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Multi-omics signature of healthy versus unhealthy lifestyles reveals associations with diseases

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Abstract

This multi-omics cross-sectional study investigated differences in metabolomics, proteomics, and epigenomics profiles between two groups of adults matched for age but differing in lifestyle factors such as body composition, diet, and physical activity patterns. Data from prior studies were utilized for a comprehensive integrative analysis. The study included 52 participants in the lifestyle group (LIFE) (28 males, 24 females) and 52 in the control group (CON) (27 males, 25 females). Using multi-omics integration software (OmicsNet and Pathview), 96 significantly ($p < 0.05$) enriched pathways were identified that differentiated the LIFE and CON groups. Top pathways significantly ($p < 2.63 \times 10^{-5}$) influenced by group status included fatty acid degradation, fatty acid elongation, glutathione metabolism, Parkinson disease, and central carbon metabolism in cancer. This study identified a distinct metabolic signature comprised of metabolites, proteins, and gene methylation sites associated with a healthy lifestyle. These findings provide unique, but complementary, results to previous single-omics analyses using metabolomics and proteomics procedures which showed that the LIFE group exhibited lower plasma bile acid levels, higher levels of beneficial fatty acids, reduced innate immune activation, enhanced lipoprotein metabolism, and increased HDL remodeling. The current multi-omics analysis builds on these previous results by providing a more holistic view of how metabolites, proteins, and methylation sites associated with a healthy lifestyle, providing a larger, more comprehensive list of altered pathways. Additionally, the integrated analysis revealed connections between lifestyle factors and conditions such as cancer and insulin resistance beyond what identified in the single-omics approaches, highlighting the broader metabolic impact of lifestyle on health. Overall, the signatures identified by this multi-omics approach provide a basis for developing more translational biomarkers, such as those that defined the cancer and insulin resistance pathways that can be used to assess one's state of health and provide guidance on behavior modifications that should be taken to lower disease risk.

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Introduction

Advances in multi-omics technologies, including those that combine proteomics, metabolomics, and epigenomics, have enabled detailed explorations into the biological underpinnings of health and disease, including how lifestyle behaviors influence physiology and contribute to disease risk or disease severity. Proteomics is the study of structure and function of proteins, which have been used for biomarker discovery and drug development [1]. Metabolomics is the profiling of metabolites, which are the substrates, co-factors, and products of metabolism that drive essential cellular functions [2]. Epigenomics is the study of changes in gene activity which occur without altering DNA sequences [3]. All three fields interact closely with each other. Epigenetic modifications like DNA methylation can directly influence the expression of genes involved in biochemical processes, thereby altering the production of metabolites [4]. Together, these disciplines contribute to understanding the molecular mechanisms underlying health and disease.

Epidemiological studies show that healthy lifestyle behaviors—broadly defined as having a balanced diet, performing regular physical activity, avoidance of tobacco use, moderation of alcohol, and maintenance of a healthy weight—are linked to increased life expectancy and reduced chronic disease risk. Indeed, these behaviors have been linked with significant risk reductions for many chronic diseases including cardiovascular disease, type 2 diabetes, cancer, and other obesity-related conditions [5–10]. Li et al. showed that for 50-year-old individuals, adherence to 5 healthy lifestyle factors could increase life expectancy by 14 and 12.2 years for females and males respectively compared to individuals who adopted zero healthy lifestyle factors [8]. Rassy et al. observed that for individuals aged 40–73 years old, those who did not smoke, regularly exercised, had no/moderate alcohol use, and had a healthy diet had lower risk of hypertension, ischemic heart disease, arrythmias, heart failure, arteriosclerosis, kidney failure, gout, sleep disorders, and mood disorders [6]. Observations such as these indicate that these healthy lifestyle behaviors can have a significant impact on risk of many different chronic diseases. However, the biological pathways mediated by these beneficial lifestyle practices, individually or in combination, remain unclear, which has limited our ability to understand the biochemical mechanisms of these protective effects. Prior studies have identified unique molecular signatures linked to cardiorespiratory fitness [11–15], healthy diet [16–21], aging [22], and obesity [25–29]. However, most studies focus on individual omics and use varied designs that hinder comparability between studies. Multi-omics technologies can help identify more comprehensive molecular signatures associated with healthy lifestyle adherence and disease risk.

We have previously performed single-omics studies to better understand the molecular signatures of healthy lifestyle patterns. One of these studies was a metabolomics investigation to compare plasma metabolomes between individuals which adhered to a healthy lifestyle (LIFE) and a control group (CON) which did not adhere to a healthy lifestyle [30]. That study identified 486 significant metabolites with a Variable Importance in the Projection (VIP; a score given to each variable in a supervised multivariate analysis to assess the importance of a variable in separating groups such as LIFE and CON individuals) score ≥ 1 and 16 significant pathways (p value < 0.05). Positive lifestyle habits were linked to lower plasma levels of bile acids, an amino acid profile characterized by higher histidine and lower glutamic acid, glutamine, β-alanine, phenylalanine, tyrosine, and proline, elevated vitamin D status, higher levels of beneficial fatty acids, and reduced levels of N-glycan degradation metabolites [30]. Another previous single-omics investigation performed by our group involved a proteomics approach to analyze dried blood spots from the same individuals. This study demonstrated distinct differences between the LIFE and CON groups for 39 of 725 proteins assayed [31]. Among them, 18 proteins were downregulated in the LIFE group, indicating a lower innate immune activation signature, while 21 proteins were upregulated, reflecting greater lipoprotein metabolism and HDL remodeling. These findings suggested that only a relatively small number of proteins were associated with positive lifestyle habits [31].

The study presented herein also collected DNA methylation data on these same individuals and integrated this data with existing metabolomics and proteomics data from previous studies. The goal of this analysis was to identify molecular signatures of healthy versus unhealthy lifestyles and their links to disease. Findings from multi-omics approaches may provide clarification of how lifestyle factors influence biological mechanisms and disease outcomes to guide targeted interventions and personalized health strategies. Furthermore, the objective of this study was to identify key genes, proteins, and metabolites that, when measured, can effectively define an individual's health status. The validation of such findings has the potential to improve clinical practice by providing biomarker signatures for health that surpass the small number of measures that are taken in routine medical examinations. Furthermore, current clinical measures and medical examinations focus on the detection of disease. This is disparate from the World Health Organization's (WHO) definition of health, 'a state of complete physical, mental, and social well-being and not

merely the absence of disease or infirmity.' [32] This study takes steps towards developing biomarkers that will be critical for further aligning healthcare practices with the WHO's definition of health as multi-omic approaches can provide a more holistic view of one's state of health by measuring factors that are responsive to physical, mental, and social state in contrast to more traditional clinical measures which may not reflect all of these elements. The importance of all of these factors together is also why DNA methylation was chosen to represent the epigenome since DNA methylation has been linked with changes in physical, mental, and social environments [33]. Given the current limitations of clinical assays and the impracticality of measuring the expression levels of hundreds or thousands of genes, proteins, and metabolites, this study aimed to identify a select group of genes, proteins, and metabolites that define health. These biomarkers, when measured alongside those already included in clinical assays, could provide a more comprehensive definition of health.

Methods

Study design and methods

This investigation used existing metabolomics and proteomics data and newly collected DNA methylation data from a cross-sectional study design to compare metabolic, proteomic, or epigenomic profiles in adults adhering (LIFE, n = 52) or not adhering (CON, n = 52) to healthy lifestyle recommendations [30, 31]. Study participants were recruited into LIFE and CON groups using a stringent set of inclusion and exclusion criteria as described below.

The procedures were approved by the Appalachian State University Human Subjects Institutional Review Board (IRB), with Federal Wide Assurance (FWA) number: FWA00027456. The IRB granted review approval (#21–0054) on 10/16/2020 [31]. The human subject research was conducted in compliance with relevant guidelines and regulations, and informed consent was obtained from all participants [31]. Blood samples were collected in an overnight fasted state, body composition was assessed using anthropometric and 8-point bioelectrical impedance methods (seca, Hamburg, Germany), and questionnaires collected information on participants' lifestyle habits, physical activity patterns, mood states, estimated VO_{2max}, nutrient intake, and medical history [31]. The proteomics and metabolomics data, along with details regarding the study participants and methods from this cross-sectional study, have been published in separate papers [30, 31]. For full details, the reader is referred to those papers, with a brief overview provided herein.

Study population

Participants were recruited into LIFE and CON groups based on predefined lifestyle and health criteria. The LIFE group consisted of 28 males and 24 females, all of whom were healthy, with no current or prior history of chronic or infectious diseases. They maintained a body mass index (BMI) of less than 25 kg/m^2, engaged in high levels of physical activity (> 300 min per week of vigorous exercise), and adhered to a healthy dietary pattern characterized by recommended intakes of fruits, vegetables, whole grains, low-fat dairy products, healthy protein foods, and a low intake of salt, sugar, fats, and alcohol. Additionally, participants had been non-smokers for at least the previous three years [31]. The control group (CON) consisted of 27 males and 25 females, all of whom were healthy with no current or prior history of chronic or infectious diseases but were classified as obese (BMI ≥ 30 kg/m^2). This group exhibited a sedentary or low physical activity level (< 150 min per week) and followed an unhealthy dietary pattern [31]. The age range for all participants was between 25 and 75 years. Individuals were excluded from the study if they had been treated for heart disease, cancer (excluding skin cancer), or any medically complicated conditions [31]. Additional details of study participants can be found in our previous publication [30].

Omics data acquisition

Metabolomics and proteomics data had been previously collected and processed. For metabolomics data acquisition and data preprocessing, plasma blood samples were subjected to untargeted ultra-high-performance liquid chromatography-high-resolution mass spectrometry (UHPLC-HRMS) for metabolic profiling using a data dependent acquisition method [30]. For proteomics data acquisition and data preprocessing, dried blood spot samples were processed using data-independent acquisition mass spectrometry (DIA-MS). Protein identifications were performed against a customized spectral library [31]. For epigenomics data acquisition and data preprocessing, epigenetic modifications were assessed using DNA extracted from blood samples. Bisulfite sequencing data was generated using BSBolt to analyze DNA methylation patterns. The reader can refer to this paper for more details [34]. Data preprocessing that converts methylation site to gene symbols were performed using UCSC Genome Browser [35].

Targeted bisulfite sequencing (TBS-seq)

DNA was extracted starting from 500 µl following the protocol supplied by the manufacturer. Purified gDNA was measured by fluorometric quantification with the Qubit dsDNA BR assay (Thermo Fisher Scientific).

Between 250 and 500 ng of purified gDNA for each sample were fragmented to an average size of 250–300 bp by sonication with a BioruptorPico device (Diagenode) using the following conditions: 15 cycles, 30 s-on and 90 s-off. The size of the sheared gDNA was assessed by automated electrophoresis by running an aliquot of each sample on the 4200 Agilent TapeStation. DNA libraries were prepared using the NEBNext UltraII library prep kit (New England Biolabs) and custom, pre-methylated Dual-Unique-Index adapters from Integrated DNA Technologies (IDT) were used for adapter ligation. Groups of 16 libraries, each carrying a different adapter, were pooled together, concentrated by drying with a SpeedVac system and used for targeted enrichment with custom made 5'-biotinylated oligos (IDT). A description of the xGen Custom Hybridization probes panel can be found in a previous publication [36]. Targeted enrichments were performed using the xGen Hybridization capture of DNA libraries kit from IDT according to the instructions provided by the manufacturer. Hybridization was carried out overnight at 65 °C. Bisulfite conversion of captured DNA was performed prior to PCR amplification using the EZ-Methylation Gold kit (Zymo Research). KAPA HiFi Uracil + (Roche) was used for final PCR amplification with the following conditions: 2 min at 98 °C; 16 cycles of (98 °C for 20 s; 60 °C for 30 s; 72 °C for 30 s); 72 °C for 5 min; hold at 4 °C. PCR products were SPRI beads purified and library QC was performed using the High-Sensitivity D1000 Assay on the 4200 Agilent TapeStation. The pool of 96 libraries was sequenced on a NovaSeq6000 (Sp lane) as paired-end 150 bases. The probes used in the capture were designed based on public EWAS loci associated with aging, cell types, and metabolic disorders.

DNA methylation data processing

The processing of bisulfite sequencing data was carried out using BSBolt (v1.5.0). Demultiplexed FASTQ files were subjected to adapter removal using cutadapt (v4.1) and aligned to the GRCh38 reference genome using the BSBolt Align function. PCR duplicates were removed using the samtools markdup function (v1.15) before calling methylation with BSBolt CallMethylation function. The outputs of the CallMethylation function (CGmap files) were combined to create methylation matrices with minimum read count coverage floors across 100% of samples. The read count floor used for a particular analysis are indicated in the relevant figures and tables for epigenomic results.

Data analysis

This investigation used freely available software tools (OmicsAnalyst, OmicsNet, and Pathview) to conduct multi-omics data analysis using existing data.

OmicsAnalyst

OmicsAnalyst is a web-based platform designed to allow users to perform a variety of well-established data-driven approaches for multi-omics integration [37]. This includes correlation networks, clustering heatmaps, and dimensionality reduction techniques to explore features within and across omics layers and their relationship to study metadata [37, 38]. There are three major steps in multi-omics analysis using OmicsAnalyst: 1) individual data processing, 2) method selection, and 3) visual analytics [38]. OmicsAnalyst contains built-in annotations for transcriptomics, proteomics, and metabolomics [37, 38]. Steps involving quality and parameter control were inserted between the main steps [37, 38]. In this study, OmicsAnalyst was used for generating multivariate plots to determine whether LIFE and CON shared similar or distinct coordinated patterns of change across omics layers. Preprocessed metabolomics, proteomics, and epigenomics data were input into the software, and all data were autoscaled. Multivariate analyses of the omics data included unsupervised Principal Component Analysis (PCA) for individual omics analysis, and supervised Data Integration Analysis for Biomarker Discovery using Latent Variable Approaches for Omics Studies (DIABLO) for multi-omics analysis. DIABLO is a multi-block PLS-DA supervised technique that integrates multiple omics datasets and identifies omics variables that discriminate phenotypic groups. Due to the large number of epigenomics data that exceeded the software's capacity, only methylation sites with more than 40 reads were included in data analysis.

