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Short communication

Can Physical Exercise or Food Deprivation Cause Release of Fat-Stored Cannabinoids?

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Abstract: The aim of this study was to evaluate whether physical exercise or food deprivation may increase cannabinoid levels in serum or urine in abstinent chronic cannabis users. The study took place in a drug detoxification ward parallel to study participants receiving treatment. Six chronic, daily cannabis users (one female, five males, average age 30.0 years; BMI 20.8) were exposed to a 45-min. moderate-intensity workout and a 24-hr period of food deprivation. Serum samples were drawn prior to and after interventions and analysed for Δ9-tetrahydrocannabinol (THC) and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCCOOH) by liquid chromatography–tandem mass spectrometry (LCMSMS), and all voided urine was tested for THCCOOH by LCMSMS and normalized to the creatinine levels, yielding ng/mg ratios. There were no major differences in the measured cannabinoid levels in serum or urine before and after physical exercise or food deprivation. We conclude that exercise and/or food deprivation are unlikely to cause sufficient cannabinoid concentration changes to hamper correct interpretations in drug testing programmes.

Testing for drugs of abuse is often requested in healthcare, workplace and criminal justice settings. As punishment may be more severe if multiple drug use is established, accurately distinguishing new drug use from residual excretion is necessary [1,2]. Due to its long retention time, cannabis is especially important in this regard [2].

After smoking of a single cannabis cigarette, serum Δ9-tetrahydrocannabinol (THC) levels are typically below detection limits within 12 hr [3], whereas its primary metabolite, 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCCOOH), may be detectable in serum for about 1 week and in urine for about 2 weeks [4]. In chronic cannabis users, however, THC and THCCOOH may be detectable in blood for up to 1 month [5–7], and THCCOOH in urine for up to 3 months [1,8,9]. The delayed cannabinoid excretion in chronic cannabis users is believed to be caused by accumulation and subsequent slow release of THC from fat tissue [10,11].

The process of THC release from fat tissue is poorly understood and may in theory be influenced by lipolysis during diet, stress and exercise [9]. An Australian research group demonstrated that lipolysis in rats induced by adrenocorticotropic hormone and food deprivation increased plasma THC levels in rats pre-treated with cannabis [12]. Recently, they also published a study on 14 human subjects, showing that physical exercise for 35 min. caused a small but statistically significant rise in THC plasma levels in regular cannabis users [13].

In our routine drug testing service, we frequently experience that cannabis users claim their positive test results (i.e. increased urinary cannabinoid levels) to be caused by fasting or exercise. As urinary cannabinoid concentrations during experimental lipolytic conditions have never been scientifically investigated, we often find ourselves unable to prove these statements true or false. As most drug testing is performed in urine, the clarification of this issue is of obvious importance.

The aim of this study was to elucidate whether physical exercise and food deprivation may cause increases in serum and urine cannabinoid levels in chronic cannabis users in a naturalistic setting.

Materials and Methods

Participants. The study took place between April 2010 and April 2011. Participants were recruited consecutively among patients with predominant cannabis abuse admitted to Lade Rehabilitation Clinic Blue Cross, a drug detoxification ward in Trondheim, Norway. They were informed, interviewed and examined by a medical doctor at the time of admission, and evaluated with respect to physical and psychological suitability. All filled in a questionnaire regarding their recent use of drugs.

One at a time, the study subjects resided in a closed ward among other patients undergoing treatment for substance abuse. All had their

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personal belongings searched prior to unit entry. They had separate rooms and private bathrooms. The ward was under 24-hr surveillance to encourage drug abstinence. Study participation was unpaid. Participants were allowed to exit the study at their own discretion. The study was approved by the regional ethics committee, and all participants filled in an informed consent form.

Because of personnel changes, recruitment to the study had to be terminated earlier than intended.

Study design. Participants were recruited on admission to the ward (day 0), and urine specimens were collected for the following 7 days. Interventions were made on day 3 and 5 in a randomly assigned order and consisted of either 45 min. of physical exercise (EX), where subjects were instructed to run on a treadmill at 60–75% of their maximal pulse (estimated by 226 minus age for males and 220 minus age for females), or 24 hr food deprivation (FD), where only drinking of water was allowed. Blood samples were drawn immediately prior to and after interventions, and at the beginning and end of the study (day 1 and 6).

Specimen collection. Participants voided urine whenever they needed to and collected their own urine specimens whenever voided during the 7-day study period. To aid this, they were provided with plastic cups, urine vials (10 ml Sarstedt Urine Monovette ® with suction tips), vial labels, a pen and a watch. Urine vials were marked with date, time and study identity letter (Participant A-F) by the participants themselves. Every evening, the ward staff collected the samples and deposited them in a freezer at −20°C. Blood samples were centrifuged, serum-extracted and immediately stored in a freezer at −20°C.

