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Opioid use affects antioxidant activity and purine metabolism: preliminary results,[†,‡]

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Abstract

Objective—More must be learned about metabolic and biochemical alterations that contribute to the development and expression of drug dependence. Experimental opioid administration influences mechanisms and indices of oxidative stress, such as antioxidant compounds and purine metabolism. We examined perturbations of neurotransmitter-related pathways in opioid dependence (OD).

Methods—In this preliminary study, we used a targeted metabolomics platform to explore whether biochemical changes were associated with OD by comparing OD individuals (n = 14) and non-drug users (n = 10).

Results—OD patients undergoing short-term methadone detoxification showed altered oxidation–reduction activity, as confirmed by higher plasma levels of α- and γ- tocopherol and increased GSH/GSSG ratio. OD individuals had also altered purine metabolism, showing increased concentration of guanine and xanthosine, with decreased guanosine, hypoxanthine and hypoxanthine/xanthine and xanthine/xanthosine ratios. Other drug use in addition to opioids was associated with partly different biochemical changes.

Conclusions—This is a preliminary investigation using metabolomics and showing multiple peripheral alterations of metabolic pathways in OD. Further studies should explore the metabolic profile of conditions of opioid abuse, withdrawal and long-term abstinence in relation to agonist and antagonist treatment and investigate biochemical signatures of opioid substances and medications.

Keywords

metabolomics; metabonomics; addiction; metabolic profiling; opioid detoxification; methadone

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INTRODUCTION

Substance misuse is commonly associated with health risks and increased susceptibility to organ damage, as in the case of alcohol abuse and liver injury (Zakhari and Li, 2007). Less is known on the nature of biochemical changes and their contribution to the development of chronic toxicity and drug dependence. Metabolomics, one of the newest “omics” approaches, provides powerful tools for revealing perturbations in metabolic pathways and networks in human disease and their treatment (Kaddurah-Daouk *et al*., 2008; Kristal *et al*., 2007a; Kristal *et al*., 2007b; Paige *et al*., 2007; Rozen *et al*., 2005). The metabolome defines a metabolic state as regulated by a net of interactions between genes and environment and provides useful information to bridge the gap between genotype and phenotype. This methodology offers a simultaneous analysis of multiple low-molecular weight compounds for the interpretation of biochemical events, and has been utilized to define metabolic “signatures” of medications, such as antipsychotics (Kaddurah-Daouk *et al*., 2007). Opioid substances are known to induce biochemical alterations (Jiang *et al*., 2003; Ueda *et al*., 2003); thus the metabolomic characterization of patients who abuse opioids can be of major interest in understanding potential health risks and underlying mechanisms.

In experimental conditions, opioid administration has been associated with changes in oxidative stress mechanisms (Sharma *et al*., 2007). Oxidative damage, known as oxidative stress, is the result of accumulation of free radicals insufficiently neutralized by antioxidant agents (Sies, 1991). Reliable indices of oxidative stress include modifications of glutathione and tocopherol levels. Reduced glutathione, α- and γ- tocopherol are chain breaking antioxidant agents responsible for scavenging the free radicals and suppress peroxidation cooperatively in aqueous and lipid-soluble regions of the cell (Bandopadhyay *et al*., 1999; Chen, 1989). Experimental changes in oxidative status induced by opioid substances affect cell energy, involve nucleotide metabolism and may influence purine compounds levels (Chen *et al*., 2007; Christie, 2008).

The initial objective is to recognize whether these changes are associated with opioid abuse and dependence. In this preliminary study, we analyzed oxidation–reduction activity and purine metabolites in plasma of opioid dependent (OD) patients during methadone detoxification, using a liquid chromatography electrochemical array detection (LCECA) metabolomics platform that has been employed to map biochemical changes and identify monoaminergic signatures of several CNS disorders, including amyotrophic lateral sclerosis and Parkinson (Bogdanov *et al*., 2008; Rozen *et al*., 2005).