OmicsNet

OmicsNet is a web-based tool that specializes in exploring multi-omics data within the context of molecular interaction knowledgebases by allowing users to build, visualize, and analyze molecular networks [39]. In this study, OmicsNet was used to create and visualize 2D and 3D multi-omics networks from lists of identified molecules of interest from the different omics datasets. This analysis aimed to identify significantly different pathways between LIFE and CON by building multi-omics networks using metabolites, proteins, and genes that significantly differentiated the two groups. The input data consisted of significant metabolites ($p < 0.05$), proteins ($p < 0.05$), and gene symbols associated with significant methylation sites ($p < 0.01$) identified by Students' t-test.

The output included significant pathways with a threshold of $p < 0.05$. Multi-omics networks were built using the KEGG database [40].

Pathview

Pathview is a tool that maps, integrates, and visualizes biological data on molecular and genetic pathways [41]. In this study, Pathview was employed for pathway visualization to determine which pathway components were affected including their direction of change. Input data included significant ($p < 0.05$) metabolites (input as KEGG IDs), and proteins (input as gene symbols), to visualize pathways selected by OmicsNet. The output consisted of pathway visualizations, allowing for the interpretation of biological relevance in the observed omics data.

Results

Subject characteristics

This study used a cross-sectional design to compare 52 participants in the LIFE group with 52 in the CON group. There were no significant differences in age, education level, or height between the LIFE and CON groups. The sex distribution was also similar between the two groups, with a chi-square value of 0.039 and a p value of 0.844. However, several body composition measures, including body mass index (BMI), fat mass index (FMI), body fat percentage, and sagittal abdominal diameter, showed significant differences ($p < 0.001$) between the LIFE and CON groups. Physical fitness and activity levels differed significantly ($p < 0.001$) between LIFE and CON groups, including estimated aerobic capacity (VO_{2max}) and total physical activity calculated as MET-min/week. In LIFE, the fruit and vegetable intake was significantly higher ($p < 0.001$) and red meat intake significantly lower ($p < 0.001$) compared to CON. In LIFE, alcohol intake (grams/day) was higher, on average, than CON group (10.2 ± 14.1 vs 11.0 ± 20.0), though not significantly. The total mood disturbance was significantly (p value < 0.001) lower in the LIFE group (90.4 ± 7.1) than in the CON group (94.8 ± 8.1). None of the subjects in LIFE and only two in CON were current cigarette smokers, but 12% and 27% of LIFE and CON, respectively, reported smoking at least 100 cigarettes in their entire lifetimes ($X2 = 3.96$, $p = 0.047$) [30, 31].

Multivariate and univariate statistics

The unsupervised PCA for metabolomics, proteomics, and epigenomics data showed a noticeable overlap between the LIFE and CON groups (Fig. 1). Comparatively, metabolomics and proteomics data were more separated between LIFE and CON groups than epigenomics data, indicating more similarities in epigenomic profiles than metabolic and proteomic profiles. Supervised DIABLO, which is a multi-block PLS-DA, generated a factor scores plot (Fig. 2A), variability plot (Fig. 2B), and the 3D visualization (Fig. 2C). The factor scores plot indicated that component 1 created the greatest difference between LIFE and CON groups. The variability plot indicated that the difference created by component 1 was largely driven by metabolomics and proteomics data. The 3D DIABLO plot showed clear separation between LIFE (red) and CON (green) groups with this supervised analysis, primarily separated based on component 1. Overall, these data show that the two groups overlap in each omics layer in an unsupervised analysis (PCA,

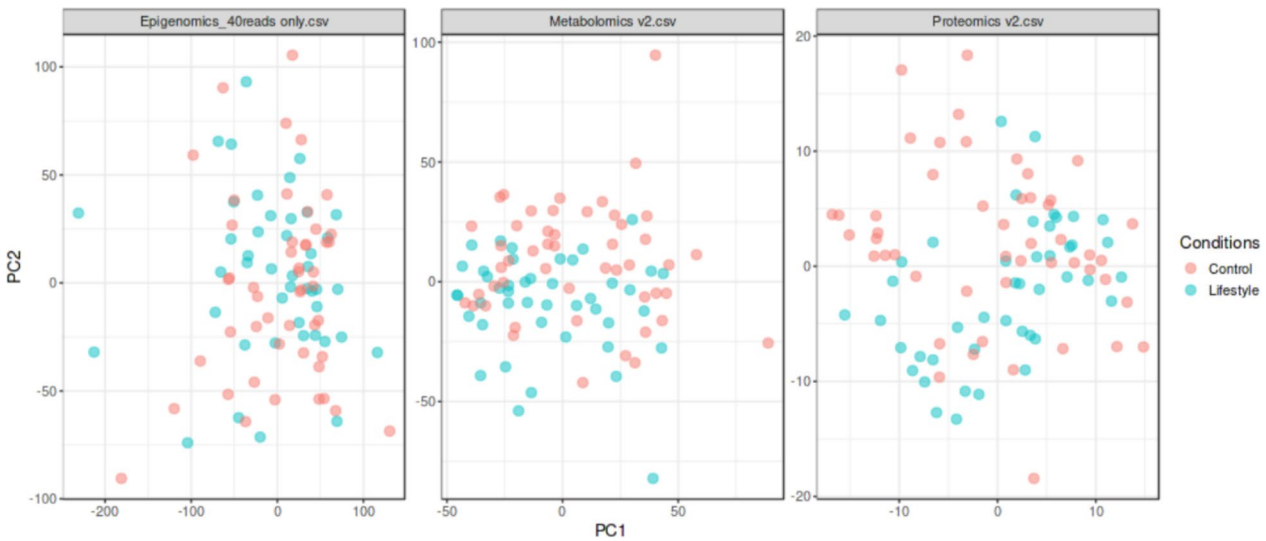

Fig. 1 PCA of metabolomics, proteomics, epigenomics data of LIFE and CON groups

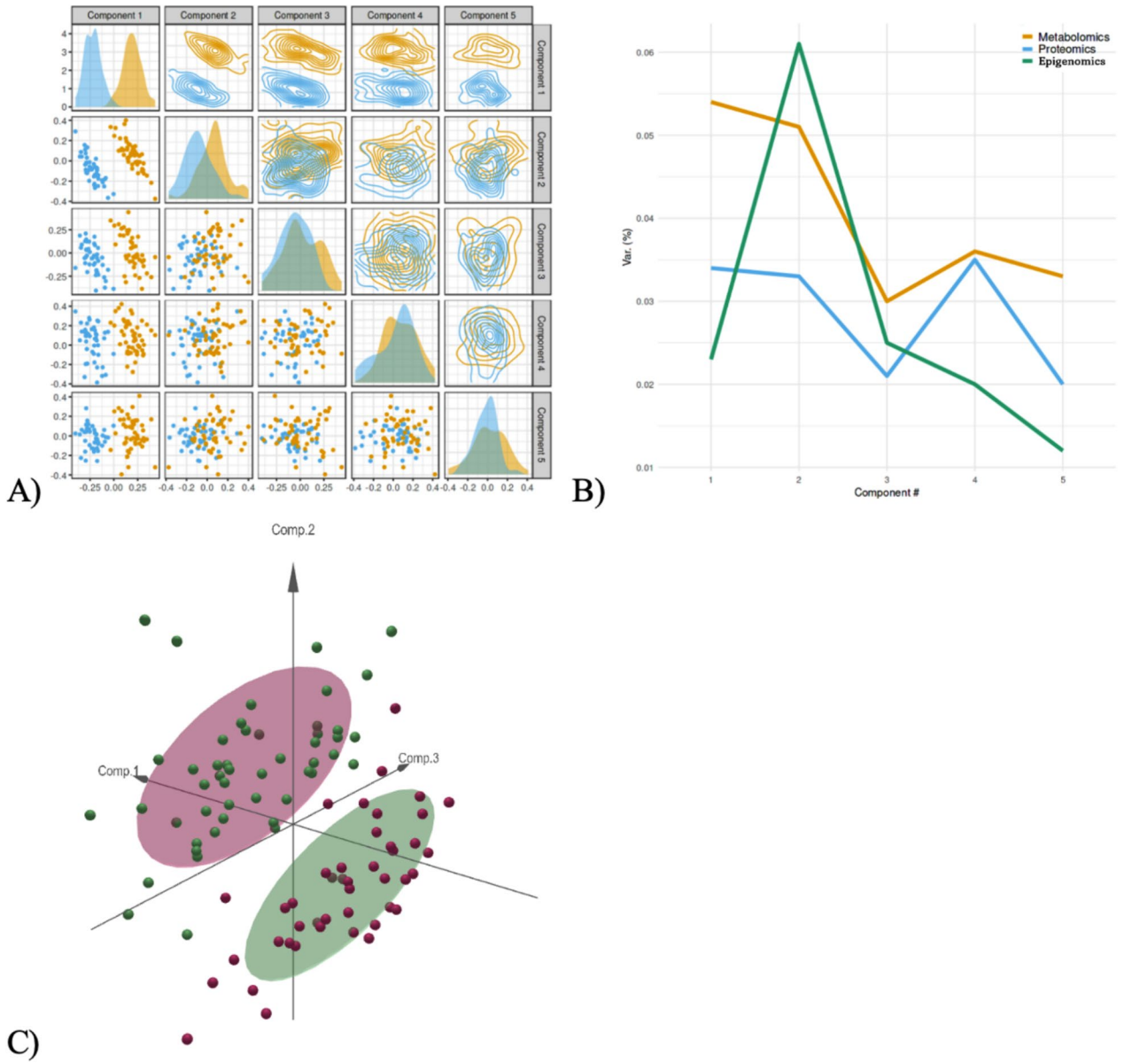

Fig. 2 Supervised DIABLO analysis, a multi-block PLS-DA technique, evaluating the difference between LIFE and CON based on multi-omics profiles. **A** The factor scores plot. Blue = CON, orange = LIFE **B** Plot of the variability in each omics layer that is explained by the integrated factor scores. **C** 3D multi-block PLS-DA plot of metabolomics, proteomics, epigenomics data of LIFE (red dots) and CON (green dots) groups. Ellipsoids represent the confidence region

Fig. 1), but an integrated, supervised analysis (DIABLO, Fig. 2) produced clear separation between the LIFE and CON groups, indicating that supervised approaches may be necessary for identifying lifestyle-related differences.

Significant pathways analysis

Two tailed, two sample equal variance students' t-test was performed to obtain significant metabolites, proteins, and methylation sites. There were 2,453 metabolite features and 105 proteins with $p < 0.05$, and 561 methylation sites with $p < 0.01$. Figure 3 shows the 2D multi-omics network between those significant metabolites, proteins, and genes of the corresponding methylation sites. There was a total of 96 pathways calculated to be significantly ($p < 0.05$) different between LIFE and CON groups by OmicsNet. The top 15 significant pathways are shown in Table 1, and the complete list of significant pathways are shown in the Online Appendix Table 1 for

Fig. 3 2D multi-omics network of significant metabolites (yellow, $p < 0.05$), proteins (red, $p < 0.05$), and genes (green) that correspond with the significant methylation sites ($p < 0.01$) in circular layout

the comparison of LIFE and CON groups. Out of the 96 significant pathways, 23 were disease/disorder pathways from the KEGG DISEASE database, a collection of pathways that are focused on molecular networks that are known to be perturbed in human diseases [42].

Discussion

This cross-sectional study focused on the multi-omics profile associated with a healthy lifestyle, using metabolomics, proteomics, and epigenomics data [30, 31]. The LIFE and CON groups were similar in sex distribution, education, and age, but differed in lifestyle factors, including physical activity, dietary patterns, and body composition. It is well-recognized that behavioral effects related to healthy lifestyles can influence molecular profiles through a variety of cellular and molecular mechanisms, and these profiles can provide insight into the risk of developing various chronic diseases [43]. The metabolomics data set previously published from this cross-sectional study showed that individuals in the LIFE group had lowered plasma levels of numerous bile acids, elevated vitamin D status, higher levels of beneficial fatty acids, reduced levels of N-glycan degradation metabolites, and an amino acid profile consistent with a reduced risk for chronic disease [30]. The proteomics dataset previously published from this study showed that individuals in the LIFE group had a lower innate immune activation signature and greater lipoprotein metabolism and HDL remodeling [31]. In this analysis, the integration of metabolomics, proteomics, and epigenomics (previously unpublished) data revealed distinct signatures between the LIFE and CON groups. Supervised, multivariate analysis of the multi-omics dataset (DIABLO) confirmed that group status (LIFE vs. CON) was strongly predicted

Table 1 The top 15 significant pathways ($p < 0.05$) analyzed and ranked by OmicsNet

Pathway	integP value
Fatty acid degradation	2.29E-15
Glutathione metabolism	1.21E-09
Fatty acid elongation	1.36E-09
Purine metabolism	7.50E-08
Oxidative phosphorylation	2.70E-07
Pyrimidine metabolism	9.16E-07
Thermogenesis	1.3E-06
Parkinson disease	4.53E-06
Aldosterone synthesis and secretion	4.57E-06
Glyoxylate and dicarboxylate metabolism	1.06E-05
GABAergic synapse	1.38E-05
Cortisol synthesis and secretion	1.64E-05
Alcoholism	2.45E-05
Central carbon metabolism in cancer	2.63E-05
Vitamin B6 metabolism	2.87E-05
Glucagon signaling pathway	0.000798

by the combined multi-omics signature. An enriched pathways analysis using OmicsNet revealed 96 pathways that were significantly different between LIFE and CON groups and twenty-three were disease-related pathways. Some of the non-disease pathways included fatty acid degradation and elongation, glutathione metabolism, and purine metabolism. Some of the disease-related pathways included Parkinson's disease, alcoholism, and central carbon in metabolism in cancer. Visualization of these pathways was generated by Pathview (Figs. 4, 5, 7, and 8). The green to red spectrum represented genes, and the blue to yellow spectrum represented metabolites [41]. Genes highlighted in red were more significantly expressed ($p < 0.05$) in LIFE group than in CON group. Metabolites highlighted in yellow had a significantly higher level ($p < 0.05$) in the LIFE group than in the CON group. This investigation was conducted as a differential profiling study, and not a quantitative assessment of metabolites, proteins, and gene expression. This study did not include the use of quantitative historical data for healthy baseline values of gene expression, proteins, or metabolites.

