, largely influenced by genes overlapping with changes in the muscle transcriptome, and a second joint intervention effect dominated by genes involved in the immune system, overlapping with changes in the SAT transcriptome. The latter associated with changes in body composition (whole-body and trunk fat%) with larger effect sizes and higher significance levels than in the original transcriptome of postprandial blood.

The effect of lifestyle interventions on the human transcriptome has mainly been reported for dietary and physical activity regimes separately, typically in metabolically unhealthy or frail participants[21,23–25,30]. This makes a direct comparison with the response of the healthy, non-diabetic, older adults[1] to the GOTO combined lifestyle intervention difficult. Nevertheless, we do find similarities to dietary interventions. Like in GOTO, nutritional interventions with a mild energy restriction (20-25%, for 4–12 weeks) hardly affected the blood transcriptome[23]. In adipose tissue, caloric restriction interventions changed the expression of genes involved in glucose and lipid metabolism, signal transduction, stem cell maintenance and vascularization[18,20,24,25,30], the former three of which were also overrepresented in the SAT transcriptome of the GOTO trial (Fig. 3). Lastly, in overweight participants or participants with obesity of a weight-loss intervention study (6 months, diet, physical activity separate and combined), genes involved in signaling pathways, translation, cell life span and ATP binding were significantly overrepresented[31], part of which we also identified in GOTO.

The effects of the muscle transcriptome to physical activity changes were reported in both anabolic and catabolic interventions, the latter being more similar to the regimen in GOTO. In line with the results found in GOTO, a systematic review investigating the effects of 21 activity interventions on the ECM in muscle, found that both types of activity interventions had a positive effect on collagen level and identified ECM adaptations related to muscle remodeling in both young and older participants[26]. Not all gene expression effects are common to all interventions. A 6-months aerobic, resistance, or aerobic + resistance training regimen in older adults with obesity changed the expression of genes involved in myogenesis, autophagy, mitochondrial biogenesis and inflammation, in all three training regimens[22], which was not observed for the muscle transcriptome in the GOTO trial. This difference may be ascribed to the better health at baseline of GOTO individuals and/or the duration and intensity of this in essence anabolic intervention. Another anabolic 6-month resistance training intervention in frail and healthy older adults, showed effects similar to GOTO; an increase in expression levels of collagen and extracellular matrix-related genes[21]. Finally, in mice a combined caloric restriction and physical activity intervention reported DEGs involved
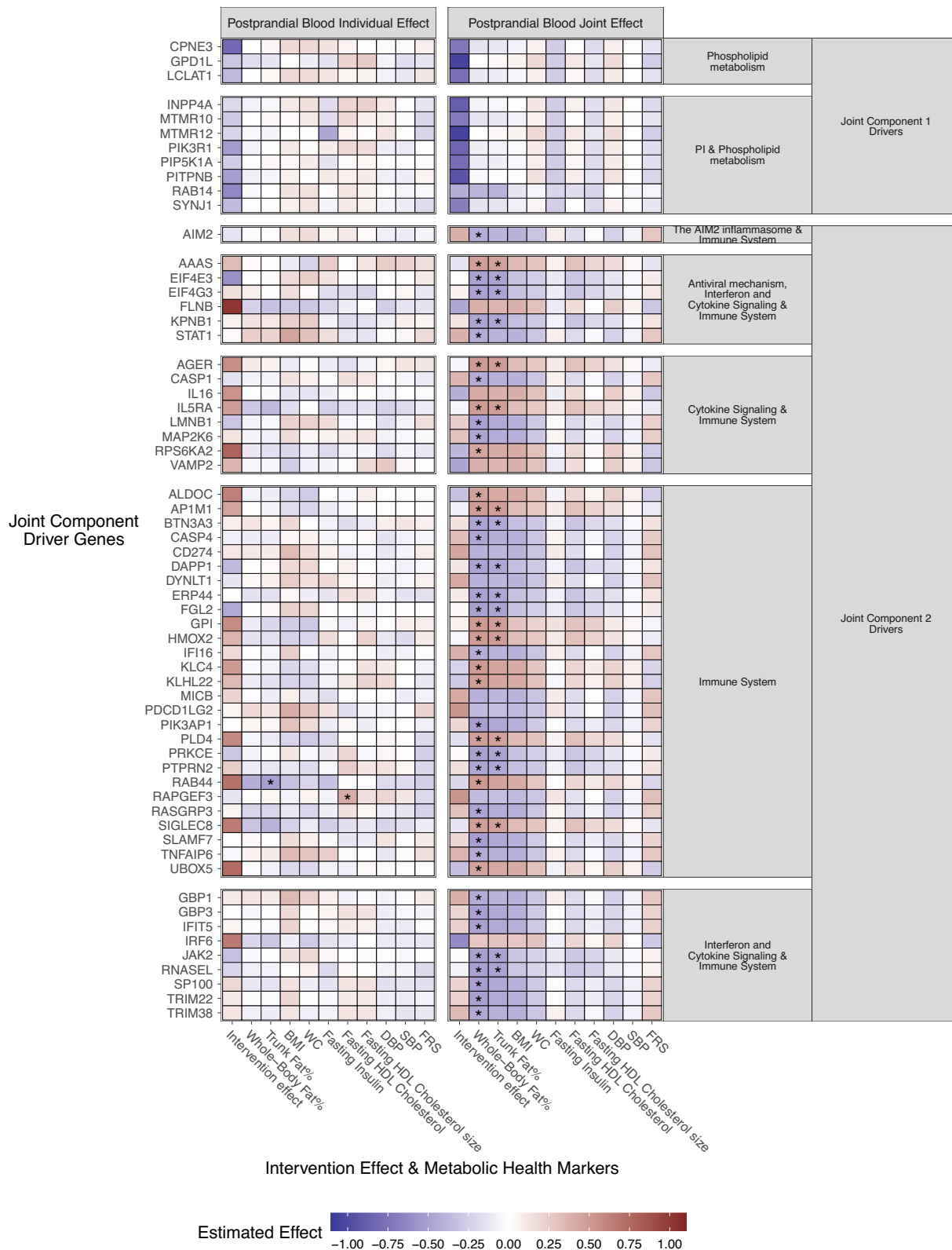
Fig. 6 | In postprandial blood, the joint component 1 drivers strongly captured the intervention effect while joint component 2 drivers were significantly associated to whole-body fat% and trunk fat% levels. Associations between the joint component drivers and the intervention effect & metabolic health markers were calculated using a two-sided linear mixed model. The genes were plotted on the y axis, the intervention effect and metabolic health markers were plotted on the x axis. Colors indicate the direction of the effect: blue, a negative effect, white; no effect, red; a positive effect, the darker the color, the stronger the effect. The P values were adjusted using the false discovery rate. Significance is indicated by the asterisks (*FDR <0.05).