METHODS

Subjects and procedures

Fourteen participants were recruited among OD individuals 18 years of age or older enrolled in a methadone detoxification study described in detail elsewhere (Mannelli *et al*., 2009), and 10 were non-drug dependent controls from an ongoing investigation in the same catchment area. The sample size was deemed sufficient based on previous investigations. We identified initial signatures of psychiatric and neurologic disorders using the same metabolomics platform in comparable samples (Rozen *et al*., Paige *et al*.). Protocols were approved by the Institutional Review Board of Duke University. All subjects provided oral and written informed consent and the study was conducted according to the principles expressed in the Declaration of Helsinki of 1975, as revised in 1983. Individuals received medical and psychiatric evaluation with routine clinical laboratory tests at screening. The diagnosis of opioid dependence (OD) with physiological dependence was confirmed by the DSM IV checklist for that disorder (APA, 2000) and by urine drug test (UDT). The condition of current dependence on substances other than opioids or nicotine was excluded.

Individuals who used or abused other substances were included only if their primary drug was an opioid drug. Subjects were also excluded in case of pregnancy, history or presence of medical or neurological disorder, suicide risk, DSM IV diagnosis of psychotic disorder, major depression and bipolar disorder. The Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (SCID), and the Addiction Severity Index (ASI) (McLellan *et al*., 1992) were used to obtain complete psychiatric and drug use history.

OD patients were participating in a 6-day inpatient detoxification and received oral methadone daily between 9 and 10 a.m., following withdrawal assessment: 30 mg on day 1, tapered by 5mg daily, with treatment completion and discharge on day 6. Severity of opioid withdrawal was evaluated using the Clinical Opiate Withdrawal Scale (COWS) (Wesson and Ling, 2003), an 11-item (0–48) interviewer-administered questionnaire. Control subjects did not receive study treatment.

UDTs were performed for opioids, cocaine, amphetamine, methamphetamine, and cannabis (UDT-5-Panel CLIA Waived Integrated Drug Testing Cup). Alcohol use was self-reported and confirmed by breathalyzer.

Blood samples for analysis were collected on days 2 or 3 of treatment from OD participants, to reduce the influence of pre-treatment differences in opioid or other drug use. All samples were collected between 10 and 11 a.m. Collection was completed over 18 months. Plasma was extracted, centrifuged, and stored frozen before shipping.

Sample preparation and analysis

Metabolomics analyses were performed by one of the authors (WM, Bedford VAMC, MA). Plasma samples (125 μl) were prepared by extraction in acidified acetonitrile and analyzed by high performance liquid chromatography (HPLC) coupled with coulometric array detection as described elsewhere (Bogdanov *et al*., 2008; Rozen *et al*., 2005). Briefly, the LCECA metabolomics platform contains 16 coulometric electrode array systems, allowing differential detection and quantification of small molecules on the basis of their oxidation–reduction potentials. The platform used for this metabolic profiling has been used for the study of the tryptophan and tyrosine pathways (Beal *et al*., 1990; Godefroy *et al*., 1990; Volicer *et al*., 1985). The robustness of the platform, its reproducibility, and sensitivity have been well documented (Rozen *et al*., 2005). During the preparation of the samples, pools were created from equal volume subaliquots of all samples. Pooled samples represent the maximum analytical complexity expected from among sample variation in the study. Aliquots of the pool were analyzed after each seven samples in the run sequence. All samples and pools were analyzed against the middle pool in the run sequence.

We selected for analysis peaks that showed good precision across replicated assays of the pool (standard deviation of peak high <10% of the main peak high). By this criterion, we identified approximately 700 peaks, of which 39 matched known compounds in our 62-component library of standard electrochemical signatures (Kristal *et al*., 2007b) and are reported in Table 1.

Data mining and statistical analysis

Baseline demographic and clinical characteristics were analyzed in three groups of subjects: controls (n = 10), OD patients with no recent drug use at admission, except for opioids and tobacco (n = 6, “opioid only,” or “OO” group), and OD patients with recent use of other drugs (n = 8, “opioid and other drugs,” or “OOD” group) (Table 2). Baseline demographic and clinical characteristics were compared among groups using (1) Kruskal–Wallis tests for continuous variables meaningful all three groups (age and weight), (2) Wilcoxon rank sum

tests for continuous variables, when the comparisons were between two groups (ASI drug score, ASI alcohol score, withdrawal score at admission, withdrawal score at evaluation, methadone dose at evaluation), and (3) Fisher's exact test, two-sided, for proportions (male %, tobacco use %).

