Exploring the Effects of Teas with Different Fermentation Degrees and Black Coffee on the Body Through Urinary Proteome

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Abstract

Tea and black coffee, as globally widely consumed beverages, play important roles in maintaining organismal health. Since urinary proteins rarely originate directly from beverages, changes in the urine proteome reflect alterations in the organism after being influenced by beverages rather than metabolites of the beverages themselves. Can the urine proteome thus reflect the effects of teas with different fermentation degrees and black coffee on the organism? This study employed both pre-post comparison and inter-group comparison approaches, collecting urine from rats before and after 7 consecutive days of consuming green tea, oolong tea, black tea, Pu-erh tea, or black coffee, and analyzed the samples using liquid chromatography-tandem mass spectrometry. The results demonstrated that the urine proteome could reflect changes in rats after one week of consuming teas with different fermentation degrees or black coffee, enriching biological processes and pathways such as adipocyte differentiation, lipid metabolism, glucose metabolism, fatty acid transport, and immune response, with the effects of differently fermented teas and black coffee on the organism showing high specificity. Furthermore, multiple differential proteins have been reported as biomarkers for cancer, cardiovascular diseases, etc., suggesting that in the clinical application of urinary disease biomarkers and related research, the influence of beverages such as tea and black coffee should be considered, and it may be necessary to develop combinations of multiple biomarkers to improve accuracy. In summary, through the urine proteome, the holistic effects of all components of tea and coffee on the organism can be comprehensively and systematically reflected, and changes in the organism after consuming teas with different fermentation degrees and black coffee can be distinguished.

Full Text

Preamble

Exploring the Effects of Teas with Different Fermentation Levels and Black Coffee on the Body via the Urine Proteome

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Abstract

Tea and black coffee, two of the most widely consumed beverages worldwide, play important roles in supporting overall health. Urinary proteins do not originate directly from beverages. Instead, changes in the urine proteome reflect the changes in the body influenced by beverage consumption, rather than their metabolites. Can the effects of teas with different fermentation levels and black coffee on the body be explored via the urine proteome? In this study, urine samples were collected from rats before and after seven consecutive days of consuming green tea, oolong tea, black tea, Pu-erh tea, or black coffee. Both before-and-after comparisons and between-group comparisons were performed, and the samples were analyzed using liquid chromatography coupled with tandem mass spectrometry. The results showed that the urine proteome reflected the changes in rats after one week of consuming teas with different fermentation levels or black coffee. Biological processes and pathways enriched from differential proteins included fat cell differentiation, lipid metabolism, glucose metabolism, fatty acid transport, and immune response. Furthermore, the effects of teas with different fermentation levels and black coffee on the body exhibited a high degree of specificity. Additionally, several differential proteins identified in this study have been reported as biomarkers for diseases such as cancer and cardiovascular diseases. This suggests that beverage consumption, including tea and black coffee, should be considered in clinical application of urine biomarkers of diseases and related studies, and the use of biomarker panels may be necessary to improve accuracy. In conclusion, the urine proteome provides a comprehensive and systematic reflection of the overall effects of all components in tea and black coffee on the body, and can distinguish changes in the body after consuming teas with different fermentation levels and black coffee.

Keywords: urine; proteomics; green tea; oolong tea; black tea; Pu-erh tea; black coffee

1 Introduction

Tea is the second most consumed beverage worldwide after water, containing various bioactive components such as polyphenols, theaflavins, thearubigins, and

caffeine, which exhibit antiviral, antioxidant, and anti-inflammatory effects [1]. Numerous studies have demonstrated that tea plays positive roles in reducing the risk of cardiovascular disease, stroke [2], type 2 diabetes [3], hypertension [4], various cancers [5], and dementia [6], while also improving mood [7], exerting anti-aging effects [8], and combating obesity [9]. Based on fermentation levels, tea can be categorized into non-fermented green tea, semi-fermented oolong tea, fully fermented black tea, and post-fermented dark tea [10], with different fermentation degrees affecting the content of bioactive components [11].

Coffee is one of the most widely consumed beverages globally, containing over a thousand components including caffeine, chlorogenic acid, diterpenes, and trigonelline [12]. It has been shown to reduce cardiovascular disease mortality and stroke incidence [13], lower the risk of cancer [14], Parkinson's disease [15], and type 2 diabetes [16], and combat obesity [17].

Proteomics reveals the composition and dynamic changes of proteins within cells or organisms by analyzing protein structure, expression, post-translational modifications, and protein-protein interactions [18]. Urine is not strictly regulated by homeostatic mechanisms, allowing it to accommodate and accumulate more extensive changes, thereby reflecting alterations in all organs and systems earlier and more sensitively [19]. Moreover, unknown components in tea and black coffee may also contribute to health maintenance. Since urinary proteins rarely originate directly from beverages, changes in the urine proteome reflect the body's response to beverage consumption rather than the beverages' metabolites. Therefore, urine proteomics research can comprehensively and systematically reflect the overall impact of all beverage components on the body.

The urine proteome is inevitably influenced by various factors including age, genetics, sex, diet, and exercise. Thus, minimizing interference from irrelevant factors is crucial in experiments. Animal models are particularly suitable as their genetic and environmental factors can be controlled [20].

Can we leverage the comprehensive, systematic, and sensitive nature of urine to reflect bodily states, thereby exploring the effects of widely consumed tea and black coffee in detail and identifying differences among teas with varying fermentation levels? This study selected commercial green tea, oolong tea, black tea, and Pu-erh tea representing four fermentation levels, along with black coffee, to explore their effects on rat bodies and distinctions among them through urine proteomics. We aim to provide new insights into their mechanisms of action and identify which biomarkers are affected by tea and black coffee consumption, offering references for clinical application of urinary disease markers and related research (Figure 1 [Figure 1: see original paper]).

2 Materials and Methods

2.1 Urine Sample Collection

Thirty healthy male Sprague Dawley rats (200$\pm$20g), $aged 6-7 weeks, were purchased from Beijing Vital River$ humidity 65%-70%). All experimental procedures were reviewed and approved by the Animal Ethics Committee of the College of Life Sciences, Beijing Normal University (Approval No.: CLS-AWEC-B-2022-003).

