Oral Fluid and Plasma Cannabinoid Ratios after Around-the-Clock Controlled Oral Δ⁹-Tetrahydrocannabinol Administration

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BACKGROUND: Oral fluid (OF) testing is increasingly important for drug treatment, workplace, and drugged-driving programs. There is interest in predicting plasma or whole-blood concentrations from OF concentrations; however, the relationship between these matrices is incompletely characterized because of few controlled drug-administration studies.

METHODS: Ten male daily cannabis smokers received around-the-clock escalating 20-mg oral Δ⁹-tetrahydrocannabinol (THC, dronabinol) doses (40-120 mg/day) for 8 days. Plasma and OF samples were simultaneously collected before, during, and after dosing. OF THC, 11-hydroxy-THC and 11-nor-9-carboxy-THC (THCCOOH) were quantified by GC-MS at 0.5-µg/L, 0.5-µg/L, and 7.5-ng/L limits of quantification (LOQs), respectively. In plasma, the LOQs were 0.25 µg/L for THC and THCCOOH, and 0.5 µg/L for 11-hydroxy-THC.

RESULTS: Despite multiple oral THC administrations each day and increasing plasma THC concentrations, OF THC concentrations generally decreased over time, reflecting primarily previously self-administered smoked cannabis. The logarithms of the THC concentrations in oral fluid and plasma were not significantly correlated ($r = -0.10; P = 0.065$). The OF and plasma THCCOOH concentrations, albeit with 1000-fold higher concentrations in plasma, increased throughout dosing. The logarithms of OF and plasma THCCOOH concentrations were significantly correlated ($r = 0.63; P < 0.001$), although there was high interindividual variation. A high OF/plasma THC ratio and a high OF THC/THCCOOH ratio indicated recent cannabis smoking.

CONCLUSIONS: OF monitoring does not reliably detect oral dronabinol intake. The time courses of THC and THCCOOH concentrations in plasma and OF were different after repeated oral THC doses, and high interindividual variation was observed. For these reasons, OF cannabinoid concentrations cannot predict concurrent plasma concentrations.

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The collection of oral fluid (OF)⁵ is noninvasive, can be readily observed without loss of privacy, and presents little risk for adulteration. Thus, OF is an important matrix for identifying drug exposure in drug treatment, pain management, the workplace, and programs for driving under the influence of drugs. The relationships between the OF and plasma concentrations of cannabinoids are incompletely characterized, however, primarily because of the scarcity of controlled drug-administration studies.

There is a close physiological relationship between OF and blood, with passive diffusion of uncharged species being the primary mechanism for drug transfer. Factors affecting disposition include the drug’s physicochemical properties, as well as OF composition, pH, and flow rate (1). Drugs that are smoked, inhaled, or ingested also may contaminate OF and the oral mucosa, increasing the likelihood of detection but reducing the correlation with blood concentrations for 30–60 min after intake (2, 3). Δ⁹-Tetrahydrocannabinol (THC) also may be ingested orally in medications, food, drinks, and hemp oil (4–8), producing lower and delayed peak blood concentrations and effects than with smoked THC (9). Oral THC, either synthetic (dronabinol, Marinol®) or plant

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² Nonstandard abbreviations: OF, oral fluid; THC, Δ⁹-tetrahydrocannabinol; 11-OH-THC, 11-hydroxy-THC; THCCOOH, 11-nor-9-carboxy-THC; LOQ, limit of quantification; OF/P, OF/plasma (ratio).
derived, is medically available in many countries around the world for analgesia, muscle relaxation, appetite enhancement, and reduction of nausea and vomiting (10). Sativex®, which is absorbed through the oral mucosa, has been approved in several countries to reduce pain and muscle spasticity (11).

The limited passage of THC from the blood into OF is suggested by studies that used intravenous administration of radiolabeled cannabinoids (12). In addition, the more polar (hydrophilic) THC metabolites 11-hydroxy-THC (11-OH-THC) and 11-nor-9-carboxy-THC (THCCOOH) were not detected [at a 0.5-µg/L limit of quantification (LOQ)] in OF after smoked THC (13). OF THCCOOH was recently quantified with 2-dimensional GC-MS or GC-MS/MS (14–16). The identification of THCCOOH in OF minimizes concerns of contamination from passive cannabis exposure, because THCCOOH is not present in cannabis smoke (17–19).