We then determined by Wilcoxon rank sum tests which compounds among those listed in Table 1 were statistically different between: (A) controls (n = 10) versus all OD patients (n = 14) and (B) controls versus the OO group (n = 6).We also compared the following compound-level ratios between groups: hypoxanthine/xanthine, xanthine/xanthosine, tryptophan/kynurenine, tryptophan/tyramine, homovanillic acid/5-hydroxyin-doleacetic acid, tyrosine/4-hydroxyphenyllactate, glutathione/oxidized glutathione (GSH/GSSG). We hypothesized that measuring product to precursor's ratios within metabolic pathways and measuring ratios of compounds influenced by common enzymatic systems may reflect the existence of metabolic alterations or homeostasic failures in a certain disease or syndrome (Rozen *et al*., 2005). In the case of OD, biochemical differences between patients and controls might reveal differences in key reactions and suggest mechanisms of disease.

We accounted for multiple hypothesis testing by estimating cumulative false discovery rates (Q-values) based on the nominal p-values (qvalue package in R; www.r-project.org/ (Storey and Tibshirani, 2003 #222). For example, for the comparison between normal controls and OD subjects (Table 1), we estimate that among the 10 compounds and ratios with the lowest p-values (hypoxanthine, hypoxanthine/xanthine, γ-tocopherol, *N*-methyl serotonin, xanthine/xanthosine, GSH/GSSG, guanosine, α-tocopherol, xanthosine, and guanine), approximately one ($0.13 \times 10 = \sim 1$, where $Q = 0.13$ for these 10 compounds and ratios) was different by chance. Similarly, in the comparison between controls and OO patients, we estimate that among the seven compounds and ratios with the lowest p-values (xanthine/ xanthosine, xanthosine, γ- tocopherol, hypoxanthine/xanthine, hypoxanthine, α- tocopherol, and guanosine), approximately one was different between the two groups by chance ($0.146 \times 7 = \sim 1$, where $Q = 0.146$ for these seven compounds and ratios).

RESULTS

Subjects

Characteristics of the sample are shown in Table 2. Subjects differed significantly only by age ($p = 0.03$, $H = 7.0$). In addition to opioids in all patients, recent use of other substances was detected in 57% of OD participants at admission. Three OD individuals (21.4%) reported use of alcohol in the 24 h preceding treatment. Six OD patients (42.9%) had positive UDTs for cocaine or cannabis. OD patients were all suffering severe opioid withdrawal at admission, according to COWS scores and definitions (Wesson and Ling, 2003). One or two days later, at the time of sample collection, they received either 20 or 25 mg of methadone and showed mild or less than mild withdrawal discomfort. In the "non-drug users" group, three subjects were tobacco smokers (30%) and no one used alcohol. No other pharmacological treatment beside methadone was recorded at the time of biological sampling.

Oxidation–reduction activity (Table 1)

Differences were found between treated OD patients and non-drug dependent volunteers in the concentration of compounds involved in the process of oxidation and reduction. The ratio of GSH/GSSG, a quotient often used to measure the condition of oxidation, was significantly higher in the OD group ($p = 0.005$, $Q = 0.032$).

The concentrations of α- and γ- tocopherol were significantly increased among drug-dependent individuals treated with methadone (respectively $p = 0.01$ and $Q = 0.05$; $p = 0.001$ and $Q = 0.02$).

The measures of redox activity that were significantly different between drug abusers and controls are shown in Figure 1.

