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Metabolic profiling revealed alterations associated with sedentary work in bus drivers

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Sedentary behavior in the workplace has emerged as a critical public health concern, with bus drivers representing a high-risk group due to prolonged compulsory sitting. This study aimed to investigate metabolic alterations associated with sedentary work in professional bus drivers compared to active controls. A total of 60 bus drivers and 60 sanitation workers (matched for age, sex, and lifestyle) as controls were enrolled. Fasting serum samples of the subjects were analyzed using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) for metabolomic profiling. Demographic data and biochemical parameters were also collected. Metabolomic analysis identified 322 metabolites, with 57 differential metabolites (52 up-regulated and 5 down-regulated) in the bus drivers relative to the controls, based on criteria of variable important for the projection value (VIP) ≥ 1, fold change ≥ 1.2 or ≤ 0.83, and $P < 0.05$. Pathway enrichment analysis revealed significant perturbations in metabolic pathways, including valine, leucine and isoleucine biosynthesis; 2-oxocarboxylic acid metabolism; and biosynthesis of amino acids. Additionally, biochemical analysis showed higher triglyceride levels in the bus drivers ($P = 0.037$). These findings highlight distinct metabolic signatures associated with prolonged sedentary work in bus drivers, providing insights into potential mechanisms linking occupational sedentary behavior to adverse health outcomes.

Keywords Sedentary, Bus driver, Metabolomics, BCAA, Metabolic pathway

Sedentary behavior is defined as any waking activity involving seated, reclined, or lying postures with low energy expenditure (≤ 1.5 metabolic equivalent units)[1]. Over the past two decades, it has emerged as a critical public health concern. Observational studies link high sedentary levels to adverse health outcomes — including poor metabolic health, musculoskeletal issues, early mortality, and increased colorectal cancer risk[2–6] — while prospective cohort studies and meta-analyses confirm that prolonged sitting independently contributes to cardiometabolic risks and all-cause mortality, regardless of leisure-time physical activity[7–11]. Sedentary behavior is also highly prevalent: Americans spend 7.7 h daily (55% of waking time) sedentary[12], 40% of Europeans' leisure time is spent watching TV[13], and 27.5% of adults and 81% of adolescents globally fail to meet aerobic exercise recommendations[14].

Sedentary time occurs in domestic, transportation, educational, and workplace settings, with work often accounting for the largest share of daily sedentary behavior among sedentary workers[15]. US full-time sedentary workers spend ~ 11 h daily in sedentary behavior (vs. 7–8 h sleeping)[16], and full-time employees typically sit for half to two-thirds of working hours[17,18].

While desk-based office workers are widely studied in sedentary behavior research, another group — bus drivers, who face compulsory sedentary work — has received insufficient attention. A cross-sectional study of East Midlands bus drivers found they spent < 3% of time stepping and accumulated high sitting time[19]. Bus drivers also face elevated health risks: 38.7% of Ghanaian bus drivers have unrecognized hypertension and 19% are obese[20]; 39.7% of Lagos long-distance bus drivers are hypertensive and 13.9% are diabetic[21]. They are at high risk of cardiovascular disease (CVD): London bus drivers had higher coronary heart disease risk than active conductors as early as 1953[22], and Stockholm bus drivers showed increased myocardial infarction incidence[23],

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with multiple studies confirming higher CVD morbidity and mortality among professional drivers[24–27]. Prolonged sitting is a key contributor here, given its established link to CVD, all-cause mortality, and type 2 diabetes — independent of physical activity[7,28] — with > 6–8 h of daily sitting associated with higher all-cause and CVD mortality[29].

Hypotheses suggest prolonged sitting impairs arterial health via hemodynamic, inflammatory, and metabolic changes, driving CVD[30]. It is also linked to metabolic syndrome (a cluster of glucose intolerance, insulin resistance, central obesity, dyslipidemia, and hypertension), which doubles cardiovascular risk[30,31]. Experimental studies show uninterrupted sitting disrupts insulin and glucose levels[30], and a meta-analysis found intermediate (4.11 h/day) and high (7.26 h/day) sedentary time increase metabolic syndrome risk[32]. However, the biological mechanisms connecting sedentary behavior to systemic metabolic changes remain poorly understood.

The critical challenge addressed in this study is: despite evidence that bus drivers' compulsory sedentary work elevates CVD and metabolic risks, the underlying metabolic changes and molecular pathways linking their sedentary behavior to adverse health outcomes are unknown. This gap limits our ability to develop targeted interventions for this high-risk occupational group.

To fill this gap, we analyzed global metabolome profiles of professional bus drivers and matched controls. Metabolomics quantifies low-molecular-weight metabolites (end products of biological and physiological processes[33], enabling identification of metabolic changes/pathways and clarification of mechanisms behind sedentary behavior's health effects. To our knowledge, this is the first study exploring the association between sedentary behavior and metabolomic changes in occupational groups. This work is important because it will uncover novel metabolic targets for mitigating health risks in bus drivers and other compulsory sedentary workers, addressing a long-overlooked public health need in occupational health.