Few controlled studies of smoked THC administration include simultaneously collected plasma and OF samples (3, 20, 21). To our knowledge, there are no paired data of plasma and OF cannabinoid concentrations after controlled oral THC administration. We compared the time courses of THC, 11-OH-THC, and THCCOOH concentrations in plasma and OF samples collected simultaneously from daily cannabis smokers: (a) after self-administered smoked cannabis; (b) after 1 oral THC dose; (c) during daily escalating multiple THC doses for 8 days; and (d) up to 22.5 h after the last THC dose. In addition, these data evaluate the ability of OF monitoring to detect oral cannabis intake and the feasibility of predicting plasma THC and THCCOOH concentrations from OF concentrations.

**Materials and Methods**

**Participants**

Participants provided written informed consent for this Institutional Review Board–approved protocol. Inclusion criteria were an age between 18 and 45 years, a self-reported cannabis smoking history ≥1 year, mean daily cannabis use ≥3 months before admission, and a urine sample positive for cannabinoids within the previous 30 days (22). Clinically important medical or psychiatric disease, cannabis-related psychosis or seizure, >6 alcoholic drinks per day ≥4 times/week, or sesame oil allergy were exclusionary. Participants resided in secure clinical research facilities under continuous medical supervision.

**Drug Administration**

Participants were admitted between 1755 and 2130 the evening before the first dose (at 1500 on day 1), for a minimum duration of cannabis abstinence of 17.5–21 h. This minimum duration was chosen to prevent cannabis withdrawal and yet ensure no acute intoxication at the time of the first dose. Dronabinol (synthetic THC) in the form of Marinol (Unimed Pharmaceuticals) was administered in 20-mg capsules with increasing frequency (every 4–8 h around the clock) for total doses of 40–120 mg/day (see Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol57/issue11). The last dose was at 0930 on day 8. This dosing regimen standardized cannabis tolerance in chronic, daily cannabis smokers. Participants were discharged 22.5 h after the last dose.

**Plasma and Of Collection and Analysis**

Peripheral venous blood and OF were collected simultaneously as follows: before and after the first oral THC dose to evaluate single-dose cannabinoid pharmacokinetics; during repeated oral THC dosing; and for 22.5 h after the last oral THC dose (see Table 1 in the online Data Supplement). Higher cannabinoid concentrations in plasma, easier analytical processing, and the availability of published data for comparison were reasons for selecting plasma over whole blood as the matrix to be evaluated in this study (23, 24). Duplicate OF samples were collected simultaneously (2 in the mouth at the same time) upon admission and 2.2 h after the last evening dose each day to evaluate the reproducibility of OF collections and to provide data for the Mandatory Guidelines for Workplace Drug Testing requirement for split samples (25). THC dosing preceded collection when both were scheduled at the same time. Venous blood was collected into sodium heparin, stored on ice, and centrifuged. The plasma was separated within 2 h and stored at 4 °C until analysis within 2 weeks. OF was collected with the Quantisal™ device (Immunalysis), which has an absorptive cellulose pad on a polypropylene stem with a volume-adequacy indicator. The pad was placed into the participant’s mouth until 1.0 mL (SD, 0.1 mL) was collected; the pad was then put into a plastic tube containing 3 mL buffer, thereby yielding a dilution of 1 part OF in 3 parts diluent. The manufacturer recommended keeping the absorbent pad in the OF–buffer mixture for a minimum of 24 h to ensure adequate recovery of the cannabinoids from the absorbent pad. This procedure also reproduces the typical collection conditions, in which samples are collected and later delivered to laboratories for analysis. The elution buffer was removed and stored at −20 °C until analysis. Lowe et al. (26) reported a variation of 0%–16% in mean plasma cannabinoid concentrations when samples analyzed immediately were compared with those stored at 4 °C for up to 16 h. Plasma cannabinoids were stable for up to 6 months at 4 °C (27); cannabinoids in...
the OF/Quantisal buffer mixture were stable (mean loss, 14.4%) for >9 months at −20 °C (28).