Purine metabolites (Table 1)

Significant changes were revealed by the analysis of purine metabolites in OD patients. Higher levels of guanine ($p = 0.03$, Q = 0.13) and xanthosine ($p = 0.01$, $Q = 0.06$) were detected compared to controls. Patients showed lower concentrations of guanosine ($p = 0.005$, $Q = 0.03$) and hypoxanthine ($p = 0.0007$, $Q = 0.01$). In addition, the ratios hypoxanthine/xanthine and xanthine/xanthosine were significantly decreased in OD individuals (respectively $p = 0.0007$, $Q = 0.01$; $p = 0.003$ and $Q = 0.03$), while no significant difference was noted for xanthine and uric acid.

Figure 2 shows purine metabolites and the ratios of purine metabolites that were significantly different between drug abusers and controls.

Monoamine pathways

There were no significant differences between OD patients in treatment and controls in a variety of monoamines and precursors from the tryptophan, tyrosine and phenylalanine pathways. However, a significant increase of *N*-methylserotonin levels was detected among OD individuals compared to controls ($p = 0.001$, $Q = 0.02$).

Biochemical alterations in “opioid-only” (OO) patients

To investigate whether the use of other drugs detected at admission influenced the pattern of metabolic response, we repeated the comparisons including only those patients (OO, $n = 6$) who did not report alcohol use in proximity of detoxification and did not show positive urine for drugs other than opioids at screening. This group showed a pattern of oxidation–reduction activity and purine metabolites not dissimilar from the whole group of drug abusers. α- and γ- tocopherol were elevated ($p = 0.01$, $Q = 0.08$; $p = 0.005$ and $Q = 0.07$, respectively) in OO patients, but the GSH/GSSG ratio was not significantly different from non-drug abusers ($p = 0.12$, $Q = 0.37$). (Figure 1). OO individuals also showed elevated xanthosine ($p = 0.001$, $Q = 0.02$), reduced guanosine ($p = 0.02$, $Q = 0.15$), and hypoxanthine ($p = 0.01$, $Q = 0.08$), while guanine concentration was not significantly different from controls ($p = 0.15$, $Q = 0.37$) (Figure 2). The ratios hypoxanthine/xanthine and xanthine/xanthosine were decreased in the OO group compared to controls (respectively $p = 0.008$, $Q = 0.08$; $p = 0.0003$ and $Q = 0.01$).

Subjects who had recently abused only opioid substances showed marginally significant higher concentrations of *N*-methylserotonin compared to controls ($p = 0.045$, $Q = 0.23$)

Comparisons of all metabolites and metabolite ratios between OO and control groups are available upon request.

DISCUSSION

OD patients undergoing methadone detoxification showed different peripheral levels of antioxidant activity and purine metabolites when compared with non-drug users. In particular, the redox status of substance abusers was characterized by increased α- and γ-tocopherol levels and GSH/GSSG ratio. In the same individuals, altered purine metabolism

was associated with increased concentration of guanine and xanthosine, decreased guanosine, hypoxanthine and hypoxanthine/xanthine and xanthine/xanthosine ratios. Whether any of these changes can serve as a biological marker of disease or response to methadone treatment needs to be studied in prospective longitudinal studies.

Redox measures

Heroin or morphine administration and withdrawal are associated with reduced antioxidant defenses in animals (Guzman *et al*., 2006; Miskevich *et al*., 2007; Pan *et al*., 2005; Payabvash *et al*., 2007), humans (Goudas *et al*., 1999; Zhou *et al*., 2000, 2001), and other systems (Coban *et al*., 2007; Gulcin *et al*., 2004). Opioid-induced oxidative stress is inconsistently modified by the administration of antioxidant agents, including tocopherol and glutathione (Mori *et al*., 2007; Pancehnko *et al*., 2004; Pan *et al*., 2005; Qiusheng *et al*., 2005; Xu *et al*., 2006; Zhang *et al*., 2004). We found increased GSH/GSSG ratio and α- and γ-tocopherol levels in substance abusers that received methadone. Elevated serum α-tocopherol and enhanced antioxidant activity were previously observed following methadone maintenance, compared with heroin abusers (Díaz-Flores Estévez *et al*., 2004; Rodriguez-Delgado *et al*., 2002). Accordingly, indices of oxidation such as reactive oxygen species were reduced in OD patients undergoing short detoxification or controls, compared with active abusers (Pereska *et al*., 2007). Although in our OD sample, the rapidly reduced withdrawal discomfort following methadone administration (Table 2) may be consistent with improved redox status, the influence of treatment cannot be determined with a single evaluation.