The 30 rats were randomly divided into six groups of five animals each. After three days of acclimatization in the standard environment, all rats were placed in metabolic cages to collect 12-hour urine samples. Rats in the experimental groups consumed commercial green tea, oolong tea, black tea, Pu-erh tea, or black coffee, while the control group received sterilized water. Pu-erh tea was prepared by dissolving 96.5 mg of tea powder in 500 mL sterilized water, and black coffee was prepared by diluting 33 mL of coffee solution with 547 mL sterilized water. Tea, black coffee, and sterilized water were provided in 250 mL drinking bottles for ad libitum consumption, with fresh beverages and water replaced daily. After seven days, all rats were placed in metabolic cages again to collect 12-hour urine samples. During urine collection, rats were fasted and deprived of water. All collected urine samples were stored at -80°C.

2.2 Urinary Protein Processing

2.2.1 Urinary Protein Extraction and Quantification Collected rat urine was centrifuged at 12,000 ×g for 40 min at 4°C. The supernatant was transferred to new tubes, and three volumes of ice-cold absolute ethanol were added. After mixing thoroughly, proteins were precipitated overnight at -20°C. The mixture was centrifuged at 12,000 ×g for 30 min at 4°C, the supernatant was discarded, and the protein pellet was resuspended in an appropriate volume of lysis buffer (8 mol/L urea, 2 mol/L thiourea, 25 mmol/L dithiothreitol, 50 mmol/L Tris). After complete dissolution, the solution was centrifuged at 12,000 ×g for 30 min at 4°C, and the supernatant was transferred to new tubes as the urinary protein extract. Protein concentration was determined using the Bradford method.

2.2.2 Urinary Protein Digestion Using the filter-aided sample preparation (FASP) method [21], 100 g of urinary protein sample was placed in a 1.5 mL tube, and 25 mmol/L NH4HCO3 solution was added to a final volume of 200 L. Dithiothreitol (DTT, Sigma) was added to a final concentration of 20 mmol/L, vortexed, and heated in a metal bath at 97°C for 10 min, followed by cooling to room temperature. Iodoacetamide (IAA, Sigma) was added to a final concentration of 50 mmol/L, vortexed, briefly centrifuged, and incubated at room temperature in the dark for 40 min. Then, 200 L of UA solution (8 mol/L urea, 0.1 mol/L Tris-HCl, pH 8.5) was added to the membrane of a 10 kD ultrafiltration tube (Pall, Port Washington, NY, USA) and washed twice by centrifugation at 14,000 ×g for 5 min at 18°C. The treated protein sample was

added and centrifuged at 14,000 ×g for 40 min at 18°C. Then, 200 L of UA solution was added, vortexed, and centrifuged at 14,000 ×g for 40 min at 18°C, with the filtrate discarded; this step was repeated once. Next, 25 mmol/L NH4HCO3 solution was added, vortexed, and centrifuged at 14,000 ×g for 40 min at 18°C, with the filtrate discarded; this step was also repeated once. The collection tube was replaced, 100 L of NH4HCO3 solution was added, and trypsin (Trypsin Gold, Promega, USA) was added at a 1:50 enzyme-to-protein mass ratio for digestion. After overnight incubation at 37°C, the peptide-containing solution was collected. Finally, peptides were desalted using an HLB column (Waters, Milford, MA) and dried using a vacuum concentrator before storage at -80°C.

2.3 Liquid Chromatography-Tandem Mass Spectrometry Analysis

Digested samples were resuspended in 0.1% formic acid. Peptide concentration was quantified using a BCA kit and diluted to 0.5 g/ L. For each sample, 3.3 L was taken to prepare a pooled peptide sample, which was fractionated using a high pH reversed-phase peptide fractionation kit (Thermo Fisher Scientific, Rockford, IL, USA). Ten fractions were collected and dried using a vacuum concentrator, then resuspended in 0.1% formic acid. iRT reagent (Biognosis, Schlieren, Switzerland) was added at a 10:1 sample-to-iRT volume ratio.

To generate the spectral library, the ten fractions were analyzed in Data Dependent Acquisition (DDA) mode. For each sample, 1 g was separated using an EASY-nLC 1200 chromatography system (Thermo Fisher Scientific, Waltham, MA, USA). Samples were loaded onto a C18 reversed-phase column (75 m$\times 2cm, 3\mu m) and analytical column (50\mu m \times$15 cm, 2 m) at a flow rate of 0.3 L/min, with gradient elution using mobile phase A (0.1% formic acid) and mobile phase B (80% acetonitrile + 0.1% formic acid) over 90 min (Table 1).

Mass spectrometry analysis was performed using an Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) with the following parameters: spray voltage 2.25 kV; full MS scan range 350-1,550 m/z at 120,000 resolution; MS/MS scans acquired in Orbitrap mode at 30,000 resolution; HCD energy 30%; top 20 most intense precursor ions selected for fragmentation; dynamic exclusion time 30 s. DDA data were searched using Proteome Discoverer software (version 2.1, Thermo Fisher Scientific) to build a Data Independent Acquisition (DIA) method, with window width and number calculated based on m/z distribution density.

Individual samples were analyzed in DIA mode, with each sample analyzed in triplicate. DIA liquid chromatography settings were identical to DDA mode. Mass spectrometry parameters were: spray voltage 2.3 kV; full MS scan range 350-1,500 m/z at 60,000 resolution; MS/MS scans acquired in Orbitrap mode with scan range 200-2,000 m/z at 30,000 resolution; HCD energy 32%. A single DIA analysis of pooled peptides was performed every 9-10 injections as a quality control throughout the analysis.

2.4 Database Searching and Data Processing

DIA data were processed using Spectronaut Pulsar software (version 19, Biognosys AG, Schlieren, Switzerland). Peptide intensity was calculated by summing the peak areas of fragment ions from MS2, and protein intensity was calculated by summing the intensities of respective peptides.

2.5 Data Analysis

This study employed both before-and-after comparisons and between-group comparisons. By comparing urinary proteins before and after tea/coffee consumption, individual variation was minimized. Differential proteins were screened using fold change (FC) $ $1.5 or $ $0.67 and two-tailed paired t-test $p < 0.05$. By comparing urinary proteins between experimental and control groups after consuming tea, black coffee, or sterilized water, the impact of short-term growth and development was avoided, with differential proteins screened using FC $ $1.5 or $ $0.67 and two-tailed unpaired t-test $p < 0.05$.

Biological analysis was performed using UniProt (https://www.uniprot.org/) and DAVID databases (https://david.ncifcrf.gov/), with literature retrieved from PubMed (https://pubmed.ncbi.nlm.nih.gov/) for functional analysis of differential proteins.