THC, 11-OH-THC, and THCCOOH were quantified in plasma (26) and OF (16) by validated 2-dimensional GC-MS methods. The linear range for plasma was 0.25–100 μg/L for all analytes except 11-OH-THC (linearity from 0.5–75 μg/L). In OF, the linear range for analytes was 0.5–50 μg/L, with the excep-

### Table 1. Pharmacokinetic parameters for cannabinoids in plasma and OF.a

<table>
<thead>
<tr>
<th></th>
<th>Predoseb</th>
<th>First dose</th>
<th>3 Days (100 mg/day)</th>
<th>3 Days (120 mg/day)</th>
<th>After last dose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of samples</strong></td>
<td>30</td>
<td>50</td>
<td>90</td>
<td>90</td>
<td>80</td>
</tr>
<tr>
<td><strong>OF</strong></td>
<td></td>
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<tr>
<td>THC</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;, μg/L</td>
<td>4.2 (ND–399)</td>
<td>1.8 (ND–6.8)</td>
<td>0.7 (ND–8.0)</td>
<td>(ND–1.1)</td>
<td>ND</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;, h</td>
<td>−18.0 (NA–0.1)</td>
<td>1.0 (NA–5.0)</td>
<td>17.0 (NA–31.0)</td>
<td>101.5b</td>
<td>NA</td>
</tr>
<tr>
<td>Range, μg/L</td>
<td>ND–399</td>
<td>ND–6.8</td>
<td>ND–8.0</td>
<td>ND–1.1</td>
<td>ND</td>
</tr>
<tr>
<td>Detection rate</td>
<td>87%</td>
<td>64%</td>
<td>11%</td>
<td>3%</td>
<td>ND</td>
</tr>
<tr>
<td>AUC, h / ng/L</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>THC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;, ng/L</td>
<td>28.5 (21.9–499)</td>
<td>52.4 (9.1–557)</td>
<td>138 (56.2–800)</td>
<td>270 (99.8–1,087)</td>
<td>319 (94.6–1055)</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;, h</td>
<td>−7.0 (−18 to 0.1)</td>
<td>3.5 (2.0–5.0)</td>
<td>61.0 (31.0–79.0)</td>
<td>103.5 (91.0–139)</td>
<td>167 (163–173)</td>
</tr>
<tr>
<td>Range, ng/L</td>
<td>ND–499</td>
<td>ND–557</td>
<td>ND–800</td>
<td>ND–1087</td>
<td>25.4–1055</td>
</tr>
<tr>
<td>Detection rate</td>
<td>93%</td>
<td>98%</td>
<td>98%</td>
<td>99%</td>
<td>100%</td>
</tr>
<tr>
<td>AUC, h / ng/L</td>
<td>374 (222–6219)</td>
<td>134 (33.6–1371)</td>
<td>4777 (1905–31 109)</td>
<td>9308 (2526–47 555)</td>
<td>3231 (1047–14 991)</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
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<tr>
<td>THC</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;, μg/L</td>
<td>5.2 (2.4–33.3)</td>
<td>9.6 (6.2–27.7)</td>
<td>25.4 (14.2–44.4)</td>
<td>31.0 (15.2–67.6)</td>
<td>14.6 (7.1–31.3)</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;, h</td>
<td>−18.0 (all participants)</td>
<td>3.0 (2.0–5.0)</td>
<td>66.0 (43.0–79.0)</td>
<td>103.5 (101.5–151.5)</td>
<td>164 (164–173)</td>
</tr>
<tr>
<td>Range, μg/L</td>
<td>1.5–33.3</td>
<td>2.3–27.7</td>
<td>3.3–44.4</td>
<td>4.2–67.6</td>
<td>1.2–31.3</td>
</tr>
<tr>
<td>Detection rate</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>AUC, h / μg/L</td>
<td>68.7 (32.2–245)</td>
<td>34.2 (16.9–51.6)</td>
<td>666 (507–1390)</td>
<td>827 (432–1611)</td>
<td>103 (66.0–195)</td>
</tr>
<tr>
<td>11-OH-THC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;, μg/L</td>
<td>2.0 (1.3–11.7)</td>
<td>5.7 (2.8–12.5)</td>
<td>16.6 (9.6–24.5)</td>
<td>19.1 (11.8–38.9)</td>
<td>16.5 (7.0–36.7)</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;, h</td>
<td>−18.0 (all participants)</td>
<td>3.0 (2.0–5.0)</td>
<td>67.0 (43.0–79.0)</td>
<td>127.5 (101.5–151.5)</td>
<td>164 (163–165)</td>
</tr>
<tr>
<td>Range, μg/L</td>
<td>0.6–11.7</td>
<td>0.8–12.5</td>
<td>2.6–24.5</td>
<td>5.1–38.9</td>
<td>1.1–36.7</td>
</tr>
<tr>
<td>Detection rate</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>AUC, h / μg/L</td>
<td>23.0 (13.9–105)</td>
<td>15.6 (9.3–31.0)</td>
<td>486 (383–765)</td>
<td>648 (447–1407)</td>
<td>107 (63.4–275)</td>
</tr>
<tr>
<td>THCCOOH</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;, μg/L</td>
<td>51.5 (24.8–136)</td>
<td>68.9 (39.2–115)</td>
<td>235 (99.1–366)</td>
<td>223 (145–441)</td>
<td>231.3 (108–427)</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;, h</td>
<td>−12.5 (−18.0 to −7.0)</td>
<td>3.0 (2.0–5.0)</td>
<td>67.0 (31.0–67.0)</td>
<td>133 (91.0–151.5)</td>
<td>164 (163–173)</td>
</tr>
<tr>
<td>Range, μg/L</td>
<td>13.3–136</td>
<td>14.3–115</td>
<td>53.2–366</td>
<td>85.5–411</td>
<td>38.4–427</td>
</tr>
<tr>
<td>Detection rate</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>AUC, h / μg/L</td>
<td>800 (341–2074)</td>
<td>232 (126–363)</td>
<td>8042 (4802–12 453)</td>
<td>10 816 (6900–19 484)</td>
<td>2913 (1620–6858)</td>
</tr>
</tbody>
</table>