in MAPK signaling, metabolic processes, cholesterol metabolism, extracellular matrix organization[36], which we also found in the GOTO trial (Fig. 3).

There is a number of other differences in study designs as compared to reported interventions. GOTO was a personalized intervention, to mimic a realistic situation when people change their lifestyle. Consequently, there was a considerable variation in the instructed lifestyle change between the participants; some changed their diet more, while other focused more in a change in physical activity. Furthermore, the transcriptome in GOTO was measured in tissues taken after a standardized meal[17], which is usually not done.

A direct association between the expression levels in adipose tissue and muscle, and classical metabolic health markers is generally not looked into, making it difficult to relate the effects identified in GOTO (Fig. 4). However, one cohort study of BMI discordant monozygotic twins (ΔBMI ≥ 2.5 kg/m^2) reported on adipose tissue and skeletal muscle transcriptome differences[42]. This study identified DEGs involved in ECM remodeling, DNA/RNA metabolism, mitochondrial metabolism (both adipose tissue and skeletal muscle), fatty acid metabolism, MAPK and AMPK signaling (adipose tissue only), and amino acid metabolism (skeletal muscle only)[42], which largely overlap with the overrepresented pathways in GOTO (Fig. 3). In addition, it was emphasized that combined differential gene expression scores of the adipose tissue transcriptome were stronger associated with classical metabolic health markers (including BMI, whole-body fat%, HDL cholesterol) than those of muscle transcriptome[42], which we also observed in GOTO (Fig. 4).