3 Results

In this experiment, rats showed no significant preference for teas with different fermentation levels or black coffee. LC-MS/MS analysis of 60 samples from experimental and control groups identified a total of 1,647 proteins, using criteria of $ $2 unique peptides per protein and protein-level false discovery rate (FDR) <1%.

3.1 Before-and-After Comparisons

3.1.1 Green Tea Group Comparison of urinary proteins before and after green tea consumption identified 61 differential proteins using criteria of FC $ $1.5 or $ $0.67 and two-tailed paired t-test $p < 0.05$ (details in Supplementary Table 1). To determine the likelihood of random generation, random grouping validation was performed on total proteins. The 10 pre- and post-green tea samples were randomly shuffled and combined into new groups, yielding 126 possible combinations. Using the same criteria (FC $ $1.5 or $ $0.67, $p < 0.05$), an average of 39.84 differential proteins were generated, indicating that at least 34.69% of differential proteins were not randomly produced.

3.1.1.1 Analysis of Differential Proteins Among differential proteins identified in the green tea before-and-after comparison, CCN family member 1 and Cadherin 26 showed complete disappearance (present in pre-consumption samples but absent in post-consumption samples). CCN family member 1 (FC = 0,

p = 1.65$×10^{-3})functionsingrowthfactorbindingandparticipatesinmulticellularorganismprocessregulation, reportedasanearly
2}$) is involved in adherens junction organization, calcium-dependent cell-cell adhesion mediated by plasma membrane adhesion molecules, CD4+ α-β T cell activation, cell migration, and cell morphogenesis.

45 kDa calcium-binding protein (Stromal cell-derived factor 4, SDF4) showed the smallest p-value and second-largest FC value (FC = 7.90, p = 6.76$×10^{-4}$), with calcium ion binding function and involvement in adipocyte differentiation. Studies show SDF4 expression is elevated in various cancer cell types, particularly those with strong proliferation and metastatic potential, with serum SDF4 levels serving as a potential diagnostic biomarker for gastric cancer and other malignant tumors [23]. SDF4 has also been reported as a potential biomarker for pancreatic cancer [24] and a potential biomarker and therapeutic target for glioblastoma [25].

N-acetylneuraminate synthase (FC = 0.16, p = 4.72$×10^{-2}$) had the third-smallest FC value, possessing N-acylneuraminate-9-phosphate synthase activity and participating in carbohydrate biosynthesis, glycosylation, CMP-N-acetylneuraminate biosynthesis, and N-acetylneuraminate biosynthesis. It has been reported as a potential prognostic biomarker for aggressive prostate cancer [26].

Tissue-type plasminogen activator (FC = 0.16, p = 4.01$×10^{-2}$) had the fourth-smallest FC value, participating in synaptic plasticity regulation, glutamatergic synaptic transmission, BDNF trans-synaptic signaling, response to cAMP, response to fatty acids, and response to hypoxia.

3.1.1.2 Enriched Biological Pathways DAVID database analysis of differential proteins revealed enrichment in biological processes including homophilic cell adhesion via plasma membrane adhesion molecules, negative regulation of oxidative stress-induced intrinsic apoptotic signaling pathway, adipocyte differentiation, UV protection, atrioventricular valve morphogenesis, and pulmonary valve morphogenesis (Figure 2 [Figure 2: see original paper]).

3.1.2 Oolong Tea Group Comparison of urinary proteins before and after oolong tea consumption identified 108 differential proteins (details in Supplementary Table 1). Random grouping analysis yielded an average of 44.60 differential proteins, indicating at least 58.70% were not randomly generated.

3.1.2.1 Analysis of Differential Proteins CCN family member 1 (FC = 0.04, p = 3.82$×10^{-2}$) was also identified in the oolong tea before-and-after comparison, with the second-smallest FC value.

Catalase (FC = 0, p = 4.60$×10^{-2}$) showed complete disappearance (present before but absent after oolong tea consumption). This protein possesses antioxidant and catalase activity, participating in cellular detoxification of hydrogen peroxide, hydrogen peroxide catabolism, response to hydrogen

peroxide, cholesterol metabolism, response to fatty acids, response to oxidative stress, and triglyceride metabolism. Studies show that oolong tea-based kombucha significantly increases catalase mRNA levels in HEK-293 cells [27].

Ly6/PLAUR domain-containing protein 3 (FC = 0.04, p = 6.54$×10^{-3})$had the third-smallest FC value and has been reported as a prognostic biomarker for thyroid cancer [29], with high PCDH8 expression also serving as a biomarker for poor gastric cancer prognosis [30].

3.1.2.2 Enriched Biological Pathways Differential proteins from oolong tea before-and-after comparison were mainly enriched in skin barrier establishment, triglyceride metabolism, positive regulation of PI3K/Akt signal transduction, UV protection, immune response, and response to oxidative stress (Figure 3 [Figure 3: see original paper]A). Oolong tea polyphenols have been reported to effectively improve circadian rhythm disorders by regulating diurnal oscillations of gut microbiota and transcription of circadian clock genes, with PI3K/Akt signaling pathway being one of the most enriched pathways for differentially expressed genes after oolong tea polyphenol intervention [31]. KEGG pathway enrichment analysis revealed significant enrichment in carbon metabolism, folate biosynthesis, 2-oxocarboxylic acid metabolism, and tryptophan metabolism (Figure 3B). Studies indicate that high oolong tea consumption during pregnancy is associated with lower serum folate levels [32].

3.1.3 Black Tea Group Comparison of urinary proteins before and after black tea consumption identified 142 differential proteins (details in Supplementary Table 1). Random grouping analysis showed an average of 40.44 differential proteins, indicating at least 71.52% were not randomly generated.

3.1.3.1 Analysis of Differential Proteins Cytoplasmic FMR1-interacting protein showed complete appearance (absent before but present after black tea consumption), reported as a potential biomarker for nasopharyngeal carcinoma diagnosis, disease progression monitoring, and treatment selection [33], as well as a potential diagnostic and prognostic biomarker for acute lymphoblastic leukemia [34].

Acid phosphatase (FC = 29.17, p = 1.51$×10^{-2})$had the second-largest FC value among differential proteins, participating i
2}$) had the smallest FC value, involved in central nervous system development, central nervous system formation, and positive regulation of IL-2 production, reported as a biomarker for early diagnosis and precision treatment of renal clear cell carcinoma [35].