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a Data are expressed as the median (range). Predose, data obtained before first oral THC dose; first dose, data obtained after first 20-mg THC dose over 5 h; 3 days (100 mg/day), data obtained over 3 days (days 2–4) of 100 mg/day THC; 3 days (120 mg/day), data obtained over 3 days (days 5–7) of 120 mg/day THC; after last dose, data obtained after the final THC dose (n = 37 doses) over 22.5 h to discharge.

b On admission, 1 OF sample was THC negative, and 2 were THCCOOH negative.

c<sub>max</sub>, median peak concentration; ND, not detected; T<sub>max</sub>, time to peak concentrations; NA, not available; AUC, area under the ROC curve.

d C<sub>max</sub> ranges include maximum concentrations for all participants.

e Negative T<sub>max</sub> values indicate times prior to the first oral THC dose.

f Only 1 participant was positive for THC in OF during days 5–7 (120 mg/day THC).
tion of THCCOOH (linearity of 7.5–500 ng/L). The increased sensitivity for THCCOOH was achieved with negative chemical ionization 2-dimensional GC-MS. Samples with concentrations outside the linear range were diluted with a blank mixture of 1 part OF and 3 parts Quantisol buffer and then reanalyzed.

DATA ANALYSIS
Cannabinoid OF/plasma (OF/P) concentration ratios were determined from simultaneously collected samples with concentrations ≥LOQ. THC/THCCOOH ratios were calculated for plasma and OF, and THC/11-OH-THC and 11-OH-THC/THCCOOH ratios were also determined for plasma. Plasma and OF THCCOOH concentrations differ by 1000-fold; therefore, OF/P THCCOOH ratios are presented in the appropriate nanogram-per-microgram units. THC/11-OH-THC and 11-OH-THC/THCCOOH ratios in OF were not determined because 11-OH-THC was not quantifiable at the 0.5-μg/L LOQ.

Median peak concentrations and the time to peak concentrations were obtained directly from the concentration–time data. The time to peak concentration denotes the time before or after the first dose. The area under the plasma or OF concentration–time curve was calculated by the linear trapezoidal method.