When investigating the intervention effect of the overrepresented pathways of one tissue in the other two tissues, we identified that the intervention had an effect on 2 out of 5 SAT overrepresented pathways in the muscle transcriptome (Fig. 4). However, the other way around, when looking into overrepresented muscle pathways expressed in SAT, the intervention only had a significant effect on only 1 out 18 pathways. This indicated that the muscle transcriptome captured some of the effects on the SAT transcriptome, possibly by a SAT component in the muscle tissue. In addition, in males, the expression of two pathways (HDL cholesterol, *NR1H3* & *NR1H2* regulate gene expression linked to cholesterol transport and efflux) and one or more of the body composition measurements (whole-body fat%, trunk fat%, WC, BMI) was significantly negatively associated in all three tissues, which could indicate a shared component between the three tissues as well (Fig. 4).

Using the JIVE data integration approach[40], we have identified a shared variation between the three tissues in the male samples represented by two joint components (Supplementary Figs. 3 and 4 and Supplementary Dataset 7). The genes that drove these two joint components were involved in phospholipid metabolism and the immune system, respectively (Fig. 5). Like the effects in the postprandial muscle transcriptome on the pathway expression levels (Fig. 4), the genes driving the first joint component (JC1) in blood showed a strong intervention effect but did not capture metabolic health effects (Fig. 6). Moreover, these genes were also more correlated to variances in the muscle transcriptome than the SAT transcriptome (Supplementary Fig. 5). Conversely, similarly to the effects in postprandial SAT, the genes driving the second joint component (JC2) in blood strongly captured the metabolic health effects of the intervention but had a weaker association to the intervention itself. This was also confirmed by a higher correlation with variances in the SAT transcriptome than the muscle transcriptome of the genes belonging to this second joint blood component (Supplementary Fig. 5). From these results, we postulated that JC1 in blood was largely driven by the muscle transcriptome, while JC2 in blood was primarily driven by the SAT transcriptome.

This trial faced several limitations, which need to be considered when interpreting the results. First, the number of participants with transcriptomics measurements of postprandial blood (88; 45 males, 24 females), postprandial SAT (78; 38 males, 40 females), and postprandial muscle (82; 48 males, 34 females), was relatively low, compared to the complete number of participants compliant to the GOTO trial (153; 75 males, 78 females). The variation in the instructed lifestyle changes in combination with the low number of samples and slight differences at baseline (Table 1), could also have led to a large variation in outcomes when comparing participants with postprandial blood, SAT, and muscle transcriptome measurements (Table 2). To circumvent part of this variation, the effects between the transcriptome and metabolic health markers were only studied within the participants with transcriptomic measurements in postprandial blood, SAT, and muscle (57; 33 males, 24 females) (Supplementary Fig. 1). Second, the group of participants with transcriptomic measurements of all three tissues was also used for the JIVE analysis. In females, the JIVE model did not identify a shared variation, this could be because of the shared effects between the tissues were too weak, which was likely attenuated by the low number of participants (24). In males, the JIVE analysis did identify two joint components between the transcriptomes of the three tissues. Lastly, after the intervention period, no further follow-up was performed on the participants of the GOTO trial, thus we cannot say whether the transcriptomic changes as result of the intervention have any long-term effects.

Overall, we were able to identify considerable effects of a 13-week moderate nutritional and physical activity intervention on the transcriptome of SAT and muscle of healthy older adults. We demonstrated that the changes in the expression of genes in metabolism pathways in SAT, and collagen and muscle remodeling pathways in muscle associated with beneficial features of overall metabolic health. These health effects were best captured by the postprandial SAT transcriptome. We identified a joint space of three tissues in postprandial blood, which was able to capture some of the effects in the other two tissues, even though postprandial blood alone did not capture this. We propose that this method could be used to estimate part of the effect of an intervention on SAT and muscle transcriptomes, when only blood expression data is available.

Methods

Trial design

The Growing Old TOgether (GOTO) trial protocol was approved by the Medical Ethical Committee of the Leiden University Medical Center before the start of the trial (P11.187). In accordance with the Declaration of Helsinki, the GOTO trial obtained informed consent from all participants prior to their entering the trial. This trial was registered at the Dutch Trial Register (https://onderzoekmetmensen.nl/en/trial/27183) as GOT NL3301 and can also be found at the international clinical trials registry platform as NL-OMON27183. The first participant was enrolled on the 11th of June 2012, while the last participant was enrolled on the 17th of January 2013.