Fibulin-1 (FC = 0.53, p = 8.93$×10^{-5}$) had the smallest p-value. Studies show Fibulin-1 is closely associated with target organ damage severity in high-risk cardiovascular disease patients and serves as a biomarker for risk stratification [36]. Plasma FBLN1 levels can also predict thyroid-associated ophthalmopathy activity [37].

3.1.3.2 Enriched Biological Pathways Differential proteins from black tea before-and-after comparison were mainly enriched in glyceraldehyde-3-phosphate metabolism, axon guidance, response to lipopolysaccharide, glycerol catabolism, response to oxidative stress, gluconeogenesis, glycolysis, lipid metabolism, glucose metabolism, and fatty acid transport (Figure 4 [Figure 4: see original paper]A). KEGG pathway enrichment analysis revealed significant enrichment in PI3K-Akt signaling pathway, fructose and mannose metabolism, carbon metabolism, inositol phosphate metabolism, glycolysis/gluconeogenesis, and tyrosine metabolism (Figure 4B). Studies demonstrate that black tea extract inhibits HepG2 cell growth and induces apoptosis via the PI3K-Akt signaling pathway [38].

3.1.4 Pu-erh Tea Group Comparison of urinary proteins before and after Pu-erh tea consumption identified 200 differential proteins (details in Supplementary Table 1). Random grouping analysis showed an average of 41.91 differential proteins, indicating at least 79.05% were not randomly generated.

3.1.4.1 Analysis of Differential Proteins Eight proteins showed complete disappearance in the Pu-erh tea before-and-after comparison: Charged multivesicular body protein 2B, SPARC-like protein 1, Syntaxin-7, Shisa family member 7, CMRF35-like molecule 1, Nebulin, Proteasome activator complex subunit 1, and Latent transforming growth factor beta binding protein 2 (present before but absent after consumption).

Charged multivesicular body protein 2B (FC = 0, p = 9.58$\times 10^{\{-4\}}) participates in autophagosome maturation, autophagy 3\}) has been reported as a marker for human glioma progression [39] and a potential prognostic biomarker for fatal C 19 pneumonia [40]. Syntaxin-7 ($FC = 0, p = 1.50\times 10^{\{-2\}}$) participates in positive regulation of receptor localization to synapse 2\}) functions in GABA receptor binding and ionotropic glutamate receptor binding, participating in GABA recepto term synaptic potentiation, and regulation of AMPA receptor clustering. CMRF35-like molecule 1 ($FC = 0, p = 2.28\times 10^{\{-2\}}$) participates in immune system processes. Nebulin ($FC = 0, p = 2.93\times 10^{\{-2\}}$) participates in cardiac thin filament assembly. Proteasome activator complex subunit 1 ($FC = 0, p = 4.02\times 10^{\{-2\}}$) has been reported as an independent prognostic biomarker for soft tissue leiomyosarcoma [41] and a biomarker for rhe 2\}$) functions in calcium ion binding and has been reported as a potential biomarker for hepatocellular carcinoma diagnosis and treatment [43], a diagnostic biomarker and potential therapeutic target for pancreatic cancer [44], with circulating LTBP-2 also reported as a biomarker for predicting adverse outcomes in dilated cardiomyopathy [45].

3.1.4.2 Enriched Biological Pathways Differential proteins from Pu-erh tea before-and-after comparison were mainly enriched in positive regulation of PI3K/Akt signal transduction, axon guidance, response to hypoxia, angiogenesis, response to lipopolysaccharide, immune response, and coronary vein morphogenesis (Figure 5 [Figure 5: see original paper]A). KEGG pathway enrichment analysis revealed significant enrichment in ECM-receptor interaction, renin-angiotensin system, focal adhesion, human papillomavirus infection, PI3K-Akt signaling pathway, cancer-related pathways, hypertrophic cardiomyopathy,

and dilated cardiomyopathy (Figure 5B). Theabrownin, a bioactive component of dark tea, has been reported to regulate glucose and lipid metabolism via the IRS-1/PI3K/Akt signaling pathway [46].

3.1.5 Black Coffee Group Comparison of urinary proteins before and after black coffee consumption identified 246 differential proteins (details in Supplementary Table 1). Random grouping analysis showed an average of 44.96 differential proteins, indicating at least 81.72% were not randomly generated.

3.1.5.1 Analysis of Differential Proteins Ten proteins showed complete disappearance in the black coffee before-and-after comparison: Tetraspanin, RAB5B, Shisa family member 7, Latent transforming growth factor beta binding protein 2, Tumor necrosis factor receptor superfamily member 4, Choline transporter-like protein 2, Glycoprotein hormone alpha-2, Protocadherin alpha-4, CCN family member 1, and Tissue-type plasminogen activator (present before but absent after consumption).

Tetraspanin (FC = 0, p = 2.39$×10^{-4})participatesinnegativeregulationofcoagulation, geneexpressionregulation, andsperma
4})possessesGDPbinding, GTPbinding, andGTPaseactivity, participatinginantigenprocessingandpresenta
0, p = 1.49×10^{-3}$) functions in GABA receptor binding and ionotropic glutamate receptor binding, participating in GABA receptor clustering, GABA signaling pathway, memory, positive regulation of long-term synaptic potentiation, and regulation of AMPA receptor clustering.

Latent transforming growth factor beta binding protein 2 (FC = 0, p = 3.95$×10^{-3}$) has been reported as a potential biomarker for hepatocellular carcinoma diagnosis and treatment [43], a diagnostic biomarker and therapeutic target for pancreatic cancer [44], with circulating LTBP-2 as a biomarker for predicting adverse outcomes in dilated cardiomyopathy [45].

Tumor necrosis factor receptor superfamily member 4 (FC = 0, p = 5.66$×10^{-3}$) participates in cellular defense response, inflammatory response, negative regulation of T cell activation-induced cell death, positive regulation of B cell proliferation, T cell proliferation, and regulation of apoptotic process, reported as a potential biomarker for endometrial cancer prognosis and immunomodulation [48] and an independent potential biomarker for pancreatic cancer prognosis prediction [49].

Choline transporter-like protein 2 (FC = 0, p = 1.31$×10^{-2})participatesincholinetransportandethanolaminetransport.G
2})participatesinadenylatecyclase−activatingGprotein−coupledreceptorsignalingpathwayandcellsurfacere
typeplasminogenactivator(FC = 0, p = 4.53×10^{-2})participatesinsynapticplasticityregulation, glutamatergicsynaptictra
2}$) has been reported as an early marker of infarct size and left ventricular dysfunction in STEMI patients [22].