Results

General characteristics and biochemical indexes

As shown in Table 1, the general characteristics of the bus drivers, including age, sex, race, body mass index, marital status, smoking, drinking and physical exercise habits, were comparable to those of the controls, except that the bus drivers had relatively higher educational levels ($P = 1.14 \times 10^{-5}$). The average work years of the bus drivers were 15.5, higher than the controls ($P = 9.48 \times 10^{-4}$), while the work hours per week were similar between the two groups. The examined, metabolism-related biochemical indexes were all comparable between the two groups except triglyceride concentration ($P = 0.037$).

Overview of the metabolomic profile

In total, 322 metabolites were identified in the serum samples of the study subjects (Supplementary Table S1 online). A typical chromatogram of the samples is provided in Supplementary Fig. S1 online. The extracted ion chromatogram (XIC) of the sample can visually show the detection of metabolites in the sample. Supplementary Fig. S2 online shows the overall distribution of the samples including QC samples. The QC samples clustered closely, indicating that the detection instrument was stable and the generated data were reproducible. We

Variables	Bus drivers ($n = 60$)	Controls ($n = 60$)	P
Age (years, median (MAD))	53.0 (4.4)	54.5 (5.2)	0.121 [a]
Sex (male, %)	100.0	100.0	---
Race (Han, %)	100.0	100.0	---
BMI (kg/m^2, median (MAD))	24.80 (3.37)	24.06 (3.42)	0.128 [a]
Education (%)			1.14×10^{-5} [b]
elementary school or uneducated	0.0	25.0	
high school	96.7	75.0	
college or postgraduate	3.3	0.0	
Marital status (married, %)	96.7	100.0	0.496 [b]
Smoker (yes, %)	0.0	0.0	---
Drinker (yes, %)	0.0	0.0	---
General physical exercise times per week (≥ 1, %)	65.0	50.0	0.140 [c]
Work year (year, median (MAD))	15.5 (6.6)	10.0 (11.1)	9.48×10^{-4} [a]
Work hours per week (> 48, %)	55.0	63.3	0.458 [c]
Serum total protein (g/L, median (MAD))	74.0 (4.4)	74.0 (3.0)	0.350 [a]
Blood glucose (mmol/L, median (MAD))	5.50 (0.52)	5.40 (0.67)	0.813 [a]
Triglyceride (mmol/L, median (MAD))	1.40 (0.59)	1.15 (0.67)	0.037 [a]
Total cholesterol (mmol/L, mean (SD))	4.82 (0.80)	4.83 (0.76)	0.935 [d]
High density lipoprotein cholesterol (mmol/L, median (MAD))	1.27 (0.21)	1.36 (0.38)	0.130 [a]
Low density lipoprotein cholesterol (mmol/L, mean (SD))	3.08 (0.57)	3.01 (0.60)	0.538 [d]

Table 1. General characteristics of the study subjects and their biochemical indexes. MAD, median absolute deviation; BMI, body mass index; SD, standard deviation. [a] Mann-Whitney test, [b] Fisher's exact test, [c] χ^2 test, [d] Student's *t*-test.