The most significant pathway differentiation (p value = 2.29E-15) between LIFE and CON groups was fatty acid degradation (Fig. 4, Table 1). Fatty acid degradation, the breaking down of fat into acetyl-CoA for energy while sparing muscle from catabolism, occurs in the mitochondria throughout the body during high energy demand states, such as trauma and exercise [44, 45]. Disrupted fatty acid degradation leads to an accumulation of elevated concentrations of metabolic intermediates in blood and organs that result in the development of chronic diseases, especially those related to metabolic dysfunction like obesity, insulin resistance, type 2 diabetes, and cardiovascular disease [46–48]. The type and amount of fat consumed directly impact the rate at which the body breaks down fatty acids for energy [49–51], with high-fat diets leading to increased fatty acid oxidation. Mitochondrial carnitine palmitoyltransferase 1 (CPT1. Figure 4, Table 1) is located in the outer membrane of mitochondria and converts acyl-CoAs into acylcarnitines, enabling transport of long-chain fatty acids into mitochondria for β-oxidation [52]. CPT1 is the rate-limiting step of this process and major regulatory mechanism of β-oxidation is inhibition by malonyl-CoA which is formed by acyl-CoA carboxylase [46, 53, 54], an enzyme that is upregulated and activated during exercise [55]. Direct CPT1 overexpression in animals and skeletal muscle cells was found to protect muscle from fatty acid induced insulin resistance and apoptosis [56–58]. Other regulators of fatty acid degradation are hormones such as insulin, glucagon, and cortisol [59–61] which are influenced by diet, exercise, and obesity [62–69]. Fatty acid elongation was the third most significant pathway that differed (p value = 1.36E-09, Fig. 5, Table 1) between LIFE and CON groups, further supporting that fatty acid metabolism is significant in defining the healthy signature. Fatty acid elongation, the process of adding carbon units to lengthen fatty acids that occur in the endoplasmic reticulum, is used for cellular metabolism, membrane structure, and cell signaling [70–72]. Fatty acid elongation imbalances are linked to a variety of diseases, including metabolic disorders, skin conditions, liver disease, cancer, and central nervous system disorders [73–78]. Our identification of fatty acid degradation and elongation as significantly altered pathways aligns with exposomics-based research, demonstrating that environmental and lifestyle exposures systematically impact lipid metabolic processes. Literature shows that lipid-related pathways — including β-oxidation and elongation of fatty acids — frequently emerge as central hubs of molecular perturbation in multi-omics exposome analyses [79]. These changes may serve as early biomarkers of cardiometabolic stress linked to lifestyle behaviors.

CPT1 and acetyl-CoA C-acyltransferase were expressed more in the LIFE group than in the CON group (Fig. 4). Trans-Dodec-2-enoyl-CoA, (S)-3-Hydroxydodecanoyl-CoA, 3-Oxo-dodecanoyl-CoA were higher in LIFE group than in CON group. Higher expression of CPT1 could improve fatty acid oxidation and reduce fatty acid buildup, which may help to prevent obesity and metabolic disorders like T2D and cardiovascular disease. [80, 81]. The LIFE group had higher CPT1 expression which implies that a healthy lifestyle decreases the risk of obesity and metabolic disorders through these fatty acid

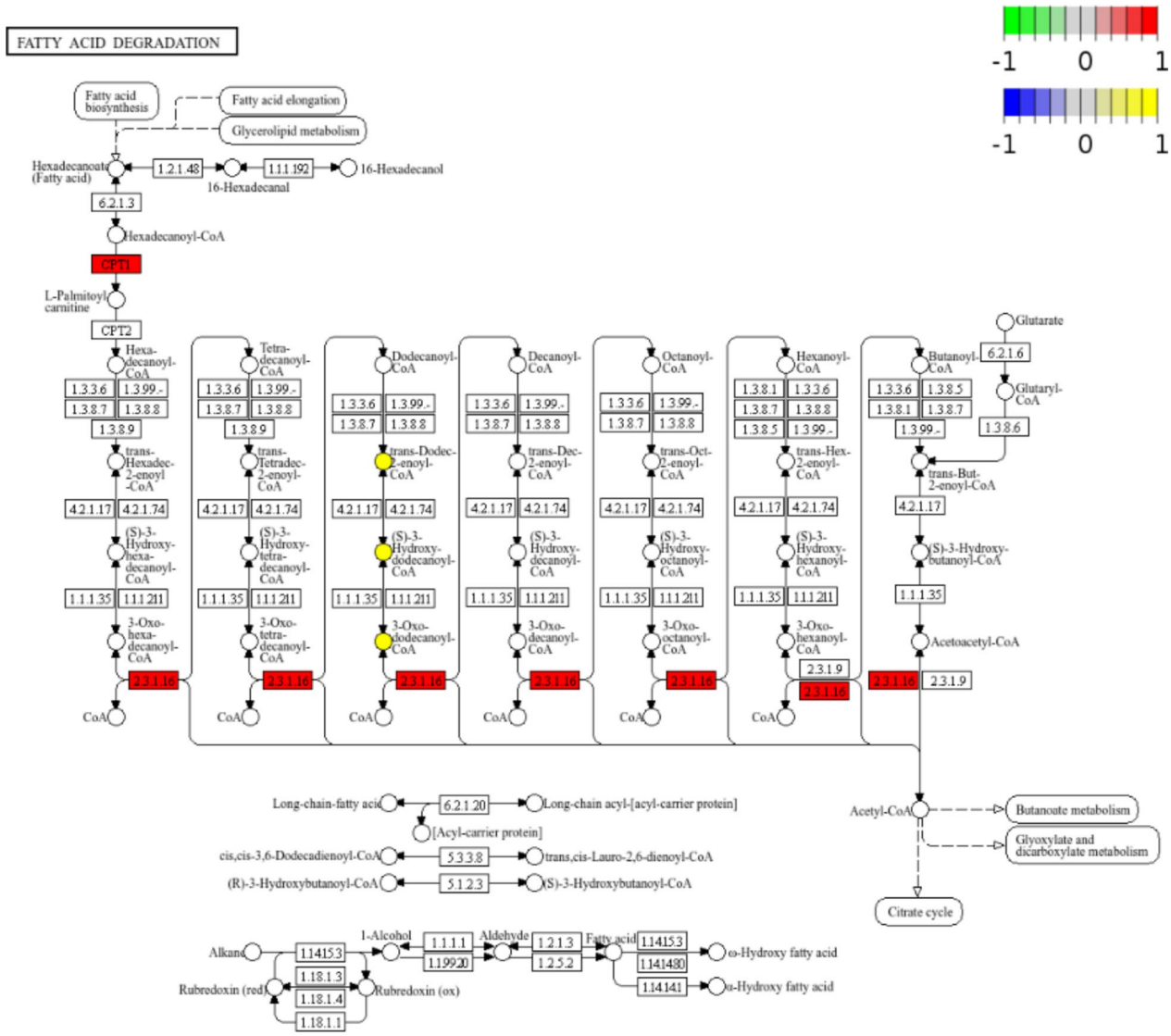

Fig. 4 Visualization of fatty acid degradation generated by Pathview. CPT1 and Acetyl-CoA C-acyltransferase were expressed more in LIFE group than CON group. Trans-Dodec-2-enoyl-CoA, (S)-3-Hydroxy-dodecanoyl-CoA, 3-Oxo-dodecanoyl-CoA were higher in LIFE group than in CON group. Genes in red and green were higher and lower in the LIFE group, respectively. Metabolites in yellow and blue were higher and lower in the LIFE group, respectively

metabolic mechanisms. Acetyl-CoA C-acyltransferase acts on 3-oxoacyl-CoAs to produce acetyl-CoA and an acyl-CoA shortened by two carbon atoms [82]. The difference in levels of intermediates before the final acetyl-CoA production and the acetyl-CoA C-acyltransferase expression might be due to differences in alcohol consumption between LIFE and CON groups. Alcohol significantly impacts CoA metabolism by increasing the production of acetyl-CoA, leading to a disruption in the normal fatty acid oxidation pathway due to an altered NADH/NAD + ratio, which contributes to the development of fatty liver disease [83–85]. Disrupted CoA metabolism can lead to a variety of issues including impaired energy production, accumulation of toxic metabolites, cellular dysfunction, and potentially contribute to the development of diseases like neurodegenerative disorders, cardiovascular diseases, and certain cancers. [86–88]

The second most significant pathway that was different between LIFE and CON groups was glutathione metabolism (p value = 1.21E-09, Table 1). Glutathione (GSH), a tripeptide made of cysteine, glutamate, and glycine, is involved in detoxification, antioxidant defense, cell cycle regulation, protein and DNA synthesis, signal transduction, and gene expression [89–95]. Depletion of GSH has

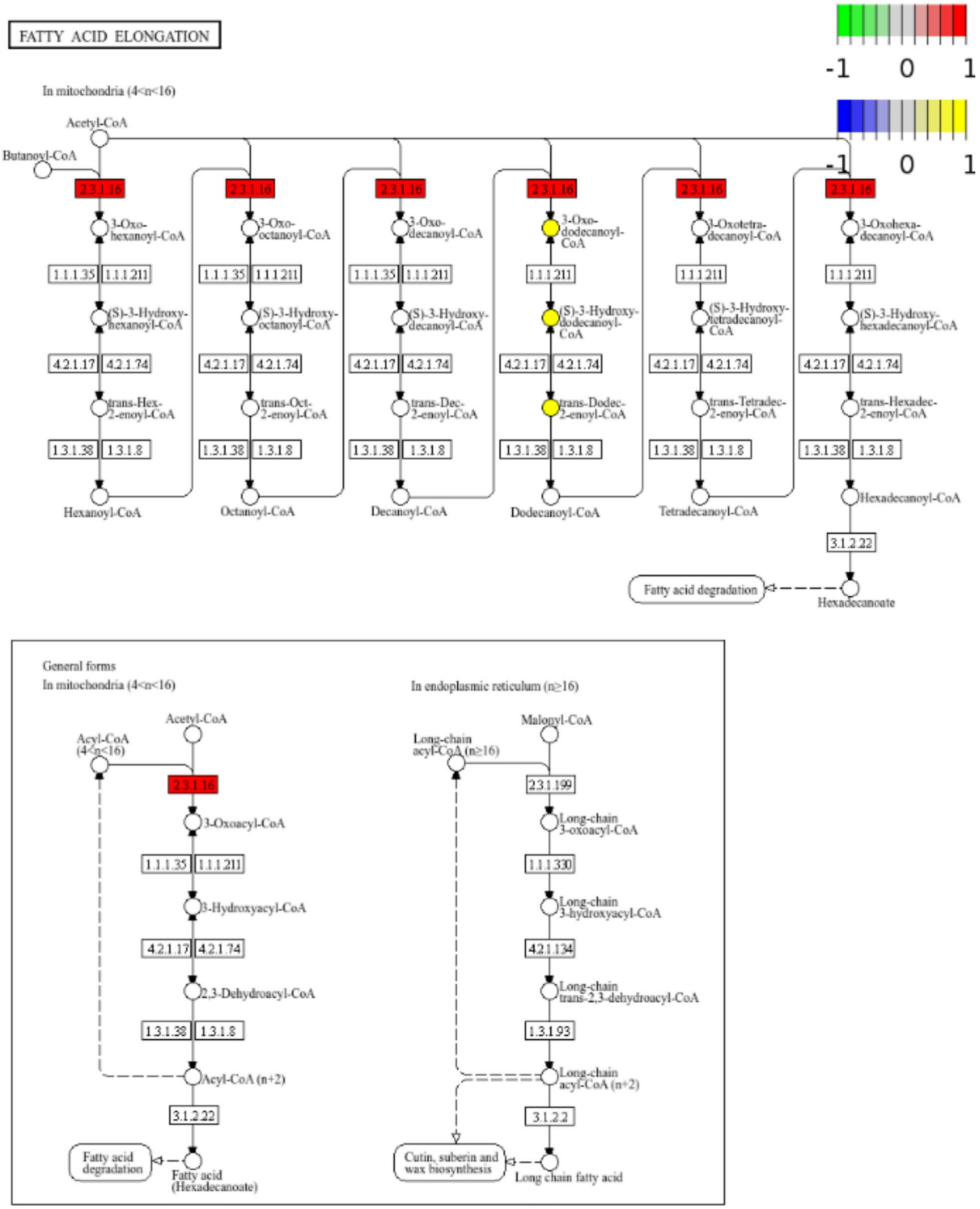

Fig. 5 Visualization of fatty acid elongation generated by Pathview. Acetyl-CoA C-acyltransferase were expressed more in LIFE group than CON group. Trans-Dodec-2-enoyl-CoA, (S)-3-Hydroxy-dodecanoyl-CoA, 3-Oxo-dodecanoyl-CoA were higher in LIFE group than in CON group. Genes in red and green were higher and lower in the LIFE group, respectively. Metabolites in yellow and blue were higher and lower in the LIFE group, respectively

been shown to be linked to multiple diseases like neurodegenerative disorders, pulmonary disease, cardiovascular diseases, immune disease, chronic age-related diseases, liver disease, and cancer [89, 96–100]. Studies have also shown that direct administration and promotion of GSH production have been used effectively in treating diseases like Parkinson's, peripheral obstructive arterial disease, cystic fibrosis, emphysema, COPD, contrast-induced nephropathy, nonalcoholic fatty liver disease, exercise-induced fatigue, and more [89, 101–107]. This study showed that the glutathione level was higher in LIFE than CON group (Fig. 6). Reduced glutathione was also one of the significant metabolites (VIP = 1.9, p value = 3.66E-05, fold difference = 2.3) found in the previous paper published using metabolomics data [30]. A diet high in saturated fat, processed sugars, and refined carbohydrates can increase oxidative stress by generating excessive amounts of free radicals [99,