Specimen analysis. Urine samples were treated by β-glucuronidase (*Escherichia coli* K12) at 45°C for 120 min. to minimize the THCCOOH-glucuronide fraction. Hundred microlitre of pre-treated urinary samples, serum sample standards and quality controls were prepared by adding 50 µl 0.1 M phosphate buffer solution (pH 6), 50 µl internal standard (50 ng/ml, d9-THCCOOH and d3-THC) and 1 ml diethyl ether/n-hexane/ethyl acetate (1:1:1) as extractants. Samples were analysed by liquid chromatography–tandem mass spectrometry (LCMSMS) using a Waters Acquity UPLC system with an autosampler and a binary solvent delivery system (Waters, Milford, MA, USA) interfaced to Waters Micromass® Quattro Premier™ XE benchtop tandem quadrupole mass spectrometer (Waters, Manchester, UK). Chromatography was performed on a 2.1 × 50 mm Waters Acquity BEH C8 1.7 mm column. The mobile phase consisted of 68% methanol in 0.34 g/l aqueous ammonium acetate with an isocratic flow rate of 0.4 ml/min. For quantitative analysis of THCCOOH and THC, the following MRM transitions were used: m/z 343→299 (quantification ion), 345→327 (qualifier ion) and m/z 315→193 (quantification ion), 315→259 (qualifier ion), respectively. MRM transition m/z 352→308, 354→336 and m/z 318→196, 318→262 were used for the internal standards d9-THCCOOH and d3-THC, respectively. The linear dynamic range ($r^2 > 0.99$) for THCCOOH and THC was 3–5000 ng/ml and 0.6–240 ng/ml, respectively. LOD for THC and THCCOOH were 0.2 ng/ml and 0.17 ng/ml, respectively. Between-day coefficients of variation (CVs) for THCCOOH calculated from quality control samples were <6% at 30 ng/ml and <4% at 3000 ng/ml. For THC, the CV was <11% at 2.5 ng/ml and <6% at 250 ng/ml.

Urine creatinine was analysed photometrically after complex formation with picric acid in an alkaline solution by a routine method (Jaffé's method) on a Cobas Integra 400 + multianalyser (Roche Diagnostics, Basel, Switzerland). The LOQ was 0.11 mg/ml, and the CV was <10%.

THCCOOH concentrations (in ng/ml) of all positive urine specimens were divided by the specimens' urine creatinine concentration (in mg/ml) to obtain the normalized THCCOOH/creatinine concentration (CC ratio, presented as ng/mg).

Results

Seven consecutively admitted cannabis users were invited to participate in the study; six accepted. None withdrew from the study after inclusion. The participants were all Caucasian, one female, five males and aged 25–34 (mean 30.0) years. Characteristics of the participants are reported in table 1. They were admitted primarily due to cannabis abuse, but four subjects also reported use of other illicit drugs at one or more occasions during the last 30 days prior to admission. All participants smoked cannabis on a daily basis (5–30 g of hashish or equivalent amounts of marihuana per week for more than 1 month) and had done so until the time of admission.

Due to phlebotic veins, only three serum samples were obtained from subject C, and due to a misunderstanding, subject E collected only one urine specimen per day for the first 4 days of the study. Otherwise, complete series of urine and serum specimens were collected for the whole period for all subjects. A total of 188 urine specimens and 34 serum specimens were collected. At the beginning of the study, THC was detected in serum of all subjects except subject C (mean 2.6, range 0–3.6 ng/ml). In three subjects, THC was still present in serum at the end of the study (day 6). At the beginning of the study, THCCOOH (mean 85.2, range 35.4–175.5 ng/ml) was measured in the serum of all participants, as was THCCOOH in their urine (mean 201.9, range 19.2–401.2 ng/ml, CC ratio

Table 1.
Participant demographics and self-reported cannabis use prior to study entry.

Participant	Sex	Age (years)	Height (m)	Weight (kg)	BMI	Self-reported cannabis use (gram of hashish per week) before study entry
A	F	26	1.67	52	19	30
B	M	30	1.82	65	20	10–15
C	M	35	1.76	63	20	5–15
D	M	30	1.83	77	23	30
E	M	25	1.84	68	20	10–15
F	M	34	1.82	74	22	15

M: male, F: female, BMI: body mass index (weight/height^2).

Table 2.
Serum and urinary cannabinoid levels in six regular cannabis users, before and after 45 min. of physical exercise.