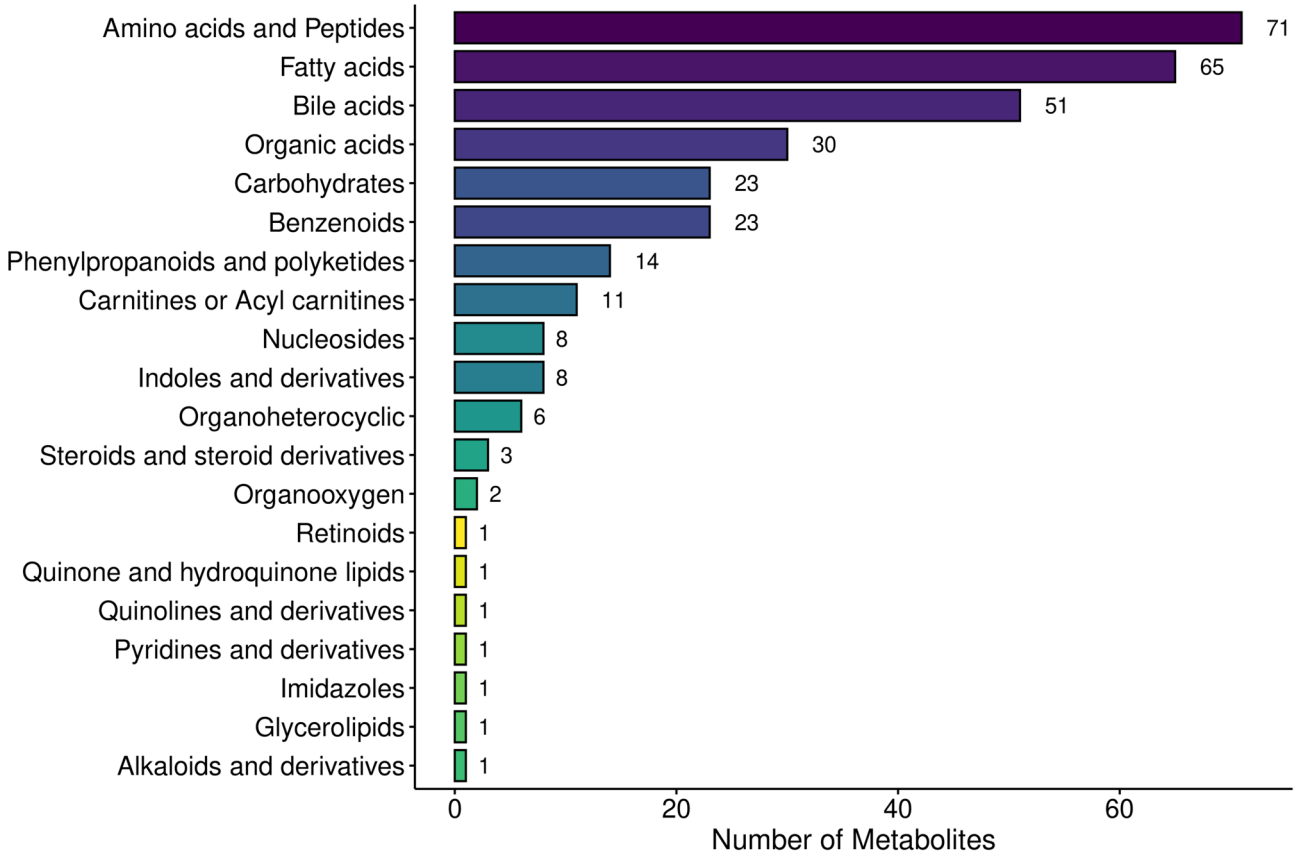

Fig. 1. Classification of the identified metabolites according to their final class.

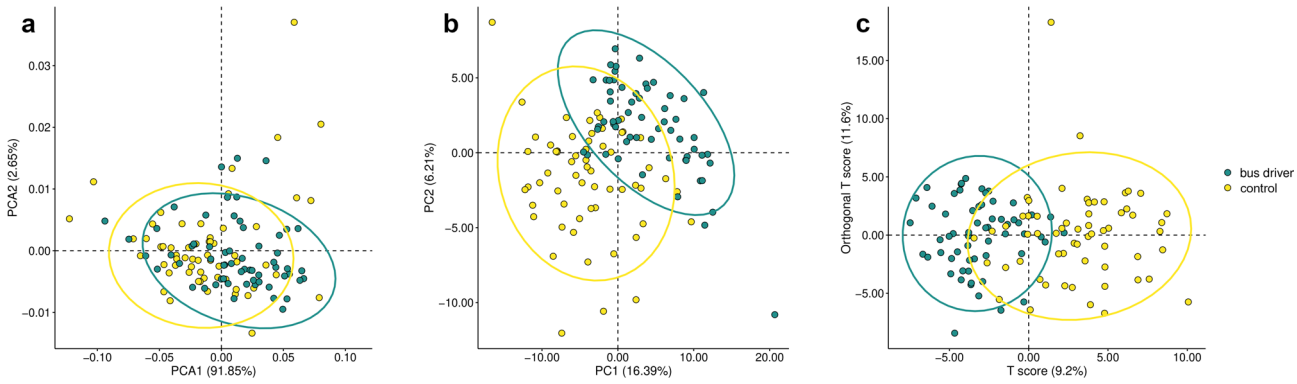

Fig. 2. Score plots on the identified metabolites of the bus drivers and controls. (**a**) principal component analysis (PCA) model, (**b**) partial least squares discriminant analysis (PLS-DA) model, (**c**) orthogonal partial least squares discriminant analysis (OPLS-DA) model.

counted and displayed the number of identified metabolites according to their final class (or sub class if the metabolite was lipid) in Fig. 1. The top three classes with the highest quantity of metabolites were amino acids and peptides, fatty acids, and bile acids.

Differential screening of metabolites

Figure 2 presents the PCA, PLS-DA, and OPLS-DA results for the subjects' metabolites, demonstrating that the metabolic patterns differ significantly between bus drivers and controls, and the subjects can be roughly divided into two groups. $R^2Y(cum)$ and $Q^2(cum)$ for the OPLS-DA were 0.59 and 0.40, respectively, indicating that the model has acceptable interpretability and predictability. The permutation test performed on the OPLS-DA (Intercepts: $R^2 = (0.0, 0.33)$, $Q^2 = (0.0, -0.33)$) indicated that the model was well fitted. Based on the following screening criteria: (a) VIP ≥ 1, (b) Fold Change ≥ 1.2 or ≤ 0.83, (c) $P < 0.05$, we identified 52 up-regulated and 5

down-regulated metabolites in the bus drivers compared with the controls (Fig. 3). Detailed information of the differential metabolites can be found in Supplementary Table S1 online.

