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Distinct Urinary Metabolomic Profiles in Cigarette vs. Next-Generation Nicotine Users

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Abstract

Background: Conventional cigarette smoking remains a leading cause of preventable morbidity and mortality, primarily due to systemic exposure to toxicants generated during combustion. Meanwhile, the rising use of electronic cigarettes (EC) and heated tobacco products (HTP) is reshaping nicotine use patterns; however, their biological effects differ from those of conventional cigarettes and remain incompletely characterized beyond targeted biomarkers. Urinary metabolomics provides a sensitive, integrative readout of host metabolism and xenobiotic processing, enabling detection of early biochemical perturbations and exposure signatures relevant to toxicological assessment and harm evaluation [1,2]. **Objective:** This study aimed to compare urinary metabolomic profiles among EC users, HTP users, conventional tobacco (CT) smokers, and non-smokers, to identify product-associated biochemical perturbations relevant to systemic toxicological effects. **Methods:** Urine samples from CT smokers, EC users, HTP users, and non-smokers (n=10 per group) were analyzed by gas chromatography-mass spectrometry (GC-MS) [3]. Metabolites were putatively identified using spectral library matching and comparison with standard compounds. Semi-quantitative data were assessed using multivariate and univariate statistical analyses. **Results:** A total of seventy-five urinary metabolites were consistently detected across all participant groups, including amino acids, organic acids, sugars, and other small polar compounds. While multivariate analyses showed no clear separation among the four groups, pairwise comparisons revealed significant metabolic differences. Compared with non-smokers, CT users showed increased levels of combustion-derived compounds (quinate, furoylglycine, guaiacol) and hippurate conjugates (2-hydroxyhippurate). EC users exhibited higher levels of amino acid metabolites (β-alanine and 3-methylhistidine) compared with both non-smokers and CT users, while energy-related intermediates were reduced (citrate) relative to CT users. HTP users showed elevated hippurate conjugates (2-hydroxyhippurate) compared with CT users, and lower levels of amino acid, carbohydrate, and nucleotide metabolites (3-methylhistidine, scyllo-inositol, 3-aminoisobutyrate, and uracil) compared with EC users. **Conclusions:** Urinary metabolomics identified product-specific metabolic signatures that distinguish CT from EC/HTP use, demonstrating its sensitivity as a tool for toxicological profiling of novel nicotine products.

Keywords: electronic cigarettes; heated tobacco products; urine; metabolomics

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