Purine metabolism

Preclinical investigations have identified multiple roles of purines in the brain, including energy metabolism, trophic functions, signaling, and neuromodulation (Boucsein C *et al*., 2003; Burnstock, 2008; Rathbone *et al*., 1999). Acute administration of morphine enhances purine catabolism lowering cell energy, a condition that reverts following drug discontinuation, but not with chronic administration in rodents (Di Francesco *et al*., 1998; Enrico *et al*., 1997; Liu *et al*., 2003, 2007). In type I diabetes, a model of chronic metabolic disease, increased mithochondrial purine catabolism is reactive to antioxidant offenses and has been measured by changes in hypoxanthine, guanine, guanosine, and xanthosine, similar to what found in plasma of OD patients (Kristal *et al*., 1999). Reduced hypoxanthine/xanthine and xanthine/xanthosine ratios confirm the existence of altered metabolic activities (Kulikowska *et al*., 2004). Uric acid and hypoxanthine levels in OD subjects were comparable or significantly lower than in controls. Diabetes, alcohol abuse, or acute cardiovascular disorders are initially associated with higher plasma levels of the final bio-products of purine pathways, which decrease in severe or chronic conditions (Kristal *et al*., 1999; Turgan *et al*., 1999; Yamamoto *et al*., 2005). Plasma guanine and xanthosine were elevated in OD patients. This could mean a potential gain of energy, through a conversion to their corresponding nucleotides via salvage pathways (Barsotti *et al*., 2005). However, accumulation can occur also in case of reduced elimination following an excessive metabolic workload, similar to what is observed in patients with chronic renal failure (Niwa *et al*., 1998). Within the multi-dimensional frame of drug-dependence, metabolomics analysis can follow patterns of biochemical changes and lead to formulate pathogenetic hypotheses at different levels. One example is offered by the neurotrophic and modulatory effects of guanine-based purines. The combination of high guanine–low guanosine levels we found in OD patients is commonly associated with brain toxic insult and increased dopamine turnover in experimental models (Ciccarelli *et al*., 1999; Loeffler *et al*., 1998, 2000; Uemura *et al*., 1991). Elevated peripheral guanine binding protein levels were previously found in OD patients receiving methadone (Linseman and Loucks, 2008; Manji *et al*., 1997). Guanine and guanine-based purines also participate in the regulation of the Gabaergic and

Glutamatergic transmission, affecting motivation, learning, memory, and anxiety (Majumder *et al*., 2007; Schmidt *et al*., 2007). This raises questions on whether increased guanine levels are equivalent to enhanced central purinergic transmission in response to oxidative damage, confirming hypotheses of purinergic neuromodulation of withdrawal and drug-seeking behaviors (Capasso and Loizzo, 2001; Majumder *et al*., 2007). To this end, future investigations should initially characterize peripheral purines patterns associated with treatment and recovery, as opposed to those modifications that accompany opioid abuse and withdrawal.

Monoamines alterations and use of other drugs

Although dopamine, serotonin, and noradrenaline contribute to the expression of OD (Espejo *et al*., 2001; Kish *et al*., 2001), no significant plasma alterations of the neurotransmitters and the majority of the metabolites were detected. Similar results adopting different evaluations methods were previously explained with the development of adaptive changes following chronic opioid use (Macedo *et al*., 1995). Only, *N*-methylserotonin levels were found elevated in drug abusers. *N*-methylserotonin is a congener of serotonin and with its derivative bufotenine has shown psychotropic and hallucinogenic effects (Takeda, 1994). Both compounds are elevated in psychiatric disorders characterized by altered perceptions and hallucinations (Takeda, 1994; Takeda *et al*., 1995).