Expression pattern and biological function analysis

The expression pattern of the 57 differential metabolites was displayed in Supplementary Fig. S3 online. The heat map shows that most of the differential metabolites in the left half part where the bus drivers mainly clustered were up-regulated (in red), while most of the differential metabolites in the right half part where the controls mainly clustered were down-regulated (in blue) (Supplementary Fig. S3 online). Most of the 52 up-regulated metabolites were significantly correlated as showed in Fig. 4, especially the metabolites from 5-aminopentanoic acid (HMDB0003355) to alpha-ketoisovaleric acid (HMDB0000019), which formed a red square in the center of Fig. 4. The 5 down-regulated metabolites from 4-Hydroxy-3-methylbenzoic acid (HMDB0004815) to trans-Piceid (HMDB0030564) in Fig. 4 were negatively related to most of the up-regulated metabolites.

Metabolic pathway enrichment analysis of the differential metabolites, based on the KEGG database, revealed significantly altered metabolic pathways, thereby aiding in the interpretation of biological phenotypes. The top 10 enriched metabolic pathways with the smallest *P* value are shown in Fig. 5. The valine, leucine and isoleucine biosynthesis pathway had the smallest *P* value and the largest rich factor, while 2-oxocarboxylic acid metabolism and biosynthesis of amino acids pathways enriched the largest number of differential metabolites.

Discussion

The present study investigated how prolonged sedentary work affects systemic metabolism by comparing professional bus drivers to physically active controls. Our targeted metabolomic analysis identified 57 serum metabolites (52 up-regulated, 5 down-regulated) that differed significantly (VIP ≥ 1, fold change ≥ 1.2 or ≤ 0.83, $P < 0.05$) between sedentary drivers and active controls. Notably, pathway enrichment analysis implicated core amino acid metabolic routes, especially the valine, leucine and isoleucine (branched-chain amino acid, BCAA) biosynthesis pathway, general biosynthesis of amino acids, and 2-oxocarboxylic acid metabolism. Biochemically, the bus drivers also had significantly higher fasting triglycerides than controls ($P = 0.037$). Together, these results suggest that chronic occupational sitting is associated with a distinct metabolic profile involving increased amino

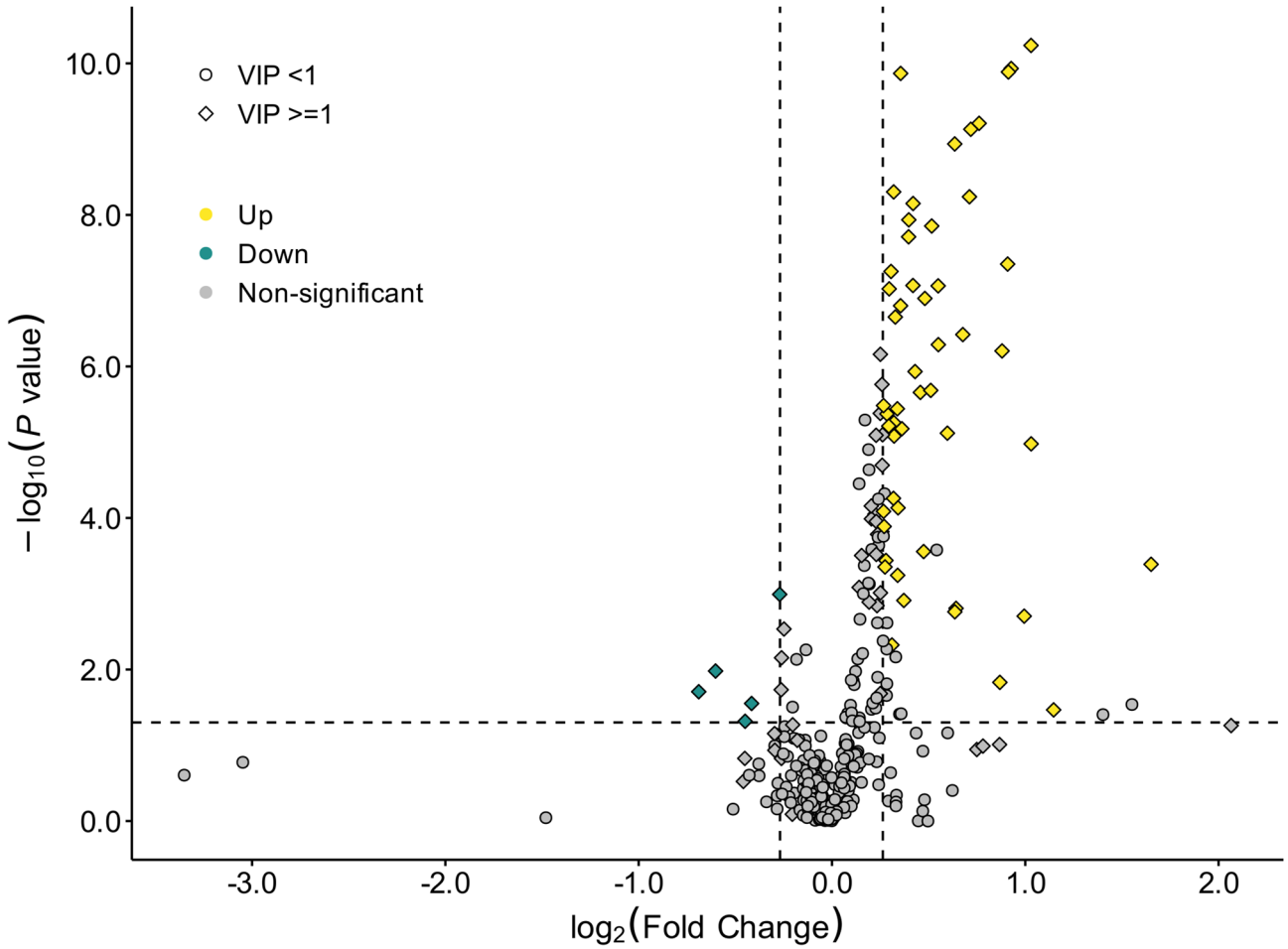

Fig. 3. The volcano plot on the differential metabolites. VIP, variable important for the projection value.