Matrilin 2 (FC = 0.26, p = 3.24$×10^{-5}$) had the smallest p-value, functioning in calcium ion binding and participating in axon guidance, dendrite regeneration, glial cell migration, neuronal migration, neurite development, and

response to axonal injury. It has been reported as a specific biomarker for distinguishing indolent from clinically aggressive pilocytic astrocytoma [50] and a prognostic biomarker for osteosarcoma [51].

3.1.5.2 Enriched Biological Pathways Differential proteins from black coffee before-and-after comparison were mainly enriched in axon guidance, positive regulation of PI3K/Akt signal transduction, response to lipopolysaccharide, Ephrin receptor signaling pathway, complement activation, heart development, negative regulation of apoptotic process, and immune response (Figure 6 [Figure 6: see original paper]A). KEGG pathway enrichment analysis revealed significant enrichment in complement and coagulation cascades, focal adhesion, PI3K-Akt signaling pathway, human papillomavirus infection, nitrogen metabolism, platelet activation, microRNAs in cancer, fluid shear stress and atherosclerosis, and cancer-related pathways (Figure 6B). Studies show coffee consumption is a risk factor for human papillomavirus (HPV) infection [52]. During early HPV infection, caffeine-mediated DNA damage response (DDR) inhibition reduces viral genome replication, while in the maintenance phase, DDR inhibition may increase viral amplicon replication [53]. Additionally, in a Parkinson's disease SH-SY5Y cell model, caffeine activates the PI3K/Akt pathway and inhibits apoptosis [54].

3.1.6 Control Group Comparison of urinary proteins before and after sterilized water consumption identified 83 differential proteins (details in Supplementary Table 1). Random grouping analysis showed an average of 40.90 differential proteins, indicating at least 50.72% were not randomly generated. These differential proteins could be enriched in development-related biological processes including skeletal system morphogenesis, heart development, and cartilage development. Although before-and-after comparison reduces individual variation, it cannot avoid developmental effects. Therefore, we performed between-group comparisons of urinary proteins from experimental and control groups after consuming tea, black coffee, or sterilized water to exclude short-term growth and development effects.

3.2 Between-Group Comparisons

3.2.1 Green Tea vs. Control Group Comparison between green tea and control groups identified 59 differential proteins (details in Supplementary Table 2). Random grouping analysis showed an average of 37.69 differential proteins, indicating at least 36.12% were not randomly generated.

3.2.1.1 Analysis of Differential Proteins Suppressor of tumorigenicity 14 protein homolog (FC = ∞, p = 2.78$×10^{-2}$) showed complete appearance (present in green tea group but absent in control group), reported to play important roles in all stages of epithelial tumorigenesis and development, with potential as an anti-cancer therapeutic target and novel diagnostic and prognostic marker [55, 56].

Myosin light chain 12A (FC = 152.50, p = 4.76$×10^{-2}$) had the second-largest FC value, functioning in calcium ion binding. MYL12A is highly expressed in smooth and skeletal muscle tissues, encoding a non-sarcomeric myosin regulatory light chain protein involved in muscle contraction regulation, representing a novel muscle strength-related candidate gene [57]. Additionally, Myl9/12 has been reported to participate in inflammatory bowel disease pathogenesis and may serve as a new therapeutic target, with plasma Myl9 levels as a biomarker for inflammatory bowel disease [58]. Studies also show cardiac-specific overexpression of MYL12A rescues the weakened cardiac contractility phenotype in necdin-deficient mice [59].

Metalloproteinase inhibitor 3 (TIMP-3) (FC = 25.42, p = 3.66$×10^{-2}$) had the third-largest FC value, functioning as a metallopeptidase inhibitor and zinc ion binding, participating in mesenchymal cell differentiation during bone development, cellular response to hypoxia, cellular response to interleukin-6, and negative regulation of vascular smooth muscle cell proliferation. TIMP-3 serves as an alternative marker for green tea polyphenols (GTP) and its main component epigallocatechin gallate (EGCG) response. GTP and EGCG inhibit prostate cancer cell migration and invasion by reactivating TIMP-3 and inhibiting MMP-2/MMP-9 activity [60]. GTP and EGCG also induce TIMP-3 expression in breast cancer cells, delaying breast cancer progression and invasion [61]. Green tea extract restores TIMP3 circadian expression disrupted by UVB irradiation [62]. TIMP-3 has been reported as a potential biomarker and therapeutic target for cancer [63]; for example, plasma TIMP3 levels are significantly lower in oral squamous cell carcinoma patients, serving as a potential biomarker for predicting tumor stage and T status [64].

Crk-like protein (FC = 23.87, p = 4.4$×10^{-2}$) had the fourth-largest FC value, participating in vascular development, B cell apoptosis, and lipid metabolism. Studies indicate CrkL can serve as a soluble serum biomarker for breast cancer patients, especially in advanced disease stages [65].

Phospholipase B1 (FC = 0.14, p = 2.51$×10^{-2}$) had the smallest FC value, participating in triglyceride catabolism, retinol metabolism, and phospholipid metabolism. It has been identified as a potential antigen for glioblastoma, highly correlated with patient survival and antigen-presenting cell infiltration, representing a potential target for glioblastoma mRNA vaccine development [66]. Additionally, PLB1 rs117512489 variant is significantly associated with non-small cell lung cancer patient survival [67]. PLB1 has also been reported as a candidate risk gene for rheumatoid arthritis [68] and may be associated with juvenile dermatomyositis [69].

Lithostathine (FC = 0.40, p = 2.52$×10^{-2}$) had the third-smallest FC value, functioning in growth factor activity, phosphatase binding, and signal receptor activity, participating in antimicrobial humoral immune response mediated by antimicrobial peptides, cellular response to chemokines, response to gastrin, liver regeneration, and response to hypoxia. It has been reported to participate in Alzheimer's disease pathophysiology, significantly increasing in early disease

stages and remaining elevated throughout the disease course [70].

3.2.1.2 Enriched Biological Pathways Differential proteins from green tea vs. control comparison were mainly enriched in axon guidance, Ephrin receptor signaling pathway, cell migration, positive regulation of ERK1 and ERK2 cascade, angiogenesis, aorta development, semaphorin-plexin signaling pathway, neural crest cell migration, heart development, positive regulation of PI3K/Akt signal transduction, and sphingosine biosynthetic process (Figure 7 [Figure 7: see original paper]A). EGCG, the main catechin in green tea, has been reported to inhibit angiogenesis through multiple mechanisms including inducing apoptosis and cell cycle arrest, regulating miRNA expression profiles, and inhibiting VEGF binding to its receptor [71]. Plasma sphingosine concentration significantly increases 2 hours after green tea extract intake [72].