Polydrug abuse is a common clinical observation and was investigated for the ability to affect metabolic pathways. Recent use of other drugs was not associated with increased drug or alcohol use severity, or more intense withdrawal discomfort in our sample (Table 2). Non-opioid drug use in the 30 days prior to detoxification was reported by 12/14 (85.7%) OD patients in the ASI interview. Plasma guanine levels and the ratio GSH/GSSG were not significantly different between controls and OD patients who did not use other drugs in proximity of detoxification (the OO patients). This is consistent with the hypothesis that the co-use of different drugs may reflect different patterns of intensity of neurobiological responses to oxidative stress (Parrott *et al*., 2007). *N*-methylserotonin was found more elevated when OD patients who abused non-opioid drugs were included in the analysis. Given the relatively small number of subjects, we could not determine if the effect was associated with non-opioid drug use.

Limitations

Although OD patients and controls were carefully characterized and the biological differences found were strong and significant, the rather small sample reduced the ability to control for confounding factors and the results should be viewed as preliminary.

Besides the discussed influence of non-opioid drug use, the number of smokers was not significantly different between groups, however individual smoking patterns were not investigated and their influence cannot be excluded. Confounding by nutritional factors has been suggested in metabolomics studies (Lenz, 2004; Walsh *et al*., 2007). The metabolomic platform we used was successfully employed to identify biochemical changes in neurologic disorders without controlling for dietary factors (Rozen *et al*., 2005). Future investigations and more stringent inclusion criteria will help determine whether diverse dietary habits and other individual factors, such as age, lifestyle, or ethnic background, constitute confounds in populations of drug abusers.

CONCLUSION

Notwithstanding the caveats, we provide evidence that metabolomics can be a valuable tool to assess biochemical changes in OD. This is a preliminary report on multiple metabolome

modifications of antioxidant activity and purine pathways in a small sample of drug-dependent individuals. Findings suggest the possibility of measuring biochemical alterations associate with the disease and its response to treatment. Further studies should characterize metabolite profiles for the conditions of opioid abuse, withdrawal and long-term abstinence in relation to different treatments and different drugs of abuse. A long-term goal is to associate metabolic perturbations with etiologic, pathogenetic, and prognostic aspects of drug dependence. The application of these principles will help formulate classification of the disease based on metabolomic profiles and identify biomarkers for drug response phenotypes, valuable tools in the process of discovering new medications, and developing new approaches to treatment.

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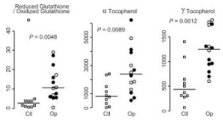

Figure 1.

Measures of oxidation–reduction activity that were significantly different between opioid dependent subjects (Op) and controls (Ctrl). Measures of oxidation–reduction activity that were significantly different between opioid dependent subjects (Op) and controls (Ctrl). In the Op group, open circles indicate patients with recent use of only opioids, while filled circles indicate individuals with other drug use in addition to opioids (see Methods). *p*-values refer to differences between all 14 opioid-dependent subjects and controls (Wilcoxon rank sum test). Horizontal lines indicate medians. Data represent means of values relative to the entire pool values for that metabolite

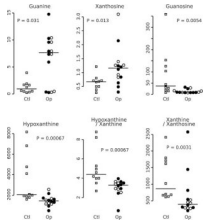

Figure 2.

Purine metabolites that were significantly different between opioid dependent patients (Op) and controls (Ctrl). Purine metabolites that were significantly different between opioid dependent patients (Op) and controls (Ctrl). In the Op group, open circles indicate patients with recent use of only opioids, while filled circles indicate individuals with other drug use in addition to opioids (see Methods). *p*-values refer to differences between all 14 opioid-dependent subjects and controls (Wilcoxon rank sum test). Horizontal lines indicate medians. Data represent means of values relative to the entire pool values for that metabolite