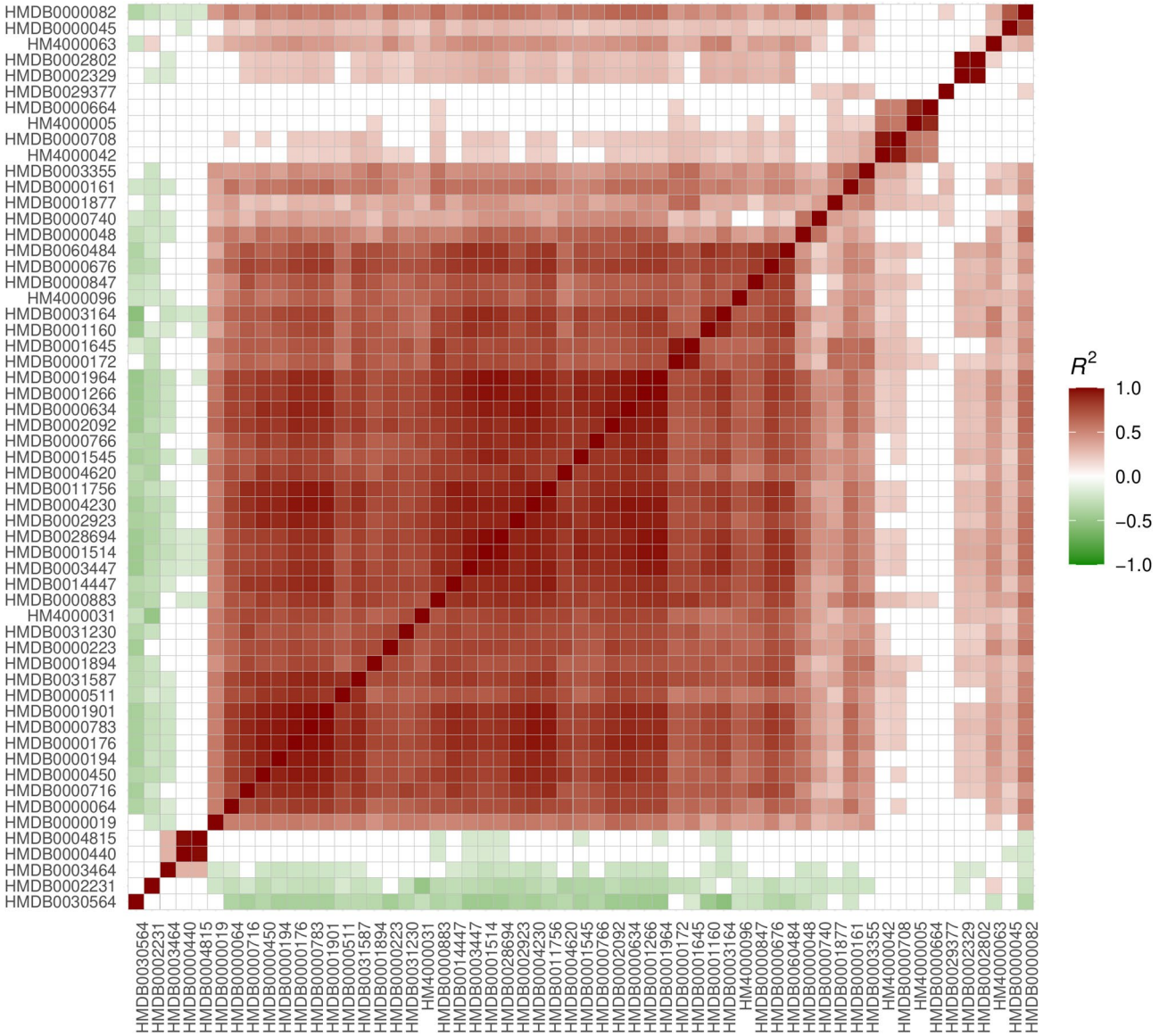

Fig. 4. The correlation heat map on the differential metabolites.

acid and lipid metabolites. Such a profile is consistent with dysregulated metabolism and elevated cardiovascular risk seen in sedentary populations[34,35].