KEGG pathway enrichment analysis revealed significant enrichment in axon guidance, microRNAs in cancer, lysosome, MAPK signaling pathway, PI3K-Akt signaling pathway, and metabolic pathways (Figure 7B). Studies show that proanthocyanidins, important polyphenols in tea, can regulate miRNAs important in cancer, glucose, and lipid homeostasis [73]. Green tea bioactive component tea polysaccharides target lysosomes, inducing apoptosis through lysosome-mitochondria pathway-mediated caspase cascade, thereby inhibiting colon cancer CT26 cell proliferation [74]. Green tea active component EGCG promotes protein kinase C-α (PRKCA) expression, alleviating LPS-induced acute lung injury and inflammatory response, possibly related to PRKCA regulation of MAPK signaling pathway and affecting macrophage release of pro-inflammatory cytokines [75]. Green tea extract inhibits HepG2 cell growth and induces apoptosis via the PI3K/Akt signaling pathway [38], and EGCG inhibits cardiomyocyte apoptosis and restores autophagic flux through PI3K/Akt signaling pathway, alleviating myocardial ischemia/reperfusion injury [76].

3.2.2 Oolong Tea vs. Control Group Comparison between oolong tea and control groups identified 60 differential proteins (details in Supplementary Table 2). Random grouping analysis showed an average of 40.48 differential proteins, indicating at least 32.53% were not randomly generated.

3.2.2.1 Analysis of Differential Proteins Selenoprotein F (FC = ∞, p = 2.99$\times$10^{-2}$) showed complete appearance (present in oolong tea group but absent in control group). SELENOF gene polymorphisms and dysregulation are closely associated with cancer and neurodegenerative diseases, and due to selenium sensitivity, SELENOF may become a target in related disease pathological processes [77].

Endoribonuclease LACTB2 (FC = 3690.17, p = 4.69$\times$10^{-2}$) had the second-largest FC value among differential proteins. LACTB2 has been reported as a biomarker for nasopharyngeal carcinoma radiotherapy prognosis and a novel therapeutic target for improving radiosensitivity [78].

Oolong tea vs. control comparison also identified Myosin light chain 12A and Phospholipase B1, with Myosin light chain 12A (FC = 487.56, p = 1.85$\times10^{\{-2\}}) having the third-largest FC value and Phospholipase B1 (FC = 0.25, p = 3.75\times10^{\{-2\}}$) the fifth-smallest FC value.

Ectonucleotide pyrophosphatase/phosphodiesterase 2 (FC = 12.84, p = 1.58$\times10\^{}\{-2\}$) had the fifth-largest FC value, reported as a potential biomarker for breast cancer occurrence and prognosis [79] and a potential diagnostic and prognostic biomarker for hepatocellular carcinoma [80]. ENPP2 also serves as an independent prognostic marker for predicting biochemical recurrence (postoperative PSA $ $0.2 ng/mL) in prostate cancer patients [81].

Type I keratin KA11 (FC = 0.04, p = 3.54$\times10^{\{-2\}}) had the smallest FC value, participating in inflammatory response, skin bar
2\}) had the fourth-largest FC value, participating in vacuole acidification. von Willebrand factor (FC = 4.94, p = 6.29\times10\^{}\{-4\}$) had the smallest p-value. Studies show von Willebrand factor serves as a biomarker for diagnosing clinically significant portal hypertension (CSPH) and severe portal hypertension (SPH) in cirrhotic patients, showing moderate correlation with hepatic venous pressure gradient (HVPG) measurements [82]. VWF also functions as a biomarker for heart valve disease, assessing disease severity, reflecting treatment intervention effects, and predicting patient prognosis [83]. Additionally, VWF is a biomarker for predicting venous thromboembolism risk and a potential target for prevention and treatment [84], as well as a novel potential prognostic biomarker for lung adenocarcinoma [85].

3.2.2.2 Enriched Biological Pathways Differential proteins from oolong tea vs. control comparison were mainly enriched in intermediate filament organization, keratinization, peptide cross-linking, organ regeneration, complement activation, epithelial morphogenesis, response to lipopolysaccharide, positive regulation of blood coagulation, and phosphatidylcholine catabolism (Figure 8 [Figure 8: see original paper]A). Studies show oolong tea polyphenols reduce serum lipopolysaccharide levels, alleviate LPS-induced microglial activation, improve neuroinflammation and neuronal damage, and reduce neurotoxic metabolite glutamate increase [86].

KEGG pathway enrichment analysis revealed significant enrichment in lysosome, complement and coagulation cascades, PI3K-Akt signaling pathway, and Staphylococcus aureus infection (Figure 8B). Studies demonstrate that PI3K-Akt signaling pathway is one of the most enriched pathways for differentially expressed genes after oolong tea polyphenol intervention [31]. Oolong tea water-soluble extracts protect Caenorhabditis elegans against Staphylococcus aureus infection [87].

3.2.3 Black Tea vs. Control Group Comparison between black tea and control groups identified 94 differential proteins (details in Supplementary Table 2). Random grouping analysis showed an average of 41.39 differential proteins, indicating at least 55.97% were not randomly generated.

3.2.3.1 Analysis of Differential Proteins Selenoprotein F, Suppressor of tumorigenicity 14 protein homolog, Dmx-like 1, and von Willebrand factor were also identified in black tea vs. control comparison. Selenoprotein F (FC = ∞, p = 9.09$×10^{-3}$) and Suppressor of tumorigenicity 14 protein homolog (FC = ∞, p = 1.87$×10^{-2}$) both showed complete appearance (present in black tea group but absent in control group). Dmx-like 1 ($FC = 12.49, p = 2$) had the fifth-largest FC value, while von Willebrand factor ($FC = 4.74, p =$ 8.26×10^{-4}$) had the fifth-smallest p-value.

Synaptobrevin homolog YKT6 (FC = 35815.21, p = 4.37$×10^{-2}$) had the third-largest FC value. Studies report YKT6 as an independent prognostic biomarker and potential immunotherapy target for oral squamous cell carcinoma [88], with upregulated expression closely associated with hepatocellular carcinoma progression, serving as a potential biomarker for poor prognosis [89]. YKT6 is also reported as a potential prognostic and immunotherapy biomarker for cervical squamous cell carcinoma and adenocarcinoma [90], and a potential prognostic and diagnostic biomarker for lung adenocarcinoma [91].