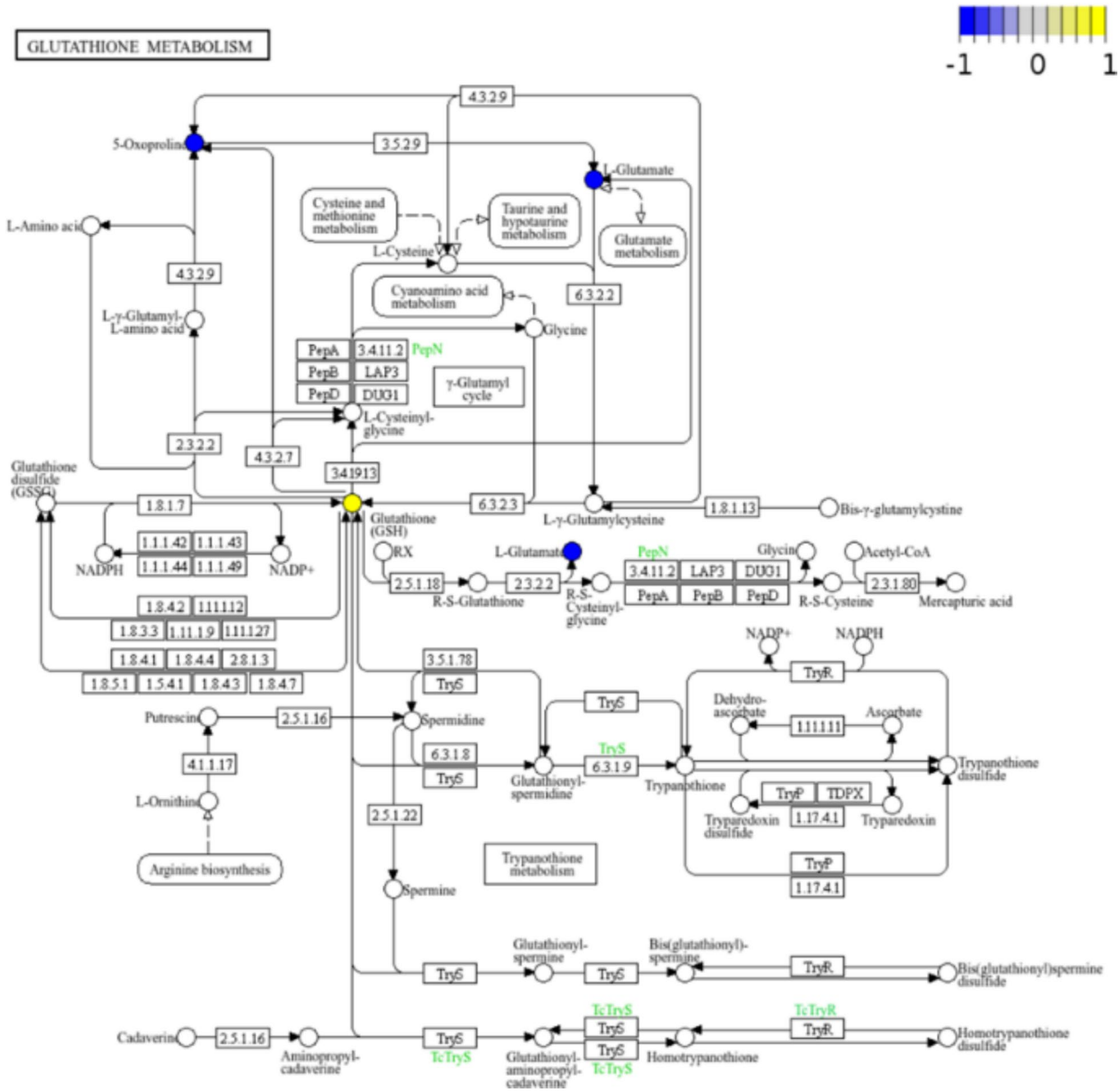

Fig. 6 Visualization of glutathione metabolism generated by Pathview. There were higher levels of GSH and lower levels of glutamate and oxoproline in LIFE group compared to CON group. Genes in red and green were higher and lower in the LIFE group, respectively. Metabolites in yellow and blue were higher and lower in the LIFE group, respectively

108–112]. Conversely, a diet rich in fruits, vegetables, and whole grains with high antioxidant content can help mitigate oxidative stress, which could explain the higher glutathione level in LIFE group. In addition, obesity, alcohol, and tobacco usage also increase oxidative stress and negatively affect levels of glutathione [112–116]. Thus, a healthy lifestyle leads to a higher glutathione level, which decreases the risk of those diseases mentioned above.

Glutamate is not only one of the amino acids involved in glutathione synthesis—it is also an excitatory neurotransmitter in the nervous system [117] and is involved in many functions like learning, memory, and pain signaling [118]. Glutamate is necessary for the synthesis of key molecules, like glutathione and GABA [119]. Glutamate also plays a key role in the tricarboxylic acid cycle during cellular metabolism to provide carbon source for synthesis of other biological molecules [120]. Glutamate is naturally found in protein-rich foods and activates umami taste receptors [119]. Its concentration is tightly controlled by many mechanisms to prevent negative effects such as excess release which could lead to excitotoxicity that has been linked to decreased neuronal regeneration [117, 121, 122]. Glutamate signaling abnormalities have also been found to be linked to neurological diseases such as Alzheimer's disease, Parkinson's disease, and schizophrenia [123]. It has also been shown that excess glutamate level facilitates tumors growth [124–126]. Our results revealed lower levels of glutamate in LIFE group compared to CON group (Fig. 6). Glutamate was the most significant metabolite (VIP = 2.6, p value = 1.30E-09, fold difference = -2.5) differentiating the two groups in the previous paper published using metabolomics data only [30]. A healthy diet limits the intake of processed foods that contain high glutamate level due to monosodium glutamate (MSG) additive [127, 128]. The highest levels of glutamate occurred in meat, fish, and eggs products [129, 130], and the LIFE group had lower red meat intake than the CON group. Extensive exercise depletes the amino acid pool, including the plasma level of glutamate [131]. However, the whole blood glutamate concentration was found to have no significant change due to glutamate being mainly confined in the red blood cell [131, 132]. Tobacco uses also significantly increases the glutamate levels in the brain through its active ingredient nicotine [133, 134], which may explain why the CON group had a higher glutamate level. The lower levels of glutamate in the LIFE group suggest that a healthy lifestyle could lead to a lower chance of developing neurological diseases or cancer that are caused by abnormally high levels of glutamate.

In addition to metabolic pathways, our analyses also revelated that disease-centric pathways were significantly enriched when differentiating LIFE and CON groups. was the most significant disease/disorder pathway (p value = 4.53E-06) that was different between LIFE and CON groups (Table 1). Parkinson's disease is a neurodegenerative disease characterized by motor and cognitive problems with early death of dopaminergic neurons that results in dopamine depletion [135]. Risk factors for increased risk of Parkinson's disease include being male, age, pesticide exposures, family history, and rural living [135, 136]. Interestingly, environmental factors that are associated with a decreased risk of developing Parkinson's disease include tobacco smoking and alcohol consumption [136–138]. The association with smoking could be due to the decreased responsiveness to nicotine during the prodromal phase of this disease [139], rather than suggesting that smoking itself is protective against Parkinson's disease. The decreased risk of Parkinson's disease associated with alcohol consumption is not well understood. One treatment for Parkinson's disease is with drugs that increase dopamine concentration or stimulate dopamine receptors [140–142].

Metabolites:

- Higher DOPA, DOPAL in LIFE
- Lower Tyr, dopamine in LIFE

Proteins:

- Less expression of CaMKII, Ubc6/7, 19S in LIFE

There were higher levels of 3,4-dihydroxyphenylalanine (DOPA) and 3,4-dihydroxyphenylacetaldehyde (DOPAL) and lower levels of tyrosine and dopamine in the LIFE group (Fig. 7). Dopamine is a neurotransmitter that plays a role in regulation of emotions, motor control, and cognitive function [143]. Exercise is found to acutely and transiently increase dopamine production in the brain due to increased serum calcium levels that activate CaMKII which activates tyrosine hydroxylase [144, 145]. However, there is little evidence that chronic physical activity lowers or elevates plasma dopamine levels when measured in the resting state. CaMKII was expressed lower in LIFE group which had higher physical activity level than the CON group, and dopamine levels were lower in the LIFE group (Fig. 7). The higher dopamine level in the CON group might be due to the higher alcohol intake and tobacco use. Alcohol consumption initially increases dopamine levels in the brain, but chronic alcohol use led to decrease in dopamine activity [146]. Nicotine in tobacco use has also been found to lead to an increase in dopamine levels [147]. DOPA is the precursor to dopamine and is synthesized in the brain from the amino acid tyrosine [148]. Tyrosine is the dietary precursor to dopamine and norepinephrine, and plays a role in

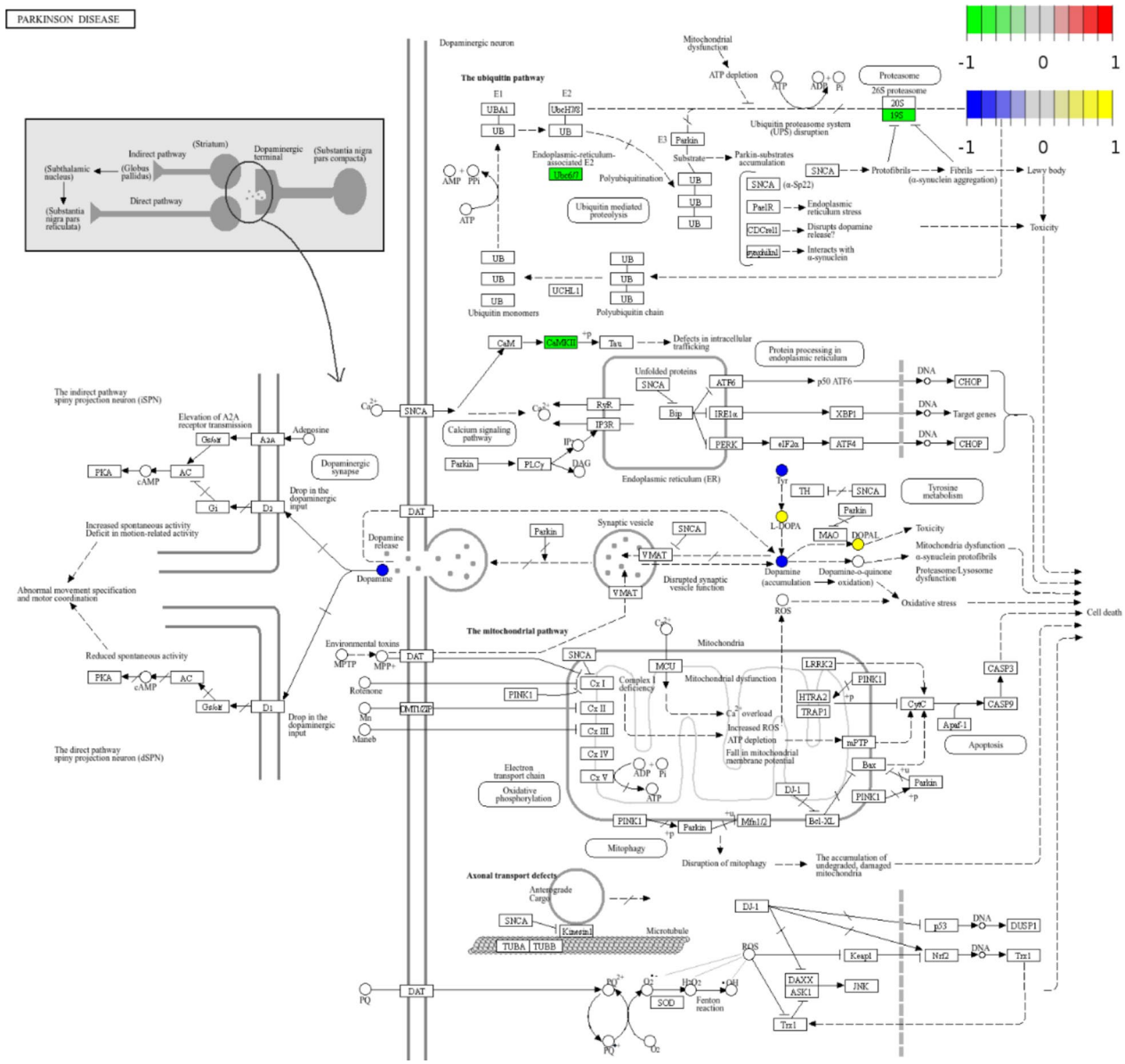

Fig. 7 Visualization of Parkinson disease generated by Pathview. There were higher levels of 3,4-dihyfroxyphenylalanine (DOPA), 3,4-dihydroxyphenylacetaldehyde (DOPAL) and lower levels of tyrosine, dopamine in the LIFE group. There were less expressions of CaMKII, Ubc6/7, 19S in LIFE group than in CON group. Genes in red and green were higher and lower in the LIFE group, respectively. Metabolites in yellow and blue were higher and lower in the LIFE group, respectively

behavior and cognitive function [149]. DOPAL is a highly reactive, toxic metabolite of dopamine breakdown, and may contribute to the development of Parkinson's disease due to its potential to damage neurons through oxidative stress and protein aggregation [150–152]. However, DOPAL was higher in the LIFE group than the CON group (Fig. 7). The reason for this remains unclear. A potential negative association between a healthy lifestyle and Parkinson's disease may be suggested, but further research is required to validate this finding.