In the Leiden Longevity study, from which the GOTO participants were recruited, sex was first self-reported and subsequently verified with birth certificates and genotype data. Inclusion criteria were as follows: middle-aged (≤ 75 years) couples consisting of offspring from long-lived siblings and his/her current partner or in incidental case as single participant, BMI levels ≥ 23 and ≤ 35 kg/m^2. Exclusion criteria: type I or type II diabetes (on diabetic medication), individuals who have lost or gained ≥ 3 kg in the 6 months prior to screening, individuals engaged in heavy/intensive physical activity (top sport or physically heavy work), any disease or condition that seriously affects body weight and/or body composition including active types of cancer, heart failure (NYHA III/VI), COPD (GOLD III/VI), recent (3 months prior to intervention) immobilization for longer than 1 week, psychiatric or behavioral problems (eg, history or clinical manifestation of any eating disorders, vegan dietary lifestyle, major depression), using one or more of the following medication: thyroid medication,

immunosuppressive drugs (e.g., prednisone, methotrexat, biologicals (TNF-alpha antagonists etc), current participation in any other intervention study or weight management program, exclusion for biopsy: use of anticoagulantia (e.g., coumarines, carbaspirin calcium), presence of exclusion criteria for MR imaging (3 tesla and 7 tesla): claustrophobia, pacemakers and defibrillators, nerve stimulators, intracranial clips, intraorbital or intraocular metallic fragments, cochlear implants, ferromagnetic implants (e.g., thoracic implant for scoliosis), inability to lie supine during for 45 min, not having a general practitioner.

Sex swaps were verified and corrected when necessary. Sex was considered in the trial design. Previous research has shown that the GOTO intervention had different effects on male and female participants[1]. Part of this was due to differences in their daily activity patterns[41]. Since the earlier results highlighted the sex-specific effects of the GOTO trial, subsequent analyses were performed in a sex-stratified manner.

The GOTO trial was a 13-week nonrandomized, noncontrolled, 1 arm open masking design lifestyle intervention trial with 164 healthy older adult participants (83 males, 81 females, mean age 62.9 + − 5.7, mean BMI 26.9 + − 2.5). The participants underwent a 25% reduction in energy expenditure, divided over 12.5% decreased caloric intake and 12.5% increased physical activity, as described in ref. 1. Before the intervention, baseline energy intake and expenditure were assessed through a 150-item food frequency questionnaire[43] and the International Physical Activity Questionnaire-Short Form (IPAQ-SF)[44]. Personalized intervention guidelines were prescribed by a dietician and a physiotherapist, in the consultation with the participant's preferences and physical abilities. The dietary guidelines were composed to be as much as possible according to the "Dutch Guidelines for a healthy diet"[1]. To stimulate better adherence to the intervention, participants were advised to increase the amount of physical activity in a way that would match their daily life pattern, with a secondary aim to become fully integrated in the participant's regular daily routine at the end as well as after the intervention. These activities generally included walking, cycling, activities in and around the house and participation in local sport activities, either alone or as a couple. The primary outcome of the GOTO intervention was a significant reduction of fasting insulin, which has been reported previously[1]. Sample size calculation has been reported in ref. 1. In short, sample size calculation was based on fasting insulin, whereby a decrease of 21% (9.75–7.69 µIU/mL) was assumed since this was observed after 3 months in the calorie restriction with exercise group of the CALERIE study[45], which was comparable to the GOTO intervention trial. As the mean fasting insulin levels (µIU/mL) in longevity family members and controls in the complete LLS cohort were 6.93 (SD = 4.3) and 8.70 (SD = 6.6), respectively, and a correlation of 0.6 between repeated insulin measurements was assumed[46], power calculation was based on an expected mean decrease of 1.65 µIU/mL, SD 4.9. With a power of 80% and an α of 5% this translates in a required sample size of 72 individuals. To account for potential differences between longevity family members and control in response to the intervention and taking into account a dropout rate of 10%, there was an aim to include 80 couples in the intervention.