PKHD1 ciliary IPT domain containing fibrocystin/polyductin (FC = 149.77, p = 2.47$×10^{-2}$) had the fourth-largest FC value, reported as a potential prognostic biomarker for colon cancer [92].

Keratin 2 (FC = 0.24, p = 2.33$×10^{-2}$) had the smallest FC value, participating in keratinocyte activation, development, migration, proliferation, intermediate filament organization, and keratinization.

Ephrin-A1 (FC = 4.30, p = 5.43$×10^{-5}$) had the smallest p-value, participating in angiogenesis, regulation of MAPK cascade, aortic valve morphogenesis, mitral valve morphogenesis, and axon guidance. It has been reported as a prognostic biomarker and potential therapeutic target for cervical cancer [93], with serum Ephrin-A1 also serving as a potential diagnostic biomarker for colorectal cancer [94].

Complement factor B (FC = 0.56, p = 4.32$×10^{-4}$) had the second-smallest p-value, participating in complement activation, positive regulation of apoptotic cell clearance, response to bacteria, response to lipopolysaccharide, and response to nutrients. It has been reported as a serum biomarker for pancreatic cancer [95] and a potential biomarker and therapeutic target for lung adenocarcinoma [96].

Interleukin 6 cytokine family signal transducer (FC = 4.47, p = 5.50$×10^{-4}$) had the fourth-smallest p-value, reported as a predictive and prognostic biomarker for breast cancer [97].

Differential protein Complement C1r subcomponent like (FC = 5.32, p = 8.53$×10^{-3}$) has been reported as a prognostic biomarker for hepatocellular carcinoma [98] and an independent poor prognostic biomarker for glioma patients, representing a potential clinical immunotherapy target [99].

3.2.3.2 Enriched Biological Pathways Differential proteins from black tea vs. control comparison were mainly enriched in blood coagulation, complement activation, proteolysis, interleukin-11-mediated signaling pathway, axon guidance, and Ephrin receptor signaling pathway (Figure 9 [Figure 9: see original paper]A).

KEGG pathway enrichment analysis revealed significant enrichment in complement and coagulation cascades, PI3K-Akt signaling pathway, viral protein interaction with cytokine and cytokine receptor, MAPK signaling pathway, Ras signaling pathway, JAK-STAT signaling pathway, and microRNAs in cancer (Figure 9B). Studies demonstrate that black tea extract inhibits HepG2 cell growth and induces apoptosis via the PI3K-Akt signaling pathway [38]. Theaflavins and thearubigins, main polyphenols in black tea, induce apoptosis in human malignant melanoma cells (A375 cells) through JNK and p38 MAPK signaling pathways [100]. Additionally, theaflavin disrupts lipid raft structure, preventing RET anchoring to the cell membrane and inhibiting downstream PI3K/Akt/Bad and Ras/Raf/ERK pathways while activating p38 MAPK/caspase-8 pathway, inducing medullary thyroid carcinoma cell apoptosis [101]. Black tea also inhibits tumor-induced thymic involution by preventing tumor-induced downregulation of IL-7Rα in thymocytes, maintaining JAK3 and STAT5 phosphorylation, and protecting the JAK-STAT signaling pathway [102].

3.2.4 Pu-erh Tea vs. Control Group Comparison between Pu-erh tea and control groups identified 95 differential proteins (details in Supplementary Table 2). Random grouping analysis showed an average of 34.63 differential proteins, indicating at least 63.55% were not randomly generated.

3.2.4.1 Analysis of Differential Proteins Suppressor of tumorigenicity 14 protein homolog, 45 kDa calcium-binding protein, Myosin light chain 12A, Crk-like protein, von Willebrand factor, Ephrin-A1, and Complement factor B were also identified in Pu-erh tea vs. control comparison. Suppressor of tumorigenicity 14 protein homolog (FC = ∞, p = 3.07$\times10^{\{-2\}})showedcompleteappearance(presentinPu-erhteagroupbutabsentincontrolgroup).45kDacalcium-bindingprotein(FC = 3\})hadthesmallestFCvalue.Myosinlightchain12A(FC = 190.12, p = 1.16\times10^{\{-2\}})hadthesecond-largestFCvalue.Crk-likeprotein(FC = 26.23, p = 4.70\times10^{\{-3\}})hadthethird-largestFCvalue.vonWillebrandfactor(FC = 3.99, p = 2.13\times10^{\{-2\}})hadthefourth-largestFCvalue.Ephrin-A1(FC = 3.62, p = 3.39\times10^{\{-4\}})hadthesixth-largestFCvalueandfourth-smallestp-value.ComplementfactorB(FC = 0.47, p = 1.10\times10\^{}\{-5\}$) had the smallest p-value.

Cadherin, EGF LAG seven-pass G-type receptor 2 (FC = 0.16, p = 3.07$\times10\^{}\{-2\}$) had the second-smallest FC value, functioning in calcium ion binding and G protein-coupled receptor activity, participating in cerebrospinal fluid secretion, motor neuron migration, neural plate regionalization, brain ventricular system development, and Wnt signaling pathway. It has been reported as a prognostic

biomarker for hepatocellular carcinoma [103].

Cytochrome b5 (FC = 0.2, p = 3.85$×10^{-2}$) had the third-smallest FC value, reported as a novel biomarker for visceral obesity intervention and safe therapeutic target [104], and a potential biomarker for distinguishing well-differentiated hepatocellular carcinoma [105].

Tissue kallikrein (FC = 1.65, p = 1.77$×10^{-6}$) had the smallest p-value, participating in positive regulation of acute inflammatory response, positive regulation of apoptotic process, and systemic arterial blood pressure regulation. It has been reported as a potential biomarker for papillary and clear cell renal cell carcinoma [106].

Acid ceramidase (FC = 1.96, p = 6.96$×10^{-5}$) had the third-smallest p-value, functioning in fatty acid amide hydrolase activity and N-acylsphingosine deacylase activity, participating in cellular response to tumor necrosis factor, ceramide biosynthetic and catabolic processes, fatty acid metabolism, regulation of steroid biosynthesis, sphingosine biosynthetic process, and regulation of programmed necrotic cell death. ASAH1 has been reported as a potential diagnostic biomarker for asthma [107], a plasma marker for recurrent glioblastoma progression [108], and a biomarker for predicting lymph node status in breast cancer patients [109].