These metabolomic alterations align with known effects of physical inactivity. Sedentary behavior is an established risk factor for cardiometabolic disease[36]. In our study, higher triglycerides in the bus drivers fit this pattern: higher physical activity is generally associated with lower triglyceride and LDL levels[35], implying that prolonged inactivity (as in drivers) predisposes to hypertriglyceridemia. In fact, previous occupational health studies have reported that professional bus drivers tend to have higher prevalence of metabolic syndrome components, including elevated triglycerides, obesity and hyperglycemia, compared to more active workers[37,38]. For example, Iranian bus drivers were found to have significantly higher rates of obesity and serum triglycerides than bus conductors or non-drivers[37]. Thus, our finding of dyslipidemia in the bus drivers is consistent with epidemiological data linking sedentary occupations to metabolic dysfunction. Elevated triglycerides are a hallmark of impaired lipid metabolism and may contribute to the increased CVD risk in this group. While other metabolic parameters (e.g., blood glucose, total cholesterol) did not differ significantly, the observed triglyceride elevation, combined with metabolomic perturbations, suggests early metabolic dysfunction that precedes overt disease.

The specific perturbations in amino acid metabolism are particularly noteworthy. We observed enrichment of the BCAA biosynthesis pathway (valine, leucine, isoleucine), along with altered aminoacyl-tRNA and general amino acid biosynthesis pathways. Elevated circulating BCAAs have been repeatedly associated with insulin resistance and metabolic disease. For instance, dysglycemic (prediabetic) individuals have been shown to exhibit significantly higher plasma BCAA concentrations compared to healthy controls[39]. In the MyoGlu trial, men

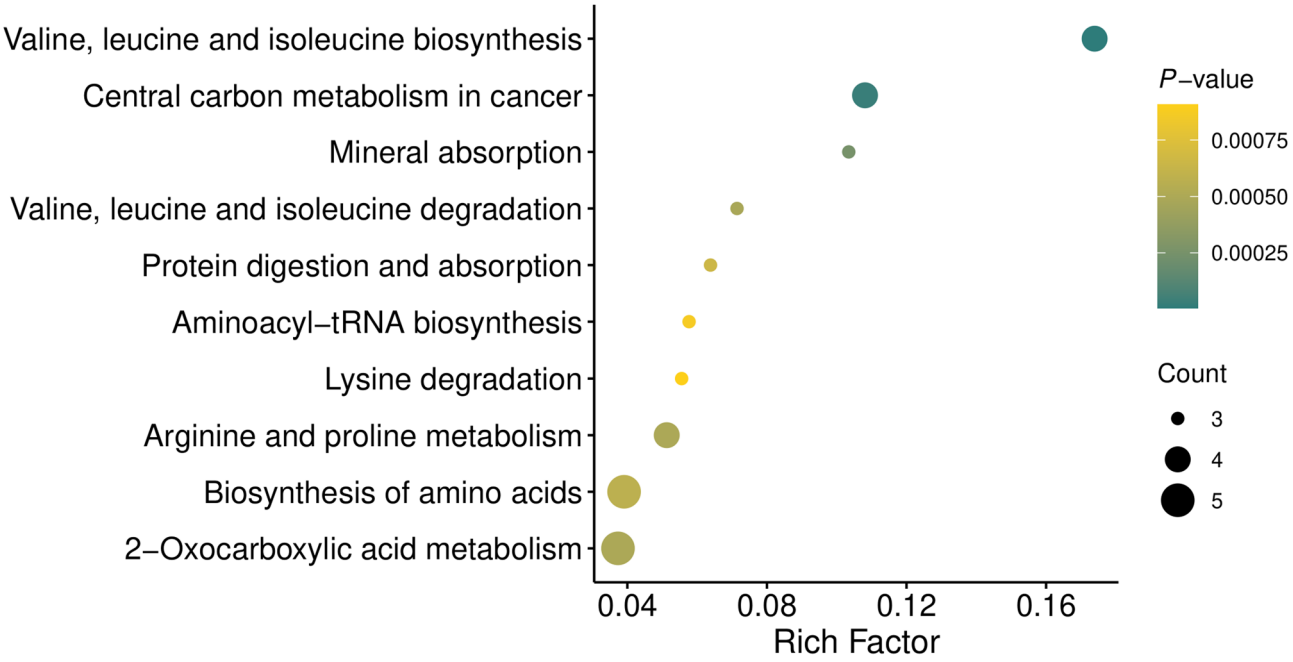

Fig. 5. Metabolic pathway enrichment on the differential metabolites.

with dysglycemia had ~ 14% higher baseline BCAAs than normoglycemic men, and these elevated BCAAs correlated with greater insulin resistance and adiposity[39]. The up-regulation of BCAA-related metabolites in the sedentary drivers may reflect impaired BCAA catabolism or increased protein breakdown — changes that are thought to contribute to metabolic risk[39]. Moreover, the enrichment of 2-oxocarboxylic acid metabolism further underscores disruptions in amino acid and energy metabolism. 2-oxocarboxylic acids are intermediates in the degradation of BCAAs and other amino acids, and their dysregulation may impair mitochondrial function—a key mechanism linking sedentary behavior to metabolic decline[40–42]. Similar pathway alterations have been reported in other metabolomic studies of metabolic interventions. For example, weight-loss treatments significantly modulated pathways of central carbon metabolism, amino acid biosynthesis, and 2-oxocarboxylic acids[43]. Additionally, pathways related to protein digestion and absorption were enriched, potentially reflecting altered nutrient processing due to prolonged sitting. In sum, our data suggest that prolonged sitting disrupts fundamental amino acid and energy metabolic pathways, which may underlie the higher cardiometabolic risk of sedentary workers.