Central carbon metabolism in cancer was the third most significant disease/disorder pathway (p value = 2.45E-05) that was different between the LIFE and the CON groups (Table 1). Cancer is a disease that is characterized by uncontrolled cellular proliferation which often leads to metastasis if the disease is allowed to progress [153]. Risk factors for cancer include aging, tobacco use, environmental exposures, family history of cancer, alcohol use, poor diet, lack of physical activity, and obesity [154–158]. Studies have already found that a

healthy lifestyle has a preventative effect on cancer development [158–162]. The results of our study support that this may be through metabolic mechanisms, particularly through central carbon pathways. Cancer cells are known to have an altered carbon metabolism, such as the Warburg effect, where the cells take up large amounts of glucose and produce high amounts of lactate even when oxygen is present [163, 164]. Hyperglycemia can contribute to an imbalance of hormones and promote cell proliferation, adhesion, migration, and invasion of cancer cells [165–169]. Blocking glucose uptake into cells can induce apoptosis in cancer cells [170, 171]. The release of lactate from tumor cells acidifies the tumor microenvironment, which can promote further tumor growth, metastasis, and immunosuppression [172–176]. Lactate levels are also found to predict a patient's overall survival and likelihood of metastasis [177, 178], and targeting lactate production is a potential cancer treatment [179]. There was lower glucose and lactate levels in the LIFE group compared to the CON group (Fig. 8) which implied that a healthy lifestyle decreases risks of cancer development through preventing excess glucose and lactate levels, which is supported by previous studies.

This study also found a lower RAS expression in LIFE group (Fig. 8). RAS is an oncogene that's part of the MAPK signaling pathway in malignant cells [180]. RAS plays a role in cell growth, proliferation, migration, signal transduction, cell cycle regulation, and wound healing and tissue repair [181–189]. This gene is found in all types of cells but found most frequently mutated oncogenes in human malignancy [190]. Mutations in the RAS genes or upstream or downstream signaling components are abnormal in most human tumors [187, 191, 192]. The lower RAS expression in the LIFE group further suggest healthy lifestyle factors decrease risk of cancer development by potentially reducing the activity of signaling pathways involved in cellular proliferation such as RAS. Additionally, there was higher expression of L-type amino acid transporter 1 (LAT1) in the LIFE group (Fig. 8). LAT1 facilitates the transport of essential

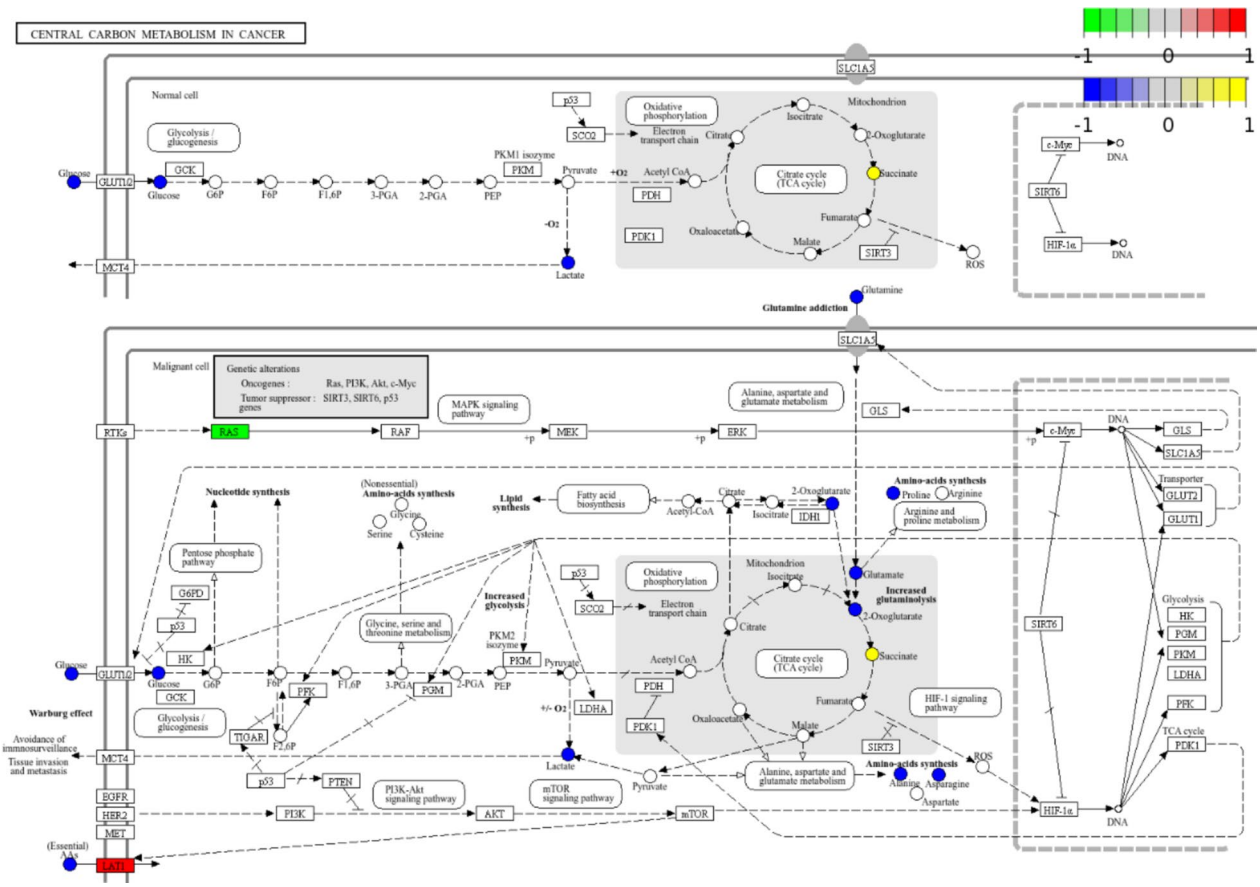

Fig. 8 Visualization of Central Carbon Metabolism in Cancer generated by Pathview. There was lower RAS expression, higher LAT1 expression, lower levels of glucose, lactate, glutamine, glutamate, proline, oxoglutarate, alanine, asparagine, and higher level of succinate in the LIFE group compared to CON group. Genes in red and green were higher and lower in the LIFE group, respectively. Metabolites in yellow and blue were higher and lower in the LIFE group, respectively

amino acids like leucine and phenylalanine, and certain drugs and hormones across the blood–brain barrier and other cell membranes [193–198]. LAT1 is found to be upregulated in various types of cancer, contributing to the growth and proliferation of cancer cells, and higher LAT1 is associated with poor prognosis of patients with multiple tumors [199–204]. This transporter is a potential target for cancer diagnosis and treatment, because inhibiting LAT1 can suppress the growth of tumor cells by downregulating the mTORC1 signaling pathway [205, 206]. The higher LAT1 expression in the LIFE group might initially seem counterintuitive, as it could suggest that healthy lifestyle factors are associated with a higher risk of cancer due to elevated LAT1 expression. However, a potential explanation lies in the rigorous physical activity levels of the LIFE group. Resistant exercise training has been shown to increase LAT1 expression to enhance muscle protein synthesis and promote muscle growth [207, 208] [23, 24]. Since the precise level of LAT1 expression was not measured, it is unclear whether the LAT1 expression in the LIFE group fell within the healthy range. Therefore, the LIFE group should not be considered to have a higher cancer risk based solely on the higher LAT1 expression compared to the CON group. Indeed, the roles of LAT1 in human physiology are varied, with some effects linked to healthy states and some effects linked to disease states. Because of this, LAT1 levels should be considered in the context of other molecular factors such as the other significant markers that we identified in this study. This further highlights the need to use multiple markers to assess one's state of health because the relationship of many molecules with disease risk is often context dependent. Further research is needed to investigate the relationship between lifestyle factors, LAT1 expression, and the development of cancer or other chronic diseases.

Conclusion

This study provided a multi-omics signature of health. Ninety-six significant pathways ($p < 0.05$) were identified, with 23 of them being disease related. These findings offer valuable insights into the molecular mechanisms that link lifestyle behaviors with health and disease, emphasizing the complex interaction between biological pathways and lifestyle factors. This highlights potential targets for the development of interventions to treat or prevent chronic disease, as well as potential novel biomarkers to define one's state of health. In particular, the top pathways we identified in this study (listed in Table 1) could be used to guide potential interventions to reduce chronic disease risk through nutritional, pharmacological, or behavioral approaches. Because these pathways provide potential molecular mechanisms for lifestyle-related disease development, targeting these processes specifically may provide more effective approaches than what is currently used clinically.

Several pathways in the integrative analysis had not been previously identified through the analysis of the metabolomics or proteomics data alone [30, 31]. The pathways that were significant in both the integrative and metabolomics-only analyses were pyrimidine biosynthesis, histidine metabolism, beta-alanine metabolism, tyrosine metabolism, and butanoate metabolism. Bile acid synthesis from metabolomics and fatty acid degradation and elongation from integrative analysis demonstrated some overlap. When comparing integrative analysis with proteomics-only, lipoprotein processing was the only shared pathway. In the proteomics-only analysis, numerous proteins are involved in immune responses, which may be related to immune response pathways and disease pathways identified in the integrative analysis, such as central carbon metabolism in cancer, breast cancer, influenza A, necroptosis, and human T-cell leukemia virus 1 infection. These comparisons demonstrate the value of integrating multiple omics types together to generate novel results that may not be identified in single-omics analyses.

The limitations of the current study include a relatively small sample size (n = 52 per group), unidentified metabolomic and proteomic peaks, software limitations such as Pathview, which only accepts the input of genes and metabolites, and the fact that all the software focuses solely on the curation of endogenous pathways. As software tools become developed to handle additional measurement types and incorporate pathways of exogenous agents (which are detectable with metabolomics methods), these data can be further mined to give a more comprehensive view of how internal and external factors are influenced by lifestyle habits. For future studies, long-term follow-up with participants to assess whether they develop diseases predicted in this study could provide valuable insights. Future studies should also aim to increase the sample size and diversity of participants, expanding the scope to include individuals from across the United States or globally. Additionally, our study used participants which span a large age range which may have introduced additional variability into the omics data due to this heterogeneity of the population. Future studies with larger sample sizes should also stratify by different age ranges to determine if different age groups have shared or unique lifestyle-linked molecular profiles. This information would potentially uncover novel aspects of precision health approaches and also potentially determine optimal times in one's lifespan to implement healthy lifestyle modifications. Ultimately, this study aims to enhance clinical practice by providing healthcare

professionals with a more comprehensive understanding of health signatures, and incorporating the measurement of genes, proteins, and metabolites such as glutathione, glutamate, RAS, and CPT1, alongside the standard biomarkers typically assessed during a routine medical visit to evaluate a patient's health status.

Supplementary Information

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Additional file 1.

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Availability of data and materials
All data is available upon request.

Declarations

Competing interests
The authors declare no competing interests.