No significant differences in THC (P = 0.331) and THCCOOH (P = 0.060) concentrations were found for 80 pairs of duplicate OF samples evaluated by paired t-test analysis (22); hence, mean values were used for data analysis. Means were used for calculations when the data were normally distributed, and medians and ranges were used when they were not. Median (range) concentrations include positive (≥LOQ) and negative (<LOQ) results. For comparative statistical tests, OF samples with concentrations <LOQ were assigned values of 0.5× LOQ (0.25 μg/L for THC and 3.75 ng/L for THCCOOH). Concentration differences before the first dose, after the first and last THC doses, and between each of days 2–7 were evaluated by nonparametric Wilcoxon tests. Because THC and THCCOOH concentrations were not normally distributed, we used the commonly accepted approach of log-transforming the data for OF–plasma correlation analysis (29, 30). Statistical analyses used SPSS software (version 14; SPSS).

Results

PARTICIPANT CHARACTERISTICS
Ten males participated in the study [mean (SD) age, 23.9 (4.0) years (range, 18–32 years); body mass index, 26.0 (4.5) kg/m² (range, 17.8–30.6 kg/m²)]. These individuals self-reported first cannabis smoking at a mean of 13.6 (1.8) years, with mean daily smoking of 1–24 joints or blunts. All participants self-reported smoking cannabis within 24 h before admission and had a positive cannabinoid urine test result at admission. Thirty-six plasma samples and 44 OF samples (including 8 duplicates) were collected from each participant. There were no statistically significant correlations between the cannabinoid concentration at any time point and the participants’ age, body mass index, or cannabis use characteristics (data not shown).

THC CONCENTRATIONS IN PLASMA AND OF
THC was present in all 360 plasma samples at concentrations up to 67.6 μg/L (day 4.3) and was present at admission in 76 OF samples (21.1%) at up to 399 μg/L (admission day) (Table 1). All 10 plasma samples and 9 of 10 OF samples were THC positive at admission, presumably because of previously self-administered smoked cannabis. The median THC concentration for the first collection (n = 10) at admission was 5.2 μg/L (range, 2.4–33.3 μg/L) in plasma and 3.3 μg/L (range, not detected to 399 μg/L) in OF (Fig. 1). The median THC OF/P ratio was 0.5 (range, 0.03–12.0); 4 of 10 participants had ratios ≥1 (Fig. 2). THC concentra-
tions significantly decreased in plasma (Z = −2.81; P = 0.005) and OF (Z = −2.19; P = 0.028) from the time of admission to the first oral THC dose.

After the first oral THC dose, OF was THC positive in 8 participants (median, 1.0 μg/L; range, not detected to 8.4 μg/L). THC concentrations in OF decreased after dosing, whereas plasma concentrations increased significantly (Z = 2.55; P = 0.011) (Fig. 1).

At combined doses of 100 and 120 mg oral THC per day, there were no significant (P > 0.05) changes in daily THC concentrations on days 2–7. THC was measurable in only 13 (7.2%) of 180 OF samples from 6 participants, with concentrations of up to 8.0 μg/L.

There were no THC-positive OF samples after the last THC dose. In contrast, plasma THC was measurable in all 10 participants, with concentrations decreasing significantly (Z = −2.81; P = 0.005) from a median of 6.7 μg/L (range, 4.6–14.2 μg/L) at the time of the last oral THC dose to a median of 3.2 μg/L (range, 1.2–5.2 μg/L) at discharge 22.5 h later. The logarithms of THC concentrations in OF and plasma were not significantly correlated (r = −0.10; P = 0.065) (Fig. 3). The median OF/P THC ratio throughout was 0.3 (range, 0.03–12.0).

THCCOOH CONCENTRATIONS IN PLASMA AND OF
All 360 plasma samples were THCCOOH positive, with the highest concentration being 498 μg/L. 6.7 days after the first dose. THCCOOH was quantified in 354 of 360 OF samples, at concentrations of up to 1088 ng/L. Concentration–time curves of median plasma and OF THCCOOH concentrations are illustrated in Fig. 4. A significant linear correlation was found between plasma and OF in the log-transformed THCCOOH concentration (r = 0.63; P < 0.001). Scatter plots and trend lines for correlations are shown in Fig. 3. THCCOOH pharmacokinetic parameters for OF and plasma are shown in Table 1.