Table 1

Metabolic comparisons between opioid dependent (OD) individuals and controls

			Percentiles						
			Control			Opioid-dependent			
Metabolic pathway and compound or ratio	**Nominal *P***	***Q***	**25th**	**Median**	**75th**	**25th**	**Median**	**75th**	**Difference in medians**
One-CþGSH-redox-coupling									
Glutathione(reduced)/glutathione(oxidized)	0.0048	0.032	1.07	2.62	3.78	6.17	10.6	16.9	7.93
Glutathione(oxidized)	0.064	0.22	8.34	14.4	27.7	3.06	5.38	12.5	−8.97
Glutathione(reduced)	0.472	0.555	15.2	42.2	94.9	31.7	88.9	104	46.7
Tocopherol									
γ-tocopherol	0.0012	0.015	306	434	620	949	1250	1630	815
α-tocopherol	0.0089	0.046	373	812	1410	1410	2400	3690	1580
Purine									
Hypoxanthine	0.0007	0.014	1900	2060	4600	1180	1460	1660	−599
Hypoxanthine/xanthine	0.0007	0.014	3.98	4.38	5.18	2.88	3.27	3.5	−1.11
Xanthine/xanthosine	0.0031	0.025	671	863	1690	292	399	539	−464
Guanosine	0.0054	0.032	21.1	37.6	121	8.11	9.28	15.7	−28.3
Xanthosine	0.013	0.059	0.501	0.672	0.711	0.833	1.16	1.27	0.486
Guanine	0.031	0.13	0.476	0.94	1.68	1.84	7.64	9.59	6.7
Xanthine	0.108	0.279	455	614	886	364	450	660	−164
Guanine back wave	0.136	0.311	1.07	1.61	6.35	0	0.623	2.15	−0.987
Uric acid	0.977	0.875	10 200	11 100	14 000	9710	11 900	12 800	837
Phenylalanine									
2-Hydroxyphenylacetic acid	0.108	0.279	3.33	5.01	5.69	4.05	5.65	6.96	0.639
4-Hydroxyphenylacetic acid	0.212	0.381	37.8	55.2	59	44.2	63.3	107	8.03
4-Hydroxybenzoic acid	0.931	0.875	193	208	254	166	262	288	54.4
4-Hydroxyphenyllactic acid	0.977	0.875	60.7	93.4	135	76.9	93	106	−0.362
Tryptophan									
N-methylserotonin	0.0014	0.015	0.0492	0.0762	0.14	0.152	0.191	0.318	0.115
3-Hydroxyanthranilic acid	0.089	0.28	0.0277	0.0977	0.133	0.0747	0.165	0.3	0.067

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Metabolic pathway and compound or ratio	Nominal *P*	*Q*	Percentiles						Difference in medians
			Control			Opioid-dependent			
			25th	Median	75th	25th	Median	75th	
Kynurenine	0.122	0.296	278	360	488	364	443	612	83.4
Tryptophan/kynurenine	0.154	0.334	11.3	13.4	15.6	8.88	11.7	12.7	−1.73
5-Hydroxyindoleacetic acid	0.235	0.403	1.97	2.38	4.08	3.36	3.6	4.35	1.23
Anthranilic acid	0.259	0.427	8.62	9.93	15.5	4.84	7.21	13.6	−2.72
5-Hydroxytrptophan	0.312	0.494	0.578	0.785	1.63	0.512	0.607	1.13	−0.178
N-acetylserotonin	0.472	0.555	2.5	7.68	13.4	4.1	7.49	8.61	−0.192
Tryptophan	0.508	0.581	4060	4470	4830	3860	5630	5790	1170
Tryptophol	0.682	0.759	0.503	0.734	0.918	0.486	0.74	0.796	0.00678
3-Hydroxykynurenine	0.886	0.875	0.919	1.19	1.44	0.643	0.808	1.73	−0.383
Serotonin	0.977	0.875	106	129	183	73.7	164	200	34.9
Tyrosine									
L-Dopa	0.192	0.381	0.979	1.52	2.25	0.674	0.894	1.38	−0.626
Homovanillic acid back wave	0.212	0.381	6.62	9.79	17.2	9.98	16.3	36.7	6.55
Vanillylmandelic acid	0.341	0.52	1	1.77	2.2	0.847	1.3	1.69	−0.469
3-*O*-methyldopa	0.371	0.539	0.248	0.731	0.805	0.408	0.874	1.01	0.143
3,4-Dihydroxyphenylacetic acid	0.40	0.539	0.343	0.692	1.21	0.595	0.737	1.16	0.0453
Tyramine	0.406	0.539	1.16	1.16	3.19	1.16	1.94	4.34	0.783
Methoxy-hydroxyphenil glycol	0.437	0.555	2.7	3.5	3.93	2.95	4.17	4.35	0.67
Homogentisic acid	0.464	0.555	0.214	0.667	0.979	0.34	1.54	2.85	0.874
Tyrosine	0.80	0.841	2650	3090	4040	2160	3170	3840	82.2
Homovanillic acid	0.841	0.866	1.8	2.37	5.22	1.36	3.91	6.37	1.54
Nor epinephrine	0.93	0.875	0.0552	0.193	0.446	0.118	0.207	0.283	0.0145
Tryptophan and phenylalanine									
Tryptophan/4-hydroxyphenyllactic acid	0.40	0.539	39.4	45.5	67.3	43.6	61.7	74.3	16.3
Tyrosine and tryptophan									
Homovanillic acid/5-hydroxyindoleacetic acid	0.752	0.815	0.81	1.04	1.85	0.339	1.2	2.03	0.158
Tryptophan/tyrosine									
Tryptophan/tyrosine	0.10	0.28	1.23	1.41	1.62	1.48	1.66	1.86	0.247