Lysosomal acid phosphatase (FC = 1.77, p = 4.62$×10^{-4}$) had the fifth-smallest p-value, reported as a biomarker for neuronal ceroid lipofuscinosis in children [110].

Differential protein Cadherin-13 (FC = 0.66, p = 7.23$×10^{-4}$) has been reported to interfere with adipocyte differentiation potential, serving as a marker of adipose tissue plasticity and reflecting adipose tissue health status [111]. It also functions as a prognostic biomarker and therapeutic target for clear cell renal cell carcinoma [112].

3.2.4.2 Enriched Biological Pathways Differential proteins from Pu-erh tea vs. control comparison were mainly enriched in homophilic cell adhesion via plasma membrane adhesion molecules, inflammatory response, complement activation, cellular response to tumor necrosis factor, lipid metabolism, negative regulation of lipid storage, systemic arterial blood pressure regulation, glycoside catabolism, hyaluronic acid metabolism, and mitral valve morphogenesis (Figure 10 [Figure 10: see original paper]A).

KEGG pathway enrichment analysis revealed significant enrichment in lysosome, complement and coagulation cascades, ECM-receptor interaction, PI3K-Akt signaling pathway, glycosaminoglycan degradation, and sphingolipid metabolism (Figure 10B). Studies show that theabrownin, a bioactive component of dark tea, regulates glucose and lipid metabolism via the IRS-1/PI3K/Akt signaling pathway [46], and Pu-erh tea extract regulates alcohol-induced metabolic disorders by modulating sphingolipid metabolism

[113].

3.2.5 Black Coffee vs. Control Group Comparison between black coffee and control groups identified 46 differential proteins (details in Supplementary Table 2). Random grouping analysis showed an average of 37.21 differential proteins, indicating at least 19.11% were not randomly generated.

3.2.5.1 Analysis of Differential Proteins Adhesion G protein-coupled receptor F5 (FC = 0.18, p = 4.47$×10^{-2}$) had the smallest FC value, functioning in G protein-coupled receptor activity, participating in energy reserve metabolism, adipocyte differentiation, glucose homeostasis, macrophage activation and negative regulation, and phospholipid biosynthesis. It has been reported as a prognostic biomarker for clear cell renal cell carcinoma [114], with plasma ADGRF5 levels as a potential biomarker for proliferative diabetic retinopathy [115].

14-3-3 protein gamma (FC = 0.21, p = 1.23$×10^{-2}$) had the second-smallest FC value, participating in cellular response to insulin stimulus, negative regulation of TORC1 signaling, regulation of synaptic plasticity, regulation of neuron differentiation, and cellular response to glucose starvation. It has been reported as a prognostic biomarker for lung adenocarcinoma and potential drug response marker [116], potential prognostic biomarker and therapeutic target for oral squamous cell carcinoma [117], prognostic biomarker related to shunt responsiveness in idiopathic normal pressure hydrocephalus patients [118], potential cerebrospinal fluid biomarker for Alzheimer's disease [119], and diagnostic marker for cognitive impairment in Parkinson's disease patients [120].

ADAMTS-like protein 4 (FC = 0.23, p = 6.85$×10^{-3}$) had the third-smallest FC value, participating in apoptosis, reported as a biomarker for primary glioblastoma multiforme [121] and prognostic biomarker for Burkitt lymphoma [122].

Enhancer of mRNA-decapping protein 4 (FC = 3.90, p = 2.99$×10^{-2}$) had the largest FC value, participating in deadenylation-independent decapping of nuclear-transcribed mRNA and nervous system development.

Lymphocyte cytosolic protein 1 (FC = 2.92, p = 2.53$×10^{-2}$) had the second-largest FC value, functioning in calcium ion binding, GTPase binding, and integrin binding, participating in protein kinase A signaling and T cell activation in immune response. Studies show LCP1 as a potential target for treating obesity-related metabolic disorders [123], biomarker for oral squamous cell carcinoma [124], and potential diagnostic and prognostic biomarker for triple-negative breast cancer [125].

3.2.5.2 Enriched Biological Pathways Differential proteins from black coffee vs. control comparison were mainly enriched in wound healing-related angiogenesis, protein glycosylation, lysosome organization, regulation of

lipopolysaccharide-mediated signaling pathway, lipid catabolism, immune response, positive regulation of calcium ion import, and midbody abscission (Figure 11 [Figure 11: see original paper]A). KEGG pathway enrichment analysis revealed significant enrichment in lysosome, metabolic pathways, glycosphingolipid biosynthesis, sphingolipid metabolism, and glycosaminoglycan degradation (Figure 11B).

4 Discussion

The results demonstrate that the urine proteome can reflect changes in rats after seven consecutive days of consuming teas with different fermentation levels or black coffee. The study also compared urinary proteins in control rats (consuming sterilized water) before and after the 7-day period, finding differential proteins enriched in development-related biological processes including skeletal system morphogenesis, heart development, and cartilage development. This indicates the urine proteome can reflect short-term growth and development changes in rats, consistent with our laboratory's previous findings [126]. Therefore, to minimize various influencing factors, this study employed both before-and-after and between-group comparisons, using the former to reduce individual variation and the latter to avoid short-term growth and development effects, thereby exploring the effects and distinctions of tea and black coffee in detail.

Venn diagrams were used to display the overlap of differential proteins identified from before-and-after and between-group comparisons across the four tea groups and black coffee group (Figure 12 [Figure 12: see original paper]), revealing few shared and many unique differential proteins among groups. Venn diagrams of biological processes (Figure 13 [Figure 13: see original paper]) and KEGG pathways (Figure 14 [Figure 14: see original paper]) enriched by differential proteins from the five experimental groups also showed few shared and many unique biological processes and pathways. This demonstrates that the urine proteome can distinguish the effects of different fermented teas and black coffee on the body, with highly specific impacts.

Furthermore, this study found that multiple differential proteins have been reported as biomarkers for cancer, cardiovascular disease, and other diseases, particularly those showing substantial changes in response to tea and black coffee. This provides valuable information for urinary disease biomarker research, suggesting that beverage consumption, including tea and black coffee, should be considered in future clinical applications of urinary disease biomarkers and related studies. Clinical urine sample collection may need to consider beverage restrictions, and single biomarkers may produce false positives, necessitating biomarker panels to improve accuracy.

5 Conclusion

The urine proteome can comprehensively and systematically reflect changes in rats after seven consecutive days of consuming four types of fermented teas or black coffee, with highly specific effects from different fermented teas and black coffee on the body.

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