On admission, the median plasma THCCOOH concentration was 48.7 μg/L (range, 24.8–137), and 8 of 10 participants had THCCOOH-positive OF samples (median, 21.9 ng/L; range, not detected to 161 ng/L). The median OF/P THCCOOH ratio was 0.5 ng/μg (0.1–1.8 ng/μg) at admission and increased after admission to a median of 0.7 ng/μg (range, 0.3–3.0 ng/μg) (Fig. 2). Plasma THCCOOH concentrations decreased significantly until the first dose (Z = −2.80; P = 0.005); there was no significant change in OF THCCOOH concentrations during this time (Z = −0.15; P = 0.878) (Fig. 1B). OF and plasma log-transformed THCCOOH concentrations were significantly correlated during this period (r = 0.62; P < 0.001).

After the first dose, the plasma and OF samples of all participants were THCCOOH positive, with median concentrations of 31.3 μg/L (range, 13.3–73.2 μg/L) and 19.2 ng/L (range, 7.6–211 ng/L), respectively (Fig. 1B). All plasma samples and 98.0% of OF samples remained THCCOOH positive for 5 h after the first dose, and log-transformed THCCOOH concentrations were significantly correlated (r = 0.62; P < 0.001).

During total dosages of 100 and 120 mg oral THC per day, THCCOOH results were positive for all 180 plasma samples collected on days 2–7, whereas 98.3%
of OF samples were positive. There was a significant correlation \( (r = 0.45; P < 0.01) \) between plasma and OF THCCOOH concentrations on days 2–7. Median OF/P THCCOOH ratios are shown in Fig. 2.

All 80 samples collected up to 22.5 h after the last THC dose were THCCOOH positive. In plasma, median THCCOOH concentrations decreased significantly \( (Z = -2.84; P = 0.004) \) from 210 \( \mu g/L \) (range, 0–720 \( \mu g/L \)) on day 1 to 80 \( \mu g/L \) (range, 0–400 \( \mu g/L \)) on day 7.

**Fig. 3.** Linear (A, B) and logarithmic (C, D) relationships and 95% CIs for THC and THCCOOH concentrations in simultaneously collected OF and plasma samples.

**Fig. 4.** THCCOOH concentrations in simultaneously collected OF and plasma samples from 10 daily cannabis users, before and after the first 20-mg oral THC dose, during 8 days of around-the-clock oral THC dosing, and up to 22.5 h after the last oral THC dose.

Data are expressed as the median (n = 10); arrows indicate the number of 20-mg THC doses ingested between collections.
90.1–427 μg/L) at the last dose to 134 μg/L (range, 38.4–347 μg/L) at discharge, whereas no significant change (Z = −0.42; P = 0.673) was observed in THCCOOH OF concentrations during this period. There was a significant correlation (r = 0.41; P < 0.01) between plasma and OF THCCOOH concentrations over this period. The median OF/P ratio increased from 0.5 (range, 0.1–4.7) at the last dose to 0.7 (range, 0.6–7.9) at discharge.

The median OF/P THCCOOH ratio was 0.7 ng/g; however, there was great intra- and interindividual variation, with ratios ranging from 0.05 to 8.7 throughout the study (Fig. 2).

THC/METABOLITE AND 11-OH-THC/THCCOOH RATIOS IN PLASMA AND OF
THC/THCCOOH ratios in plasma were low at admission (median, 0.11; range, 0.06–0.24), for 5 h after the first oral THC dose (median, 0.12; range, 0.07–0.15), throughout 7 days of repeated dosing (medians ranging from 0.03 to 0.13) (Fig. 5), and after the last oral THC dose (median, 0.04; range, 0.01–0.11). Median THC/11-OH-THC and 11-OH-THC/THCCOOH ratios throughout the study were 2.2 (range, 1.5–3.3) and 0.05 (range, 0.03–0.09), respectively, at admission; 1.6 (range, 1.2–2.9) and 0.06 (range, 0.04–0.11) for 5 h after the first oral THC dose; 0.8–1.7 and 0.04–0.09 throughout repeated dosing; and 1.3 (range, 0.8–2.0) and 0.02 (range, 0.01–0.04) after the last oral THC dose.