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References

1. Al-Amrani S, Al-Jabri Z, Al-Zaabi A, Alshekaili J, Al-Khabori M. Proteomics: concepts and applications in human medicine. World J Biol Chem. 2021;12(5):57–69.
2. Johnson CH, Ivanisevic J, Siuzdak G. Metabolomics: beyond biomarkers and towards mechanisms. Nat Rev Mol Cell Biol. 2016;17(7):451–9.
3. Epigenomics and Epigenetics Research | EGRP/DCCPS/NCI/NIH [Internet]. epi.grants.cancer.gov. Available from: https://epi.grants.cancer.gov/epigen/
4. Suhre K, Zaghlool S. Connecting the epigenome, metabolome and proteome for a deeper understanding of disease. J Intern Med. 2021;290(3):527–48.
5. Nyberg ST, Singh-Manoux A, Pentti J, Madsen IEH, Sabia S, Alfredsson L, et al. Association of healthy lifestyle with years lived without major chronic diseases. JAMA Intern Med. 2020;180(5):760.
6. Rassy N, Van Straaten A, Carette C, Hamer M, Rives-Lange C, Czernichow S. Association of healthy lifestyle factors and obesity-related diseases in adults in the UK. JAMA Intern Med. 2023;6(5):e2314741-1.
7. Li Y, Schoufour J, Wang DD, Dhana K, Pan A, Liu X, et al. Healthy lifestyle and life expectancy free of cancer, cardiovascular disease, and type 2 diabetes: prospective cohort study. BMJ. 2020;368(8228).
8. Li Y, Pan A, Wang DD, Liu X, Dhana K, Franco OH, et al. Impact of healthy lifestyle factors on life expectancies in the US population. Circulation. 2018;138(4):345–55.
9. Colpani V, Baena CP, Jaspers L, van Dijk GM, Farajzadegan Z, Dhana K, et al. Lifestyle factors, cardiovascular disease and all-cause mortality in middle-aged and elderly women: a systematic review and meta-analysis. Eur J Epidemiol. 2018;33(9):831–45.
10. Veronese N, Li Y, Manson JE, Willett WC, Fontana L, Hu FB. Combined associations of body weight and lifestyle factors with all cause and cause specific mortality in men and women: prospective cohort study. BMJ. 2016;24: i5855.
11. Carrard J, Guerini C, Appenzeller-Herzog C, Infanger D, Königstein K, Streese L, et al. The metabolic signature of cardiorespiratory fitness: a systematic review. Sports Med. 2021;52(3):527–46.
12. Castro A, Duft RG, Silva LM, Ferreira MLV, Andrade ALL, Bernardes CF, et al. Understanding the relationship between intrinsic cardiorespiratory fitness and serum and skeletal muscle metabolomics profile. J Proteome Res. 2021;20(5):2397.
13. Contrepois K, Wu S, Moneghetti KJ, Hornburg D, Ahadi S, Tsai MS, et al. Molecular choreography of acute exercise. Cell. 2020;181(5):1112-1130.e16.
14. Kistner S, Döring M, Krüger R, Rist M, Weinert CH, Bunzel D, et al. Sex-specific relationship between the cardiorespiratory fitness and plasma metabolite patterns in healthy humans—results of the KarMeN study. Metabolites. 2021;11(7):463–3.
15. Weiss A, Alack K, Klatt S, Zukunft S, Schermuly R, Frech T, et al. Sustained endurance training leads to metabolomic adaptation. Metabolites. 2022;12(7):658.
16. Noerman S, Landberg R. Blood metabolite profiles linking dietary patterns with health—toward precision nutrition. J Intern Med. 2022;293(4):408–32.
17. Lépine G, Fouillet H, Rémond D, Huneau JF, Mariotti F, Polakof S. A Scoping review: metabolomics signatures associated with animal and plant protein intake and their potential relation with cardiometabolic risk. Adv Nutr. 2021.
18. Rafiq T, Azab SM, Teo KK, Thabane L, Anand SS, Morrison KM, et al. Nutritional metabolomics and the classification of dietary biomarker candidates: a critical review. Adv Nutr. 2021;12(6):2333–57.
19. Andraos S, Beck KL, Jones MB, Han TL, Conlon CA, de Seymour JV. Characterizing patterns of dietary exposure using metabolomic profiles of human biospecimens: a systematic review. Nutr Rev. 2022.
20. Kim H, Rebholz CM. Metabolomic Biomarkers of healthy dietary patterns and cardiovascular outcomes. Curr Atheroscler Rep. 2021;23(6).
21. LeVatte M, Keshteli AH, Zarei P, Wishart DS. Applications of metabolomics to precision nutrition. Lifestyle Genom. 2021;15(1):1–9.
22. Pieter Giesbertz, Brandl B, Volkert D, Hauner H, Skurk T. Age-related metabolite profiles and their relation to clinical outcomes in young adults, middle-aged individuals, and older people. FASEB J. 2023;37(6).
23. Chashmniam S, Hashemi Madani N, Nobakht Mothlagh Ghoochani BF, Safari-Alighiarloo N, Khamseh ME. The metabolome profiling of obese and non-obese individuals: metabolically healthy obese and unhealthy non-obese paradox. Iran J Basic Med Sci. 2020;23(2):186–194. https://doi.org/10.22038/IJBMS.2019.37885.9004.
24. Tan HC, Hsu JW, Tai ES, Chacko S, Kovalik JP, Jahoor F. The impact of bariatric surgery on glutathione synthesis in individuals with severe obesity. Antioxidants (Basel). 2024;13(8):967. https://doi.org/10.3390/antiox13080967.
25. Cirulli ET, Guo L, Leon Swisher C, Shah N, Huang L, Napier LA, et al. Profound perturbation of the metabolome in obesity is associated with health risk. Cell Metab. 2019;29(2):488-500.e2.
26. Ho JE, Larson MG, Ghorbani A, Cheng S, Chen MH, Keyes M, et al. Metabolomic Profiles of Body Mass Index in the Framingham Heart Study Reveal Distinct Cardiometabolic Phenotypes. Meyre D, editor. PLOS ONE. 2016;11(2):e0148361.

27. Carayol M, Leitzmann MF, Ferrari P, Zamora-Ros R, Achaintre D, Stepien M, et al. Blood metabolic signatures of body mass index: a targeted metabolomics study in the EPIC Cohort. J Proteome Res. 2017;16(9):3137–46.
28. Bellot PENR, Moia MN, Reis BZ, Pedrosa LFC, Tasic L, Barbosa F, et al. Are phosphatidylcholine and lysophosphatidylcholine body levels potentially reliable biomarkers in obesity? A review of human studies. Mol Nutr Food Res 2023;67(7).
29. Pathmasiri W, Rushing BR, McRitchie S, Choudhari M, Du X, Smirnov A, et al. Untargeted metabolomics reveal signatures of a healthy lifestyle. Sci Rep. 2024;14(1).
30. Nieman DC, Sakaguchi CA, Matteo Pelleigrini, Thompson MJ, Sumner S, Zhang Q. Healthy lifestyle linked to innate immunity and lipoprotein metabolism: a cross-sectional comparison using untargeted proteomics. Sci Rep. 2023;13(1).
31. World Health Organization. Constitution of the World Health Organization [Internet]. World Health Organization. 2025. Available from: https://www.who.int/about/governance/constitution.
32. Mitchell C, Schneper LM, Notterman DA. DNA methylation, early life environment, and health outcomes. Pediatr Res. 2015;79(1–2):212–9.
33. Protti G, Rubbi L, Gören T, Sabirli R, Civlan S, Kurt Ö, et al. The methylome of buccal epithelial cells is influenced by age, sex, and physiological properties. Physiol Genomics. 2023;55(12):618–33.
34. Perez G, Barber GP, Benet-Pages A, Casper J, Clawson H, Diekhans M, et al. The UCSC genome browser database: 2025 update. Nucleic Acids Res. 2024;53(D1):974.
35. Protti G, Rubbi L, Gören T, Sabirli R, Civlan S, Kurt Ö, et al. The methylome of buccal epithelial cells is influenced by age, sex, and physiological properties. Physiol Genom. 2023;55(12):618–33.
36. Zhou G, Ewald J, Xia J. OmicsAnalyst: a comprehensive web-based platform for visual analytics of multi-omics data. Nucleic Acids Res. 2021;49(W1):W476–82.
37. Ewald JD, Zhou G, Lu Y, Kolic J, Ellis C, Johnson JD, et al. Web-based multi-omics integration using the Analyst software suite. Nat Protoc. 2024;19(5):1467–97.
38. Zhou G, Xia J. Using OmicsNet for Network Integration and 3D Visualization. Current Protoc Bioinform 2018;65(1).
39. Kanehisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res. 2000;28(1):27–30.
40. Luo W, Brouwer C. Pathview: an R/Bioconductor package for pathway-based data integration and visualization. Bioinformatics. 2013;29(14):1830–1.
41. Kanehisa M, Goto S, Furumichi M, Tanabe M, Hirakawa M. KEGG for representation and analysis of molecular networks involving diseases and drugs. Nucleic Acids Res. 2009;38(suppl_1):D355–60.
42. Marabita F, James T, Karhu A, Virtanen H, Kettunen K, Stenlund H, et al. Multiomics and digital monitoring during lifestyle changes reveal independent dimensions of human biology and health. Cell Systems. 2022;13(3):241–55.
43. Talley JT, Mohiuddin SS. Biochemistry, Fatty Acid Oxidation. PubMed. Treasure Island (FL): StatPearls Publishing; 2022.
44. Houten SM, Violante S, Ventura FV, Wanders RJA. The biochemistry and physiology of mitochondrial fatty acid β-oxidation and its genetic disorders. Annu Rev Physiol. 2016;78(1):23–44.
45. Abel ED. Free fatty acid oxidation in insulin resistance and obesity. Heart Metab: Manag Coron Patient. 2010;48:5–10.
46. Serra D, Mera P, Malandrino MI, Mir JF, Herrero L. Mitochondrial fatty acid oxidation in obesity. Antioxid Redox Signal. 2013;19(3):269–84.
47. Baruteau J, Sachs P, Broué P, Brivet M, Abdoul H, Vianey-Saban C, et al. Clinical and biological features at diagnosis in mitochondrial fatty acid beta-oxidation defects: a French pediatric study of 187 patients. J Inherit Metab Dis. 2013;36(5):795–803.
48. Schrauwen P, Wagenmakers AJ, van Marken Lichtenbelt WD, Saris WH, Westerterp KR. Increase in fat oxidation on a high-fat diet is accompanied by an increase in triglyceride-derived fatty acid oxidation. Diabetes. 2000;49(4):640–6.
49. DeLany JP, Windhauser MM, Champagne CM, Bray GA. Differential oxidation of individual dietary fatty acids in humans. Am J Clin Nutr. 2000;72(4):905–11.
50. Softic S, Meyer JG, Wang GX, Gupta MK, Batista TM, Lauritzen HPMM, et al. Dietary sugars alter hepatic fatty acid oxidation via transcriptional and post-translational modifications of mitochondrial proteins. Cell Metab. 2019;30(4):735-753.e4.
51. Bonnefont J. Carnitine palmitoyltransferases 1 and 2: biochemical, molecular and medical aspects. Mol Aspects Med. 2004;25(5–6):495–520.
52. Schreurs M, Kuipers F, van der Leij FR. Regulatory enzymes of mitochondrial β-oxidation as targets for treatment of the metabolic syndrome. Obes Rev. 2010;11(5):380–8.
53. McGarry JD, Brown NF. The mitochondrial carnitine palmitoyltransferase system - from concept to molecular analysis. Eur J Biochem. 1997;244(1):1–14.
54. Tunstall RJ, Mehan KA, Wadley GD, Collier GR, Bonen A, Hargreaves M, et al. Exercise training increases lipid metabolism gene expression in human skeletal muscle. Am J Physiol-Endocrinol Metab. 2002;283(1):E66-72.
55. Bruce CR, Hoy AJ, Turner N, Watt MJ, Allen TL, Carpenter K, et al. Overexpression of carnitine palmitoyltransferase-1 in skeletal muscle is sufficient to enhance fatty acid oxidation and improve high-fat diet-induced insulin resistance. Diabetes. 2008;58(3):550–8.
56. Henique C, Mansouri A, Fumey G, Lenoir V, Girard J, Bouillaud F, et al. Increased mitochondrial fatty acid oxidation is sufficient to protect skeletal muscle cells from palmitate-induced apoptosis. J Biol Chem. 2010;285(47):36818–27.
57. Sebastián D, Herrero L, Serra D, Asins G, Hegardt FG. CPT i overexpression protects L6E9 muscle cells from fatty acid-induced insulin resistance. Am J Physiol-Endocrinol Metab. 2007;292(3):E677–86.
58. Park EA, Mynatt RL, Cook GA, Kashfi K. Insulin regulates enzyme activity, malonyl-CoA sensitivity and mRNA abundance of hepatic carnitine palmitoyltransferase-I. Biochem J. 1995;310(3):853–8.
59. Bhathena SJ. Relationship between fatty acids and the endocrine and neuroendocrine system. Nutr Neurosci. 2006;9(1–2):1–10.
60. Litwack G. Human biochemistry. S.L.: Elsevier Academic Press; 2021. p. 441–74.
61. Trefts E, Williams AS, Wasserman DH. Exercise and the regulation of hepatic metabolism. Prog Mol Biol Transl Sci. 2015;135:203–25.
62. Goodwin ML. Blood glucose regulation during prolonged, submaximal, continuous exercise: a guide for clinicians. J Diabetes Sci Technol. 2010;4(3):694–705.
63. Ludwig DS, Ebbeling CB, Rimm EB. Carbohydrates, insulin secretion, and "precision nutrition." Diabetes Care. 2022;45(6):1303–5.
64. Schuppelius B, Schüler R, Pivovarova-Ramich O, Hornemann S, Busjahn A, Machann J, et al. Alterations in glucagon levels and the glucagon-to-insulin ratio in response to high dietary fat or protein intake in healthy lean adult twins: a post hoc analysis. Nutrients. 2024;16(22):3905.
65. Stern JH, Smith GI, Chen S, Unger RH, Klein S, Scherer PE. Obesity dysregulates fasting-induced changes in glucagon secretion. J Endocrinol. 2019;243(2):149–60.
66. Baudrand R, Vaidya A. Cortisol dysregulation in obesity-related metabolic disorders. Curr Opin Endocrinol Diabetes Obes. 2015;22(3):143–9.
67. Pearlmutter P, DeRose G, Samson C, Linehan N, Cen Y, Begdache L, et al. Sweat and saliva cortisol response to stress and nutrition factors. Sci Rep. 2020;10(1):19050.
68. Hackney AC, Walz EA. Hormonal adaptation and the stress of exercise training: the role of glucocorticoids. Trends Sport Sci. 2013;20(4):165–71.
69. Wang X, Yu H, Gao R, Liu M, Xie W. A comprehensive review of the family of very-long-chain fatty acid elongases: structure, function, and implications in physiology and pathology. Eur J Med Res. 2023 ;28(1).
70. Kyselová L, Vítová M, Řezanka T. Very long chain fatty acids. Prog Lipid Res. 2022;87:101180.
71. Zhi K, Yehia NA, Semnani-Azad Z, Mejia SB, Boucher BA, Malik V, et al. Lifestyle factors associated with circulating very long-chain saturated fatty acids in humans: a systematic review of observational studies. Adv Nutr. 2023;14(1):99–114.
72. Bogie JFJ, Haidar M, Kooij G, Hendriks JJA. Fatty acid metabolism in the progression and resolution of CNS disorders. Adv Drug Deliv Rev. 2020;159:198–213.
73. de la Garza Puentes A, Montes Goyanes R, Chisaguano Tonato AM, Torres-Espínola FJ, Arias García M, de Almeida L, et al. Association of maternal weight with FADS and ELOVL genetic variants and fatty acid levels- The PREOBE follow-up. Rosenfeld CS, editor. PLOS ONE. 2017;12(6):e0179135.