Secondary outcome measures are blood parameters (metabolic/metabolomics), parameters measured in biopsies of muscle and fat (transcriptomics/proteomics/epigenetics), anthropometrics, body composition, blood pressure, 24-h glucose monitoring, energy metabolism (resting metabolic rate), magnetic resonance imaging (leg, brain, cartilage of the knee) and psychological factors (cognitive performance, mood, quality of life, sleep, hunger). The GOTO intervention trial significantly improved blood parameters, changed anthropometrics (notably weight and waist circumference), significantly reduced blood pressure, significantly reduced resting energy expenditure, significantly improved quality of life in the female participants[1], significantly reduced total body and trunk fat%[14]. The effects of the

GOTO intervention trial on the fasting blood proteome, epigenetic measurements in blood, subcutaneous adipose tissue and muscle tissue are currently being prepared for publication. The 24-h glucose monitoring have been analyzed previously, but are currently not published, since the effects of the intervention on non-continuous glucose measurements have been reported in ref. 1. Currently, the magnetic resonance imaging results are not analyzed.

To stimulate adherence and proper following of the intervention, the participants had weekly contact with the dietician and physiotherapist, either through telephone, email or at the participant's residence. For each day of the intervention, a participant's dietary and activity compliance to the trial program was self-reported in an intervention diary (Supplementary Dataset 8). The compliance was subsequently summed for each week (ranging from 0 to 7, according to the amount of days compliant to the intervention regimen during a given week). Lastly, the mean activity and dietary compliance over the 13 weeks was calculated. Participants with a mean physical activity or dietary compliance equal to or above 3.5, were considered as compliant to the intervention guidelines. Participants with an average self-reported compliance below 3.5 for both physical activity and diet, were considered as non-compliant, and subsequently excluded from the data analysis. Using these thresholds, 153 out of 164 participants, were compliant to the intervention. Analysis investigating the effect of the intervention on metabolic health markers of compliant and non-compliant participants showed that the compliant participants responded stronger to the intervention than the non-compliant participants (Supplementary Fig. 6)[12].

Both before and after the intervention, the participants were subjected to a nutrient challenge, consisting of an overnight fast and an intake of standardized nutrient shake, as described in ref. 17. Thirty minutes after the nutrient challenge, samples of postprandial whole blood, postprandial subcutaneous adipose tissue (SAT) (*subcutaneous abdominal fat pad*) and postprandial muscle (*vastus vascularis*) were taken. Out of 153 compliant participants, postprandial transcriptomics were measured in three tissues: 88 (45 males, 43 females) in blood, 78 (38 males, 40 females) in SAT and 82 (48 males, 34 females) in muscle. Fifty-seven participants (33 males, 24 females) had postprandial transcriptomic measurements in all three tissues (Supplementary Fig. 1).

All statistical analyses were performed in R (version 4.2.2). When appropriate, correction for multiple tests were adjusted using the false discovery rate (FDR). In the differential gene expression analysis, an FDR-adjusted P value below 0.01 was considered significant. In all other analyses, an FDR-adjusted P value below 0.05 was considered significant.

Diagnostic measurements
All measurements were performed in fasting serum collected through venipuncture. Insulin was measured using a Immulite 2000 xPi (Siemens, Eschborn, Germany). HOMA2-IR was calculated using the publicly available HOMA calculator (https://www.dtu.ox.ac.uk/homacalculator/)[47]. Fasting HDL cholesterol and HDL cholesterol size were measured using the previously described Nightingale platform[48]. Complete methods of diagnostic measurements are described in ref. 1.

DEXA measurements
Method for the DEXA measurements is described in ref. 14. In short: whole-body DEXA (Discovery A, Hologic Inc., Bedford, MA, USA) was used to measure eleven body composition components, including whole-body fat mass and trunk fat mass. Whole-body fat% was calculated by dividing whole-body fat mass by whole-body mass. Trunk fat% was calculated by dividing the trunk fat mass by the total trunk mass.

Investigation of health marker effects
The intervention effect of each health marker was calculated using a linear mixed model, adjusted for age (fixed effects) and individual

(random effects). The effect for males and females were calculated separately. The significance was adjusted for multiple testing using the Bonferroni correction method.