In OF, THC/THCCOOH ratios were 100-fold to 1000-fold higher than in plasma on admission (median, 212; range, 38.0–3184), rapidly decreasing to 74.8 (range, 10.5–131) by the first oral THC dose and then to 45.2 (range, 16.4–92.2) 5 h later (Fig. 5). The last OF THC/THCCOOH ratio was 0.7 for participant I, who was intermittently positive until day 7.

Lastly, 11-OH-THC was not detected in OF at the 0.5-μg/L LOQ, whereas 11-OH-THC was quantifiable in all plasma samples, but at relatively low concentrations (0.6–38.9 μg/L).

Discussion

Our previously published data (22, 31) revealed distinct differences in the disposition and time course of THC in plasma and OF, limiting the ability to predict plasma THC concentrations from OF results after oral THC administration and contradicting the strong correlation observed after the administration of smoked cannabis (29). Surprisingly, OF and plasma were similar in the disposition of THCCOOH.

THC CONCENTRATIONS IN PLASMA AND OF
We believe that this study constitutes the first characterization of THC and metabolites in paired plasma and OF samples after controlled oral THC administration. Simultaneously collected plasma and OF samples had different THC concentration patterns over time. Plasma results were continuously THC positive, predominantly because of the oral mode of THC administration. OF THC concentrations rapidly decreased after admission despite repeated oral THC doses, suggesting that THC was derived primarily from previously self-administered smoked cannabis. THC was detected at ≥1 μg/L in all plasma samples for 22.5 h after the last THC dose. In contrast, all OF samples were consistently negative for THC during this period. This pattern of findings suggests that THC testing of OF will detect recent smoked but not recent oral THC intake.

THC concentrations (1.9–9.6 μg/L) in plasma 18.5 h after admission were considerably higher than the highest plasma THC concentration (0.7 μg/L) observed 12 h after smoking one 33.8-mg (3.55%) THC cigarette (32), but they were consistent with findings for chronic users after 1 day of abstinence (0.5–9.0 μg/L) (33).
THCCOOH concentrations in plasma and of

Maximum plasma THCCOOH concentrations were 10-fold higher (146–498 µg/L) than in previous studies that administered 10–15 mg of oral THC daily for 4 days (74.5–244 µg/L) (34) or 7.5 mg for 1 day (10.4–43.0 µg/L) (35). These higher maximum concentrations were not unexpected, considering the larger daily doses, the longer dosing duration, and the possible contribution from released THC body stores accumulated during participants’ chronic daily cannabis self-administration.

The OF THCCOOH concentrations observed in the present study of repeated oral THC dosing were much higher, up to 1088 ng/L, than previously reported. OF THCCOOH concentrations from 2–352 ng/L were reported for 109 (76.2% positive) randomly admitted during participants’ chronic daily cannabis self-administration.

Our study has also provided the first data on the THCCOOH OF/P ratio. Plasma THCCOOH concentrations after oral and smoked administration have been well studied (31, 37, 39), yet only recently has it been possible to detect OF THCCOOH at nanogram-per-liter concentrations (14–16). Large variations in THCCOOH OF/P ratios were observed between participants and over time.