These patterns are consistent with large-scale observations of activity-related metabolomic changes. A recent Chinese cohort study found that higher sedentary time was associated with adverse lipid and amino acid markers: physical inactivity correlated with increased alanine, glucose, lactate, and triglycerides, whereas active individuals had higher levels of large HDL particles[36]. Our findings mirror this trend: sedentary drivers had a lipid profile and amino acid metabolite signature (e.g., elevated BCAAs) that aligns with the metabolite changes linked to physical inactivity.

Methodologically, the use of metabolomics allowed for a comprehensive assessment of low-molecular-weight metabolites, capturing subtle changes that may not be detected by standard biochemical tests. The strong clustering of QC samples in PCA (Supplementary Fig. S2 online) confirms the reliability of the metabolomic data. The study's strengths also include rigorous matching of controls for age, sex, race, smoking and drinking habits, minimizing confounding variables. However, several limitations should be noted. First, the cross-sectional design cannot establish causality, and the sample size, while sufficient for initial discovery, may limit generalizability of the results. Further validation should be conducted in larger cohort studies. Second, sedentary time was not objectively and quantitatively measured by instruments like accelerometer, therefore dose-response relationships with differential metabolites could not be established. In future cohort studies, the dose-response relationship should be modeled to help accurately evaluate the health effect of sedentary work. Third, unmeasured factors such as diet or stress could potentially influence the metabolic profiles, so caution should be taken when interpreting differentially regulated metabolites such as caffeic acid and chlorogenic acid, which are typically of dietary origin, and diet and nutrition should be necessarily considered in future investigations.

In summary, the observed metabolic alterations provide a potential mechanistic link between prolonged sedentary work and adverse health outcomes in bus drivers. By pinpointing specific pathways (amino acid biosynthesis, BCAA metabolism, 2-oxocarboxylic acid metabolism) that differ with sedentary behavior, this study extends theoretical understanding of the molecular effects of inactivity and suggests potential biomarkers for early detection of risk. Targeting these pathways could inform interventions to mitigate metabolic risk in this occupationally vulnerable population.

Conclusions

This study demonstrates that professional bus drivers, a group with prolonged occupational sedentary behavior, exhibit distinct metabolic profiles compared to active sanitation workers. The identified 57 differential metabolites and perturbed pathways, particularly valine, leucine and isoleucine biosynthesis, and 2-oxocarboxylic acid metabolism, highlight specific metabolic disruptions associated with sedentary work. Additionally, elevated triglyceride levels in the bus drivers further support the link between occupational sitting and early metabolic dysfunction.

These findings contribute to our understanding of the biological mechanisms underlying the health risks of sedentary behavior, emphasizing the need for targeted interventions to reduce sitting time and improve metabolic health in bus drivers and other sedentary workers. Future longitudinal studies are warranted to confirm causality and explore the potential of these metabolic signatures as biomarkers for early detection of metabolic impairment. These insights may ultimately guide strategies to mitigate the health consequences of sedentary occupations.

Methods

Study subjects

This study was initially conducted in 2022, aiming to preliminarily investigate the relationship between occupational sedentary behavior and metabolic syndrome. It was approved by the Medical Ethics Committee of Shenzhen Prevention and Treatment Center for Occupational Diseases (No. LL-202034), and conducted in accordance with the Helsinki Declaration on ethical principles for medical research involving human subjects. Written informed consent was provided by all participants or their legal guardians. Sixty professional bus drivers were randomly selected from a public transportation company in Shenzhen as study cases, who had been working as bus drivers for more than a year. Then, 60 sanitation workers, matched on age, sex, race, smoking, drinking and physical exercise habits, were recruited as controls. The controls also worked in the same city for more than a year, and their daily work involved walking along the city streets and cleaning up. Demographics and other basic characteristics of all subjects were investigated using structured questionnaires. Fasting blood samples were collected for biochemical examination and subsequent metabolomic profiling.

Biochemical examination

Serum was separated from approximately 2 mL of fasting blood from each subject via centrifugation at 2564 × *g* for 8 min. Then serum total protein, glucose, triglyceride, total cholesterol, high density lipoprotein cholesterol (HDL-C), and low density lipoprotein cholesterol (LDL-C) concentrations were measured using an AU5800 Chemistry Analyzer (Beckman Coulter, California, US).