RNA isolation and sequencing

The method for RNA isolation and sequencing is described in ref. 17. In short: libraries were prepared using Illumina TruSeq version 2 library preparation kits. Data processing was performed the in-house BIOPET Gentrap pipeline, described in ref. 49. The following steps were part of the data processing: low-quality trimming using sickle version 12.00. Cutadapt version 1.1 was used to perform the adapter clipping. The reads were aligned to GRCh37, while masking for SNPs common in the Dutch population (GoNL[50] MAF > 0.01), using STAR version 2.3.0e. Picard version 2.4.1. was used to perform sam-to-bam conversion and sorting. Read quantification was performed using htseq-count version 0.6.1.p1 using Ensemble gene annotations version 86 for gene definitions. In postprandial blood, the sequencing resulted in an average of 37.2 million reads per sample, 97% (+ − 0.4%) of which were mapped. In postprandial SAT, samples had an average of 11.4 million sequenced reads, 95% (+ − 1.6%) of which were mapped. In postprandial muscle, an average of 36.9 million sequence reads per sample, 98% (+ − 0.4%) of which were mapped.

Differential gene expression

In all tissues, the normalizations and differential gene expression analysis were performed in a sex-stratified manner. Genes that had less than 2 counts on average, were removed from the dataset. Tissue-specific confounders were identified by associating them with the principal components that represent 90% of the variation of TMM-CPM normalized gene expression.

Postprandial blood. There were 88 participants (45 male, 43 females) with RNA-seq measurements pre- and post intervention. We adjusted for the following confounders: (1) technical effects: median 5' bias, median 3' bias, and flow cell, mean insert size in female samples only, (2) cell type percentages: eosinophiles, monocytes, lymphocytes, basophiles), and (3) personal details (age at baseline, and person ID as a random effect).

Postprandial SAT. 78 participants (38 male, 40 female) had RNA-seq measurements pre-and post intervention. We adjusted for the following confounders: (1) technical effects: molarity of sample, isolation date, and plate number in female samples only; and (2) personal measures: age at baseline, and person ID as a random effect.

Postprandial muscle. There were 82 participants (48 male, 34 female) with RNA-seq measurements pre- and post intervention. In male samples we adjusted for the following confounders: (1) technical effects: total yield, flow cell, isolations series, 28S/18S ratio, plate number, mean insert size, total number of aligned bases, RNA integrity number, median 5' bias, median 3' bias; and (2) personal details: age at baseline, and person ID as a random effect. In female samples, we adjusted for the following confounders: (1) technical effects: RNA 260/280 ratio, 28S/18S ratio, biopsy number, hand grip strength, RNA integrity number, mean insert size, isolation series, sequence % that passed quality control, total yield, total number of aligned bases, median 3' bias; and (2) personal details: age at baseline, and person ID as a random effect.

The differential gene expression analysis was performed using linear mixed model analysis (limma version 3.52.2) in combination with VOOM normalization[51]. We adjusted for multiple testing using the false discovery rate. An FDR-adjusted *P* value <0.01 was considered significant. Genes with an absolute log2 fold change ≥0.25 were considered differentially expressed.

Functional enrichment

The pathway overrepresentation analysis was performed using the enricher function of clusterProfiler (version 4.4.4)[52]. Up- and down-regulated significant DEGs were used as an input, all expressed genes that passed the quality control were used as a background. The Reactome database (version 81)[53] was used for the functional enrichment. The tests were adjusted for multiple testing using the Bonferroni correction method. An adjusted *P* value below 0.05 was considered significant.

Pathway expression-level analysis

To investigate the effect of the intervention on the pathway expression levels in the different tissues, the mean expression levels of the differentially expressed genes in the pathways were calculated and subsequently scaled to mean 0 and standard deviation (sd) 1. The associations between the intervention (0 for baseline, 1 for post intervention) and the pathway expression was calculated using the function *lmer* of the package *lmerTest* (version 3.1-3)[54], adjusting for the same covariates as in the differential gene expression analysis. The significance level of the association was adjusted for multiple testing, using the false discovery rate. Similarly, the association between the pathway expression levels expression levels and metabolic health markers were calculated. Metabolic health marker measurements were scaled to mean 0 and SD 1. Outliers with more than 3 SD values from the mean were removed. Only participants with transcriptome measurements of all three tissues were used in the health marker association analysis (57, 33 male, 24 female).