THC and THCCOOH OF/P concentration ratios

THC OF/P ratios were highly variable in our study, results consistent with those of previous reports (20, 21). The wide ratio range (0.1–12.0) on admission most likely reflected the recency of last cannabis smoking. Two previous studies found much less variation in the ratio: 0.2–3.1 (32) and 0.5–2.2 (3) in 6 marijuana users. Others have found a much wider range in the ratio and higher maxima in OF/serum (21) and OF/whole blood (30) ratios. These inconsistent data emphasize the need for investigations of controlled cannabis and THC administration by different routes. Furthermore, the determination of pharmacokinetic phenotype might help explain concentration differences and improve the interpretation of OF cannabinoid test results. The route of cannabinoid administration influences THC metabolism. After oral THC administration, first-pass metabolism increases, with the drug absorbed in the gastrointestinal tract delivered directly through the portal circulation to the liver. Increased first-pass metabolism leads to lower THC bioavailability because of the rapid metabolism of THC to 11-OH-THC and THCCOOH, and the formation of glucuronide and sulfate conjugates after phase II metabolism. After smoking, 11-OH-THC concentrations are approximately 5%–10% of those of THC, because the drug absorbed in the lung is delivered directly into the circulation, bypassing initial metabolism by the liver. After oral cannabinoid dosing, hepatic microsomal THC oxidation yields approximately equivalent 11-OH-THC concentrations (37, 38). Thus, differences in OF/P ratios are partially explained by the oral route of THC administration, but additional factors include differences in OF-collection methods (expectoration vs collection device, spontaneous vs stimulated OF production) and timing after exposure.

Increased OF/P THC ratios are expected immediately after cannabis smoking because of direct contamination of the oral mucosa by the THC in smoke. We administered doubly encapsulated oral THC, which minimized oral mucosa contamination. The occasional positive THC samples we have noted appear to be from the release of THC from tissue stores after chronic daily cannabis smoking, although low-level THC contamination from oral THC cannot be excluded.

The plasma ratios of THC/11-OH-THC (range, 0.24–1.0) in samples collected up to 5 h after the first oral THC dose (Fig. 1). The OF THC/THCCOOH concentration peaked later than the plasma THCCOOH concentration in 8 of 10 participants after the first oral THC dose (Fig. 1), suggesting a delay in plasma and OF THC equilibration. This equilibration delay contributes to OF/P variation and the poor prediction of plasma cannabinoid concentrations from OF results.

Plasma THC/11-OH-THC, THC/THCCOOH, and 11-OH-THC/THCCOOH ratios

The plasma ratios of THC/11-OH-THC (range, 0.47–4.4), THC/THCCOOH (range, 0.01–0.61), and 11-OH-THC/THCCOOH (range, 0.005–0.19) were consistent with previously published data obtained after oral THC administration (31). The range of plasma ratios were 0.34–4.0 for the THC/11-OH-THC ratio and ≤0.29 for both the THC/THCCOOH and 11-OH-THC/THCCOOH ratios after oral THC administration of 7.5 mg/day for 5 days (35) or after a single 20-mg oral THC dose (38).

In the current study, the 11-OH-THC/THC ratio ranged from 0.22 to 0.76 during the predose period, results consistent with the source of cannabinoids being cannabis smoking before admission. We noted ratios <1 (range, 0.24–1.0) in samples collected up to 5 h after the first oral THC dose. Ratios >1 were not observed until day 3, and many ratios <1 were observed during the subsequent days of repeated oral THC dosing. This pattern of findings is not consistent with a previously proposed rule (40) of distinguishing oral THC from smoked cannabis intake based on 11-OH-THC/THC ratios >1 within 2 h of intake, or >1.5 thereafter.

Of THC/THCCOOH ratios

THC/THCCOOH ratios >10 occurred only at admission and up to 12 h after the first oral THC dose. These findings are consistent with the participants’ self-
reports of (smoked) cannabis use within 1 day of admission. THC was rapidly eliminated from OF, yielding samples with THC/THCCOOH ratios <5 by 1 day after the first oral THC dose. Thus, OF samples with high THC/THCCOOH ratios suggest recent cannabis ingestion.

In summary, our data demonstrate that THC detected in OF primarily reflects smoked cannabis, not oral administration, and that high OF/P THC and OF THC/THCCOOH ratios (>1000) may be good indicators of recent cannabis smoking. Our report presents for the first time a detailed evaluation of the relationship between OF and plasma THCCOOH. The similar profiles and moderate correlation between OF and plasma THCCOOH concentrations after oral THC administration suggest the likelihood of a physiological link between these fluids. Dose, route, and frequency of cannabis exposure; smoking topography; time since last use; and OF-collection method also influence cannabinoid OF concentrations. Because of the high inter-individual variation, the required equilibration time for cannabinoids in plasma and OF, and the differences in cannabinoid disposition in these 2 matrices, predicting plasma cannabinoid concentrations from OF concentrations cannot be scientifically supported.

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