Metabolomic profiling

Metabolomics detection was based on previously published research[44]. Metabolites were first extracted as follows: **(a)** Add 120 µL of sample release agent to 20 µL of sample or 20 µL of standard, shake the mixture at 1200 rpm for 30 min, and centrifuge at 18,000 × *g*, 4℃ for 30 min; **(b)** Transfer 30 µL of the supernatant to a 96-well plate, and add 20 µL of derivatization reagent and 20 µL of EDC working solution; **(c)** Cover the plate with an aluminum film, place it in a constant temperature shaker, and let the reaction proceed at 1200 rpm, 40℃ for 60 min; **(d)** Centrifuge again at 4000 × *g*, 4℃ for 5 min, transfer 30 µL to a new 96-well plate, add 90 µL of sample diluent to each well, and mix at 600 rpm for 10 min; **(e)** Centrifuge at 4000 × *g*, 4℃ for 30 min, and seal the film.

The sample extracts were then analyzed using Waters UPLC I-Class Plus (Waters, USA) equipped with QTRAP 6500 Plus (SCIEX, USA). Chromatography conditions are as follows. The column used is BEHC18 (2.1 mm × 10 cm, 1.7 µm, Waters, USA). The mobile phase is water containing 0.1% formic acid (solvent A) and acetonitrile containing 30% isopropanol (solvent B). Elution was performed using the following gradient: 0–1.00 min, 5% B; 1.00–5.00 min, 5% B; 5.00–9.00 min, 70% B; 9.00–11.00 min, 50% B; 11.00–13.50 min, 22% B; 13.50–14.00 min, 95% B, the flow speed above is 0.400 mL/min; 14.00–16.00 min, 100% B, the flow speed is 0.600 mL/min; 16.00–18.00 min, 5% B, the flow speed is 0.400 mL/min. The column temperature is 40℃.

Mass spectrometry conditions: For a QTRAP 6500 Plus equipped with an ESI Turbo ion spray interface, ion source parameters are as follows. Ion source temperature: 400℃; ion spray voltage: 4500 V (positive mode) and −4500 V (negative mode); ion source gas 1 (GS1), ion source gas 2 (GS2), and curtain gas were set to 60, 60, and 35 psi, respectively. The MRM method was set in MRM mode, including MRM parent-daughter transition information of target metabolites, collision energy (CE), declustering potential (DP), and retention time.

Quantile normalization was adopted for XIC data normalization because it ensures that the signal intensity distributions of all samples are consistent, which is particularly suitable for our dataset. For missing values in XIC data, k-nearest neighbor (KNN) method was used for imputation, because it leverages the signal similarity of samples from the same group to avoid introducing artificial bias. To address potential batch effects, we integrated batch correction into the normalization workflow. Before normalization, we included QC samples in each batch, ensuring that batch-related variations were initially controlled at a low level. During normalization, we further applied the ComBat algorithm to adjust for residual batch effects.

Skyline v21.1.0.146 (MacCoss Lab Software) was used for metabolite identification and quantification. Then a data matrix containing information such as metabolite identification results and quantitative results was obtained, and the data were further processed for information analysis.

Data analysis and plotting

Data analysis and plotting were performed using R v4.2.3 software (R Development Core Team, Vienna, Austria). Continuous variables (including metabolite levels) were compared between groups using Student's *t*-

test or Mann-Whitney U test, depending on their distribution; categorical variables were compared using χ^2 test or Fisher's exact test. The Benjamini-Hochberg procedure was used to control the false discovery rate (FDR) in multiple comparisons of metabolites. The corrected *P*-values were presented as q-values in Supplementary Table S1 online. After using Probabilistic Quotient Normalization and QC-RLSC to pre-process the metabolomic data, we applied principal component analysis (PCA) to all samples including QC samples to observe the overall distribution of samples in each group and the stability of the whole analysis process. Pathway functional annotation was conducted using the KEGG Pathway Database to identify the key biochemical metabolic pathways associated with the metabolites. The overall metabolite difference between the two groups was analyzed by PCA, partial least squares discriminant analysis (PLS-DA), and orthogonal partial least squares discriminant analysis (OPLS-DA). Thereafter, we used the variable important for the projection (VIP) value of metabolites in OPLS-DA, fold change and *P*-value to screen differential metabolites, and mapped a volcano plot. The expression patterns of differential metabolites were further analyzed by clustering analysis and correlation clustering, and pathway enrichment analysis was performed using right-tailed Fisher's exact test based on the hypergeometric distribution, with the Benjamini-Hochberg method applied to control the FDR.

Data availability

All data generated or analyzed during this study are included in this published article (and its Supplementary Information files).

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Author contributions

DL : conception, design of the work, interpretation of data, writing - original draft. SW : acquisition, analysis, interpretation of data, writing - original draft. DW : acquisition, analysis, interpretation of data. PL : acquisition, interpretation of data. ZX : analysis, interpretation of data. LZ : analysis, interpretation of data. WG : analysis, interpretation of data, writing - original draft. NZ : conception, design of the work, writing - review & editing. All authors reviewed and approved the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Ethics approval and consent to participate

All procedures performed in the study involving human participants were approved by the Medical Ethics Committee of Shenzhen Prevention and Treatment Center for Occupational Diseases (No. LL-202036) and were conducted in accordance with the 1975 Declaration of Helsinki and its later amendments or comparable ethical standards. Written informed consent for participating in the study was obtained from all participants or their legal guardians.

Consent for publication

Not applicable.

Additional information

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