Joint and individual variation explained (JIVE) analysis

The Joint and Individual Variation Explained (JIVE) was calculated using the R package *r.jive* (version 2.4)[40,55]. Analysis was performed on all genes that passed the quality control across 57 participants (33 male, 24 female). Prior to JIVE, the gene expression levels were Frobenius normalized and subsequently scaled to a mean of 0 and a SD of 1. For each tissue matrix $X^t \in \mathbb{R}^{p_t \times n}$ (where t represents the tissue, p_t represents the number of genes measured for tissue t, and n represents the number of samples), the JIVE analysis linearly decomposes the input matrix (X^t) into three matrices representing: (1) the shared data across the tissue: $J^t \in \mathbb{R}^{p_t \times n}$ explaining the joint variation across the three tissue types, (2) a data matrix representing the variation unique to a tissue: $A^t \in \mathbb{R}^{p_t \times n}$, and (3) a data matrix representing the residual noise, i.e the remaining unexplained variation in a tissue: $R^t \in \mathbb{R}^{p_t \times n}$, with: $X^t = J^t + A^t + R^t$ (Supplementary Fig. 3). J^t is further decomposed into sub-matrices $J^t = U^t S$, where $U^t \in \mathbb{R}^{p_t \times r}$ represents a matrix containing the p_t gene loading vectors for the r joint components, and S is a sample scoring matrix of joint effects across all tissues (S is the same for all tissues). The number of joint components, r, is selected using permutation testing as described in ref. 40. Likewise, A^t is decomposed, and for each tissue, $A^t = W^t S^t$, where $W^t \in \mathbb{R}^{p_t \times r_t}$ represents a matrix containing the p_t gene loading vectors for the r_t individual tissue components (selected by permutation testing), and $S^t \in \mathbb{R}^{r_t \times n}$ is a sample scoring matrix of individual components. For each tissue, we can identify the genes that contribute most to the joint and individual effects based on the ranking of genes by each column of U^t and W^t, respectively.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The individual-level data from the Growing Old TOgether (GOTO) trial are protected by Dutch personal integrity laws and other (privacy) regulations. As such, restrictions apply to the availability of the GOTO trial data. All summary statistics and data, underlying the main figures,

are available in the source data file at: https://git.lumc.nl/fabogaards/goto_transcriptomics_analysis.git (https://doi.org/10.5281/zenodo.10980162). Data will be available immediately following publication, no end date. Additional summary statistics of the GOTO trial are available upon request to Fatih Bogaards (corresponding author; f.a.bogaards@lumc.nl). The GOTO data is available for replication purposes upon request to P. Eline Slagboom (p.slagboom@lumc.nl) and if replication is conducted within the secure Leiden University Medical Center network environment. Initial responses for data requests will be within 2 weeks. Research requests for commercial use will not be considered. The protocol of the GOTO lifestyle intervention trial is accessible upon request to Marian Beekman (m.beekman@lumc.nl). Source data are provided with this paper.

Code availability

The scripts containing the code for the data pre-processing and data analysis can be freely downloaded at: https://git.lumc.nl/fabogaards/goto_transcriptomics_analysis.git (https://doi.org/10.5281/zenodo.10980162).

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Author contributions

P.E. Slagboom and C.P.G.M. de Groot designed the trial. M. Beekman collected and curated the health data. N. Lakenberg and H.E.D. Suchiman generated the transcriptome data. F.A. Bogaards T. Gehrmann, M.J.T. Reinders, and P.E. Slagboom designed the data analysis approach, F.A. Bogaards performed data analysis, and F.A. Bogaards, T. Gehrmann, M.J.T. Reinders, C.P.G.M. de Groot, and P.E. Slagboom performed the research and interpreted the data. All authors were involved in drafting and revising the manuscript.

Competing interests

The authors declare no competing interests.

Ethics statement

The Growing Old TOgether Trial protocol was approved by the Medical Ethical Committee of the Leiden University Medical Center before the start of the trial (27/06/2012) (P11.187). In accordance with the Declaration of Helsinki, the Growing Old TOgether Trial obtained informed consent from all participants prior to their entering the trial. This trial was registered at the Dutch Trial Register (https://onderzoekmetmensen.nl/en) as GOT NL3301.

Additional information

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