74. Macášek J, Zeman M, Žák A, Staňková B, Vecka M. Altered indices of fatty acid elongases ELOVL6, ELOVL5, and ELOVL2 activities in patients with impaired fasting glycemia. Metab Syndr Relat Disord. 2021;19(7):386–92.
75. Matsuzaka T, Kuba M, Koyasu S, Yamamoto Y, Motomura K, Arulmozhiraja S, et al. Hepatocyte ELOVL fatty acid elongase 6 determines ceramide acyl-chain length and hepatic insulin sensitivity in mice. Hepatology. 2020;71(5):1609–25.
76. Armel C, Ebenhöh O, Bakker BM, Adélaïde Raguin. Kinetic data for modeling the dynamics of the enzymes involved in animal fatty acid synthesis. Biosci Rep 2023 ;43(7).
77. Kessler SM, Simon Y, Gemperlein K, Gianmoena K, Cadenas C, Zimmer V, et al. Fatty acid elongation in non-alcoholic steatohepatitis and hepatocellular carcinoma. Int J Mol Sci. 2014;15(4):5762–73.
78. Sarigiannis D, Karakitsios S, Anesti O, Stem A, Valvi D, Sumner SC, et al. Advancing translational exposomics: bridging genome, exposome and personalized medicine. Hum Genomics. 2025;19:48.
79. Bruce CR, Hoy AJ, Turner N, Watt MJ, Allen TL, Carpenter K, et al. Overexpression of carnitine palmitoyltransferase-1 in skeletal muscle is sufficient to enhance fatty acid oxidation and improve high-fat diet-induced insulin resistance. Diabetes. 2008;58(3):550–8.
80. Schlaepfer IR, Joshi M. CPT1A-mediated fat oxidation, mechanisms, and therapeutic potential. Endocrinology. 2020;161(2):bqz046.
81. Barkley LB, Farrar MW, Knowles WS, Raffelson H. A synthesis of dl-cortisone acetate. J Am Chem Soc. 1953;75(16):4110–1.
82. Cederbaum AI. Alcohol metabolism. Clin Liver Dis. 2012;16(4):667–85.
83. You M, Arteel GE. Effect of ethanol on lipid metabolism. J Hepatol. 2019;70(2):237–48.
84. Jeon S, Carr R. Alcohol effects on hepatic lipid metabolism. J Lipid Res. 2020;61(4):470–9.
85. Czumaj A, Szrok-Jurga S, Hebanowska A, Turyn J, Swierczynski J, Sledzinski T, et al. The pathophysiological role of CoA. Int J Mol Sci. 2020;21(23):9057.
86. Jackowski S, Leonardi R. Deregulated coenzyme A, loss of metabolic flexibility and diabetes. Biochem Soc Trans. 2014;42(4):1118–22.
87. Xie J, Chen X, Zheng M, Zhu J, Mao H. The metabolism of coenzyme a and its derivatives plays a crucial role in diseases. Front Biosci. 2024;29(4):143–53.
88. Pizzorno J. Glutathione! Integr Med (Encinitas, Calif). 2014;13(1):8–12.
89. Marí M, Morales A, Colell A, García-Ruiz C, Fernández-Checa JC. Mitochondrial glutathione, a key survival antioxidant. Antioxid Redox Signal. 2009;11(11):2685–700.
90. Forman HJ, Zhang H, Rinna A. Glutathione: overview of its protective roles, measurement, and biosynthesis. Mol Aspects Med. 2009;30(1–2):1–12.
91. Bachhawat AK, Thakur A, Kaur J, Zulkifli M. Glutathione transporters. Biochim et Biophys Acta (BBA) Gen Subj. 2012;1830(5):3154–64.
92. Filomeni G, Aquilano K, Civitareale P, Rotilio G, Ciriolo MR. Activation of c-Jun-N-terminal kinase is required for apoptosis triggered by glutathione disulfide in neuroblastoma cells. Free Radical Biol Med. 2005;39(3):345–54.
93. Jozefczak M, Remans T, Vangronsveld J, Cuypers A. Glutathione is a key player in metal-induced oxidative stress defenses. Int J Mol Sci. 2012;13(3):3145–75.
94. Jones DP. [11] Redox potential of GSH/GSSG couple: assay and biological significance. Elsevier. 2002;93–112.
95. Ballatori N, Krance SM, Notenboom S, Shi S, Tieu K, Hammond CL. Glutathione dysregulation and the etiology and progression of human diseases. Biol Chem. 2009;390(3):191–214.
96. Julius M, Lang CA, Gleiberman L, Harburg E, Difranceisco W, Schork A. Glutathione and morbidity in a community-based sample of elderly. J Clin Epidemiol. 1994;47(9):1021–6.
97. Liu H, Wang H, Shenvi S, Hagen TM, Liu RM. Glutathione metabolism during aging and in Alzheimer disease. Ann N Y Acad Sci. 2004;1019:346–9.
98. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. J Nutr. 2004;134(3):489–92.
99. Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. Biomed Pharmacother. 2003;57(3–4):145–55.
100. Hauser RA, Lyons KE, McClain T, Carter S, Perlmutter D. Randomized, double-blind, pilot evaluation of intravenous glutathione in Parkinson's disease. Mov Disord. 2009;24(7):979–83.
101. Arosio E, De Marchi S, Zannoni M, Prior M, Lechi A. Effect of glutathione infusion on leg arterial circulation, cutaneous microcirculation, and pain-free walking distance in patients with peripheral obstructive arterial disease: a randomized, double-blind, placebo-controlled trial. Mayo Clin Proc. 2002;77(8):754–9.
102. Bishop C, Hudson VM, Hilton SC, Wilde C. A pilot study of the effect of inhaled buffered reduced glutathione on the clinical status of patients with cystic fibrosis. Chest. 2005;127(1):308–17.
103. Stav D, Raz M. Effect of N-acetylcysteine on air trapping in COPD. Chest. 2009;136(2):381–6.
104. Cooke RWI, Drury JA. Reduction of oxidative stress marker in lung fluid of preterm infants after administration of intra-tracheal liposomal glutathione. Neonatology. 2005;87(3):178–80.
105. Saitoh T, Satoh H, Nobuhara M, Machii M, Tanaka T, Ohtani H, et al. Intravenous glutathione prevents renal oxidative stress after coronary angiography more effectively than oral N-acetylcysteine. Heart Vessels. 2011;26(5):465–72.
106. Medved I, Brown MJ, Bjorksten AR, Murphy KT, Petersen AC, Sostaric S, et al. N-acetylcysteine enhances muscle cysteine and glutathione availability and attenuates fatigue during prolonged exercise in endurance-trained individuals. J Appl Physiol. 2004;97(4):1477–85.
107. Lu SC. Regulation of glutathione synthesis. Mol Aspects Med. 2009;30(1–2):42–59.
108. Griffith OW. Biologic and pharmacologic regulation of mammalian glutathione synthesis. Free Radical Biol Med. 1999;27(9–10):922–35.
109. Apel K, Hirt H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol. 2004;55:373–99.
110. Jiang S, Liu H, Li C. Dietary regulation of oxidative stress in chronic metabolic diseases. Foods. 2021;10(8):1854.
111. Boden G, Homko C, Barrero CA, Stein TP, Chen X, Cheung P, et al. Excessive caloric intake acutely causes oxidative stress, GLUT4 carbonylation, and insulin resistance in healthy men. Sci Transl Med. 2015;7(304):304re7.
112. Ignatowicz E, Woźniak A, Kulza M, Seńczuk-Przybyłowska M, Cimino F, Piekoszewski W, et al. Exposure to alcohol and tobacco smoke causes oxidative stress in rats. Pharmacol Rep. 2013;65(4):906–13.
113. Sba G, Choi S, Krishnan J, Ruckmani K. Cigarette smoke and related risk factors in neurological disorders: an update. Biomed Pharmacother. 2017;85:79–86.
114. Paquot N. The metabolism of alcohol. Rev Med Liege. 2019;74(5–6):265–7.
115. Institute of Medicine (US) Forum on neuroscience and nervous system disorders. glutamate-related biomarkers in drug development for disorders of the nervous system: workshop summary. PubMed. Washington (DC): National Academies Press (US); 2011.
116. Gécz J. Glutamate receptors and learning and memory. Nat Genet. 2010;42(11):925–6.
117. Brosnan JT, Brosnan ME. Glutamate: a truly functional amino acid. Amino Acids. 2012;45(3):413–8.
118. Zhang D, Hua Z, Li Z. The role of glutamate and glutamine metabolism and related transporters in nerve cells. CNS Neurosci Ther. 2024;30(2):e14617.
119. Cortese BM, Phan KL. The role of glutamate in anxiety and related disorders. CNS Spectr. 2005;10(10):820–30.
120. Swanson CJ, Bures M, Johnson MP, Linden AM, Monn JA, Schoepp DD. Metabotropic glutamate receptors as novel targets for anxiety and stress disorders. Nat Rev Drug Discov. 2005;4(2):131–44.
121. Egbenya DL, Aidoo E, Kyei G. Glutamate receptors in brain development. Child's Nerv Syst. 2021;37(9):2753–8.
122. Stepulak A, Rola R, Polberg K, Ikonomidou C. Glutamate and its receptors in cancer. J Neural Transm. 2014;121(8):933–44.
123. Takano T, Lin JHC, Arcuino G, Gao Q, Yang J, Nedergaard M. Glutamate release promotes growth of malignant gliomas. Nat Med. 2001;7(9):1010–5.
124. Rothstein JD, Brem H. Excitotoxic destruction facilitates brain tumor growth. Nat Med. 2001;7(9):994–5.
125. Stańska K, Krzeski A. The Umami taste: from discovery to clinical use. Otolaryngol Polska. 2016;70(4):10–5.