Metabolic pathway and compound or ratio	Nominal *P*	*Q*	Percentiles: Control 25th	Control Median	Control 75th	Opioid-dependent 25th	Opioid-dependent Median	Opioid-dependent 75th	Difference in medians
Sulfur aminoacids									
Cysteine	0.056	0.21	168	275	303	292	343	483	68.2
Methionine	0.212	0.381	575	767	941	720	1000	1410	236

We report median, 25th, and 75th percentile values and differences in medians of metabolites (n = 37, two represented by a second peak, indicated by “back wave”), and metabolite ratios (n = 7), Nominal p-values and Q-values are the results of Wilcoxon rank sum test comparisons between groups. Compounds are grouped by metabolic pathway. Data represent means of values relative to the entire pool values for that metabolite.

Table 2

Sociodemographic and drug use characteristics of individuals with opioid only use (OO = 6), other drug use in addition to opioids (OOD = 8), and no drug use (controls = 10)

% or mean and SD	OO *n* = 6	OOD *n* = 8	Controls *n* = 10	*p* (statistical test[a])
Age (years)	36.8 (9.5)	23.4 (4.9)	35.0 (10.8)	0.03 (K)
Weight (lbs)	156.8 (13.3)	152.6 (15.4)	168.7 (19.7)	0.09 (K)
Male	83.3	87.5	60	0.49 (F)
African American	33.3	12.5	30	0.60 (F)
Tobacco use	66.6	75	30	0.19 (F)
Alcohol use within 24–48 h	0	37.5	0	*b*
Urine tests[c]				
Opioid-positive urine test	100	100	0	*b*
Cocaine-positive urine test	0	37.5	0	*b*
Cannabis-positive urine test	0	62.5	0	*b*
ASI composite drug score	0.130 (0.05)	0.213 (0.08)	—	0.067 (W)
ASI composite alcohol score	0.050 (0.04)	0.068 (0.04)	—	0.34 (W)
Withdrawal score: admission (0–48)	40.5 (3.6)	40.1 (4.7)	—	0.80 (W)
Withdrawal score: evaluation (0–48)	12.3 (3.9)	12.5 (5.4)	—	1.0 (W)
Methadone dose at evaluation (mg)	21.0 (3.5)	19.8 (3.5)	—	0.50 (W)

— denotes not applicable.

[a] Statistical tests: K = Kruskal–Wallis rank sum test, F = Fisher's exact test, W = Wilcoxon rank sum test, two-sided, between OO and OOD groups.

[b] Different by criteria for OO, OOD, and control groups.

[c] Performed at evaluation in controls and 24–48 h before evaluation in OO and OOD subjects.