126. Conn H. "UMAMI": the fifth basic taste. Nutr Food Sci. 1992;92(2):21–3.
127. Tennant DR. Review of glutamate intake from both food additive and non-additive sources in the European Union. Ann Nutr Metab. 2018;73(Suppl. 5):21–8.
128. Composition of foods raw, processed, prepared USDA National Nutrient Database for standard reference, release 26 documentation and user guide. 2013.
129. Henriksson J. Effect of exercise on amino acid concentrations in skeletal muscle and plasma. J Exp Biol. 1991;1(160):149–65.
130. Divino Filho J, Hazel S, Furst P, Bergstrom J, Hall K. Glutamate concentration in plasma, erythrocyte and muscle in relation to plasma levels of insulin-like growth factor (IGF)-I, IGF binding protein-1 and insulin in patients on haemodialysis. J Endocrinol. 1998;156(3):519–27.
131. Watkins S, Koob GF, Athina S. Neural mechanisms underlying nicotine addiction: acute positive reinforcement and withdrawal. Nicotine Tob Res. 2000;2(1):19–37.
132. McGehee DS, Heath MJS, Gelber S, Devay P, Role LW. Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors. Science. 1995;269(5231):1692–6.
133. Kalia LV, Lang AE. Parkinson's disease. Lancet. 2015;386(9996):896–912.
134. Noyce AJ, Bestwick JP, Silveira-Moriyama L, Hawkes CH, Giovannoni G, Lees AJ, et al. Meta-analysis of early nonmotor features and risk factors for Parkinson disease. Ann Neurol. 2012;72(6):893–901.
135. Quik M. Smoking, nicotine and Parkinson's disease. Trends Neurosci. 2004;27(9):561–8.
136. Fukushima W, Miyake Y, Tanaka K, Sasaki S, Kiyohara C, Tsuboi Y, et al. Alcohol drinking and risk of Parkinson's disease: a case-control study in Japan. BMC Neurol. 2010;10(1):111.
137. Ritz B, Lee PC, Lassen CF, Arah OA. Parkinson disease and smoking revisited: ease of quitting is an early sign of the disease. Neurology. 2014;83(16):1396–402.
138. Seppi K, Weintraub D, Coelho M, Perez-Lloret S, Fox SH, Katzenschlager R, et al. The movement disorder society evidence-based medicine review update: treatments for the non-motor symptoms of Parkinson's disease. Mov Disord. 2011;26(S3):S42-80.
139. Connolly BS, Lang AE. Pharmacological treatment of Parkinson disease. JAMA. 2014;311(16):1670.
140. Barone P, Poewe W, Albrecht S, Debieuvre C, Massey D, Rascol O, et al. Pramipexole for the treatment of depressive symptoms in patients with Parkinson's disease: a randomised, double-blind, placebo-controlled trial. Lancet Neurol. 2010;9(6):573–80.
141. Klein MO, Battagello DS, Cardoso AR, Hauser DN, Bittencourt JC, Correa RG. Dopamine: functions, signaling, and association with neurological diseases. Cell Mol Neurobiol. 2019;39(1):31–59.
142. Itagaki C, Isobe T, Taoka M, Natsume T, Nomura N, Horigome T, et al. Stimulus-coupled interaction of tyrosine hydroxylase with 14–3–3 proteins. Biochemistry. 1999;38(47):15673–80.
143. Di Liegro CM, Schiera G, Proia P, Di Liegro I. Physical activity and brain health. Genes. 2019;10(9):720.
144. Chiara GD. Alcohol and dopamine. Alcohol Health Res World. 2024;21(2):108.
145. Benowitz NL. Pharmacology of nicotine: addiction, smoking-induced disease, and therapeutics. Annu Rev Pharmacol Toxicol. 2010;49(1):57–71.
146. Misu Y. Is DOPA a neurotransmitter? Trends Pharmacol Sci. 2002;23(6):262–8.
147. Hase A, Jung SE, aan het Rot M. Behavioral and cognitive effects of tyrosine intake in healthy human adults. Pharmacol Biochem Behav. 2015;133:1–6.
148. Rees JN, Florang VR, Eckert LL, Doorn JA. Protein reactivity of 3,4-Dihydroxyphenylacetaldehyde, a toxic dopamine metabolite, is dependent on both the aldehyde and the catechol. Chem Res Toxicol. 2009;22(7):1256–63.
149. Anderson DG, Florang VR, Schamp JH, Buettner GR, Doorn JA. Antioxidant-mediated modulation of protein reactivity for 3,4-dihydroxyphenylacetaldehyde, a toxic dopamine metabolite. Chem Res Toxicol. 2016;29(7):1098–107.
150. Masato A, Plotegher N, Terrin F, Sandre M, Faustini G, Thor A, et al. DOPAL initiates αSynuclein-dependent impaired proteostasis and degeneration of neuronal projections in Parkinson's disease. NPJ Parkinsons Dis. 2023. https://doi.org/10.1038/s41531-023-00485-1.
151. National Cancer Institute. What Is Cancer? [Internet]. National Cancer Institute. National Institutes of Health; 2021. Available from: https://www.cancer.gov/about-cancer/understanding/what-is-cancer.
152. CDC. Cancer Risk Factors [Internet]. Cancer. 2024. Available from: https://www.cdc.gov/cancer/risk-factors/index.html.
153. World Health Organization. Cancer [Internet]. World Health Organization; 2025. Available from: https://www.who.int/news-room/fact-sheets/detail/cancer.
154. Lewandowska A, Rudzki M, Rudzki S, Lewandowski T, Laskowska B. Environmental risk factors for cancer – review paper. Ann Agric Environ Med. 2019;26(1):1–7.
155. Rehm J, Shield KD, Weiderpass E. Alcohol consumption. A leading risk factor for cancer. Chem Biol Interact. 2020;331: 109280.
156. Ubago-Guisado E, Rodríguez-Barranco M, Ching-López A, Petrova D, Molina-Montes E, Amiano P, et al. Evidence update on the relationship between diet and the most common cancers from the European prospective investigation into cancer and nutrition (EPIC) study: a systematic review. Nutrients. 2021;13(10):3582.
157. Anand P, Kunnumakara AB. Cancer is a preventable disease that requires major lifestyle changes. Pharm Res. 2008;25(9):2097–116.
158. Stein CJ, Colditz GA. Modifiable risk factors for cancer. Br J Cancer. 2004;90(2):299–303.
159. Gonzalez CA, Riboli E. Diet and cancer prevention: contributions from the European prospective investigation into cancer and nutrition (EPIC) study. Eur J Cancer. 2010;46(14):2555–62.
160. Clinton SK, Giovannucci EL, Hursting SD. The world cancer research fund/American institute for cancer research third expert report on diet, nutrition, physical activity, and cancer: impact and future directions. J Nutr. 2019;150(4):663–71.
161. Warburg O. On the origin of cancer cells. Science. 1956;123(3191):309–14.
162. Wong TL, Che N, Ma S. Reprogramming of central carbon metabolism in cancer stem cells. Biochim Biophys Acta. 2017;1863(7):1728–38.
163. Vigneri P, Frasca F, Sciacca L, Pandini G, Vigneri R. Diabetes and cancer. Endocr Relat Cancer. 2009;16(4):1103–23.
164. Johnson JA, Carstensen B, Witte D, Bowker SL, Lipscombe L, Renehan AG. Diabetes and cancer (1): evaluating the temporal relationship between type 2 diabetes and cancer incidence. Diabetologia. 2012;55(6):1607–18.
165. Suh S, Kim KW. Diabetes and cancer: is diabetes causally related to cancer? Diabetes Metab J. 2011;35(3):193.
166. Duan W, Shen X, Lei J, Xu Q, Yu Y, Li R, et al. Hyperglycemia, a neglected factor during cancer progression. Biomed Res Int. 2014;2014:1–10.
167. Ryu TY, Park J, Scherer PE. Hyperglycemia as a risk factor for cancer progression. Diabetes Metab J. 2014;38(5):330.
168. Chan DA, Sutphin PD, Nguyen P, Turcotte S, Lai EW, Banh A, et al. Targeting GLUT1 and the warburg effect in renal cell carcinoma by chemical synthetic lethality. Sci Transl Med. 2011;3(94):94ra70.
169. Wong TL, Che N, Ma S. Reprogramming of central carbon metabolism in cancer stem cells. Biochim et Biophys Acta (BBA). 2017;1863(7):1728–38.
170. Pérez-Tomás R, Pérez-Guillén I. Lactate in the tumor microenvironment: an essential molecule in cancer progression and treatment. Cancers. 2020;12(11):3244.
171. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the warburg effect: the metabolic requirements of cell proliferation. Science. 2009;324(5930):1029–33.
172. Faubert B, Li KY, Cai L, Hensley CT, Kim J, Zacharias LG, et al. Lactate metabolism in human lung tumors. Cell. 2017;171(2):358-371.e9.
173. Dhup S, Kumar Dadhich R, Ettore Porporato P, Sonveaux P. Multiple biological activities of lactic acid in cancer: influences on tumor growth, angiogenesis and metastasis. Curr Pharm Des. 2012;18(10):1319–30.
174. San-Millán I, Brooks GA. Reexamining cancer metabolism: lactate production for carcinogenesis could be the purpose and explanation of the Warburg Effect. Carcinogenesis. 2016;38(2):119–33.
175. Walenta S, Wetterling M, Lehrke M, Schwickert G, Sundfør K, Rofstad EK, et al. High lactate levels predict likelihood of metastases, tumor recurrence, and restricted patient survival in human cervical cancers. Can Res. 2000;60(4):916–21.

176. Pennington Z, Goodwin ML, Westbroek EM, Cottrill E, Ahmed AK, Sciubba DM. Lactate and cancer: spinal metastases and potential therapeutic targets (part 2). Ann Transl Med. 2019;7(10):221–31.
177. Doherty JR, Cleveland JL. Targeting lactate metabolism for cancer therapeutics. J Clin Investig. 2013;123(9):3685–92.
178. Prior IA, Lewis PD, Mattos C. A comprehensive survey of Ras mutations in cancer. Can Res. 2012;72(10):2457–67.
179. Stacey DW. Cyclin D1 serves as a cell cycle regulatory switch in actively proliferating cells. Curr Opin Cell Biol. 2003;15(2):158–63.
180. Boonstra J, Rijken P, Humbel B, Cremers F, Verkleij A, van Bergen en Henegouwen P. The epidermal growth factor. Cell Biol Int. 1995;19(5):413–30.
181. Cary LA, Han DC, Guan JL. Integrin-mediated signal transduction pathways. Histol Histopathol. 1999;14(3):1001–9.
182. Kranenburg O, Gebbink MFBG, Voest EE. Stimulation of angiogenesis by Ras proteins. Biochem Biophys Acta. 2004;1654(1):23–37.
183. Bahar ME, Kim HJ, Kim DR. Targeting the RAS/RAF/MAPK pathway for cancer therapy: from mechanism to clinical studies. Signal Transduct Target Ther. 2023;8(1):455.
184. Zhang W, Liu HT. MAPK signal pathways in the regulation of cell proliferation in mammalian cells. Cell Res. 2002;12(1):9–18.
185. Downward J. Targeting RAS signalling pathways in cancer therapy. Nat Rev Cancer. 2003;3(1):11–22.
186. Leicht DT, Balan V, Kaplun A, Singh-Gupta V, Kaplun L, Dobson M, et al. Raf kinases: function, regulation and role in human cancer. Biochim et Biophys Acta (BBA) Mol Cell Res. 2007;1773(8):1196–212.
187. Simanshu DK, Nissley DV, McCormick F. RAS proteins and their regulators in human disease. Cell. 2017;170(1):17–33.
188. Prior IA, Hood FE, Hartley JL. The frequency of ras mutations in cancer. Can Res. 2020;80(14):2969–74.
189. Spiegel J, Cromm PM, Zimmermann G, Grossmann TN, Waldmann H. Small-molecule modulation of Ras signaling. Nat Chem Biol. 2014;10(8):613–22.
190. Abdallah Sayyed-Ahmad, Gorfe AA. How to make an undruggable enzyme druggable: lessons from ras proteins. In: Advances in protein chemistry and structural biology; 2020.
191. Singh N, Ecker G. Insights into the structure, function, and ligand discovery of the large neutral amino acid transporter 1, LAT1. Int J Mol Sci. 2018;19(5): 1278.
192. Huttunen J, Gynther M, Vellonen KS, Huttunen KM. L-type amino acid transporter 1 (LAT1)-utilizing prodrugs are carrier-selective despite having low affinity for organic anion transporting polypeptides (OATPs). Int J Pharm. 2019;571: 118714.
193. Ikotun OF, Marquez BV, Huang C, Masuko K, Daiji M, Masuko T, et al. Imaging the l-type amino acid transporter-1 (LAT1) with Zr-89 immunopet. PLoS ONE. 2013;8(10): e77476.
194. Scalise M, Galluccio M, Console L, Pochini L, Indiveri C. The human SLC7A5 (LAT1): the intriguing histidine/large neutral amino acid transporter and its relevance to human health. Front Chem. 2018;22:6.
195. Barar J, Rafi MA, Pourseif MM, Omidi Y. Blood-brain barrier transport machineries and targeted therapy of brain diseases. Bioimpacts. 2016;6(4):225–48.
196. Yan R, Li Y, Müller J, Zhang Y, Singer S, Xia L, et al. Mechanism of substrate transport and inhibition of the human LAT1-4F2hc amino acid transporter. Cell Discov. 2021;7: 16.
197. Janpipatkul K, Suksen K, Borwornpinyo S, Jearawiriyapaisarn N, Hongeng S, Piyachaturawat P, et al. Downregulation of LAT1 expression suppresses cholangiocarcinoma cell invasion and migration. Cell Signal. 2014;26(8):1668–79.
198. Zhao Y, Wang L, Pan J. The role of L-type amino acid transporter 1 in human tumors. Intractable Rare Dis Res. 2015;4(4):165–9.
199. Kanai Y. Amino acid transporter LAT1 (SLC7A5) as a molecular target for cancer diagnosis and therapeutics. Pharmacol Ther. 2021;230:107964.
200. Yanagida O, Kanai Y, Chairoungdua A, Kim DK, Segawa H, Nii T, et al. Human L-type amino acid transporter 1 (LAT1): characterization of function and expression in tumor cell lines. Biochim et Biophys Acta (BBA) Biomembr. 2001;1514(2):291–302.
201. Zhang C, Xu J, Xue S, Ye J. Prognostic value of L-type amino acid transporter 1 (LAT1) in various cancers: a meta-analysis. Mol Diagn Ther. 2020;24(5):523–36.
202. Zhang J, Xu Y, Li D, Fu L, Zhang X, Bao Y, et al. Review of the correlation of lat1 with diseases: mechanism and treatment. Front Chem. 2020;20:8.
203. Häfliger P, Charles RP. The L-type amino acid transporter LAT1—an emerging target in cancer. Int J Mol Sci. 2019;20(10):2428.
204. Cormerais Y, Giuliano S, Lefloch R, Front B, Durivault J, Tambutté E, et al. Genetic disruption of the multifunctional CD98/LAT1 complex demonstrates the key role of essential amino acid transport in the control of mTORC1 and tumor growth. Cancer Res. 2016;76(15):4481–92.
205. Drummond MJ, Glynn EL, Fry CS, Timmerman KL, Volpi E, Rasmussen BB. An increase in essential amino acid availability upregulates amino acid transporter expression in human skeletal muscle. Am J Physiol-Endocrinol Metabol. 2010;298(5):E1011–8.
206. Drummond MJ, Fry CS, Glynn EL, Timmerman KL, Dickinson JM, Walker DK, et al. Skeletal muscle amino acid transporter expression is increased in young and older adults following resistance exercise. J Appl Physiol. 2011;111(1):135–42.
207. Moore DR, Tang JE, Burd NA, Rerecich T, Tarnopolsky MA, Phillips SM. Differential stimulation of myofibrillar and sarcoplasmic protein synthesis with protein ingestion at rest and after resistance exercise. J Physiol. 2009;587(4):897–904.
208. Roberson PA, Mobley CB, Romero MA, Haun CT, Osburn SC, Mumford PW, et al. LAT1 protein content increases following 12 weeks of resistance exercise training in human skeletal muscle. Front Nutr. 2021;14:7.

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