	Serum THC concentrations (ng/ml)			Serum THCCOOH concentrations (ng/ml)			Urinary CC ratio (ng/mg)		
Participant	Before EX	After EX	Change (%)	Before EX	After EX	Change (%)	Before EX	After EX	Change (%)
A	3.1	3.6	+0.5 (16)	107.1	107.7	+0.6 (1)	157.2	190.1	+32.9 (21)
B	1.0	1.9	+0.9 (90)	23.6	44.0	+20.4 (86)	113.9	68.5	−45.4 (40)
C	<LOQ	NA[1]	–	10.4	NA[1]	–	39.2	21.0	−18.1 (46)
D	1.3	1.0	−0.3 (23)	29.9	24.8	−5.1 (17)	58.5	37.0	−21.5 (37)
E	0.7	0.8	+0.1 (14)	14.4	11.0	−3.4 (24)	62.6[2]	32.3	−30.3 (48)
F	1.9	2.5	+0.6 (23)	22.0	23.9	+1.9 (9)	53.7	27.3	−26.4 (49)
Mean	1.6	2.0	+0.4 (25)	39.4	42.3	+2.9 (7)	80.9	62.7	−18.2 (23)

THC: Δ9-tetrahydrocannabinol, THCCOOH: 11-nor-9-carboxy-Δ9-tetrahydrocannabinol, CC ratio: creatinine-normalized THCCOOH, EX: exercise, NA: not applicable, <LOQ: below the 0.6 ng/ml limit of quantification.
[1]Due to phlebotic veins, blood sampling failed in participant C after exercise.
[2]The baseline urine specimen from participant E was collected the day before the exercise intervention and not immediately prior to exercise.

Table 3.
Serum and urinary cannabinoid levels in six regular cannabis users, before and after 24 hr of food deprivation.

	Serum THC concentrations (ng/ml)			Serum THCCOOH concentrations (ng/ml)			Urinary CC ratio (ng/mg)		
Participant	Before FD	After FD	Change (%)	Before FD	After FD	Change (%)	Before FD	After FD	Change (%)
A	4.8	2.2	−2.6 (54)	88.0	101.9	+13.9 (16)	204.9	43.8	−161.1 (79)
B	<LOD	<LOD	–	13.7	15.8	+2.1 (15)	65.7	49.4	−16.3 (25)
C	<LOQ	<LOQ	–	18.9	25.1	+6.2 (33)	80.8	49.5	−31.3 (39)
D	0.7	0.6[1]	−0.1 (14)	21.1	22.4	+1.3 (6)	45.5	26.3	−19.2 (42)
E	<LOQ	<LOQ	–	6.9	7.6	+0.7 (10)	32.5	31.3	−1.2 (4)
F	2.3	2.4	+0.1 (4)	16.6	30.7	+14.1 (85)	107.5	23.9	−83.6 (78)
Mean	2.6	1.7	−0.9 (35)	27.5	33.9	+6.4 (23)	89.5	37.4	−52.1 (58)

THC: Δ9-tetrahydrocannabinol, THCCOOH: 11-nor-9-carboxy-Δ9-tetrahydrocannabinol, CC ratio: creatinine-normalized THCCOOH, FD: food deprivation, <LOD: below the 0.2 ng/ml limit of detection, <LOQ: below the 0.6 ng/ml limit of quantification.
[1]Serum THC was below LOQ in participant D immediately after FD, but was present the following day, in the concentration shown in the table.

mean 144.4, range 60.6–295.0 ng/mg). Serum and urine remained THCCOOH positive throughout the whole study period for all subjects.

All six participants completed both the EX and FD interventions. Generally, no major differences in serum or urine cannabinoid levels before and after exercise or food deprivation were apparent (fig. 1, tables 2–3).

Discussion

It has been hypothesized that conditions that may enhance lipolysis, such as food deprivation, stress, exercise or weight loss, may lead to bursts of release of stored cannabinoids from adipose tissue. An Australian research group tested this concept in rats [12] and recently also in humans [13]. The rats were pre-treated with THC and then given either adrenocorticotropic hormone (ACTH, a known stress hormone and lipolytic agent) or deprived of food for 24 hr. Both stress and fasting resulted in statistically significant increase in plasma THC and THCCOOH levels [12]. In the follow-up study in 14 regular cannabis users, the investigators found similar results: A 35-min. bicycle workout at moderate intensity resulted in slight (<40%) but statistically significant increase in plasma THC levels [13]. The exercise-induced THC rise was no longer present 2 hr post-exercise, and exercise did not significantly affect THCCOOH plasma levels, nor did overnight fasting for 12 hr affect THC or THCCOOH plasma levels.

Our study sheds further light on this subject. Firstly, we tested subjects with longer duration of exercise (45 min.) and longer duration of food deprivation (24 hr) than in the Australian study. Secondly, our study is the first to provide urine data.

In serum, we measured transient and generally minor increases in serum THC and THCCOOH levels during physical exercise and food deprivation. Compared with individual pre-challenge values, serum THC and THCCOOH levels increased by a mean of 25% and 7%, respectively, after exercise. In a single individual (B), the increments were major in the sense that serum levels increased by a factor close to 2 (table 2). The corresponding changes in serum levels after fasting were somewhat lower, but otherwise similar (table 3). Our results are in accordance with the small increase in plasma THC levels observed after exercise in the Australian study [13]. Our participants had serum THC levels in the 0–3.6 ng/ml range at the time of admission to the ward, which is similar to those described as 'baseline' in the Australian human study [13] and those previously reported in chronic

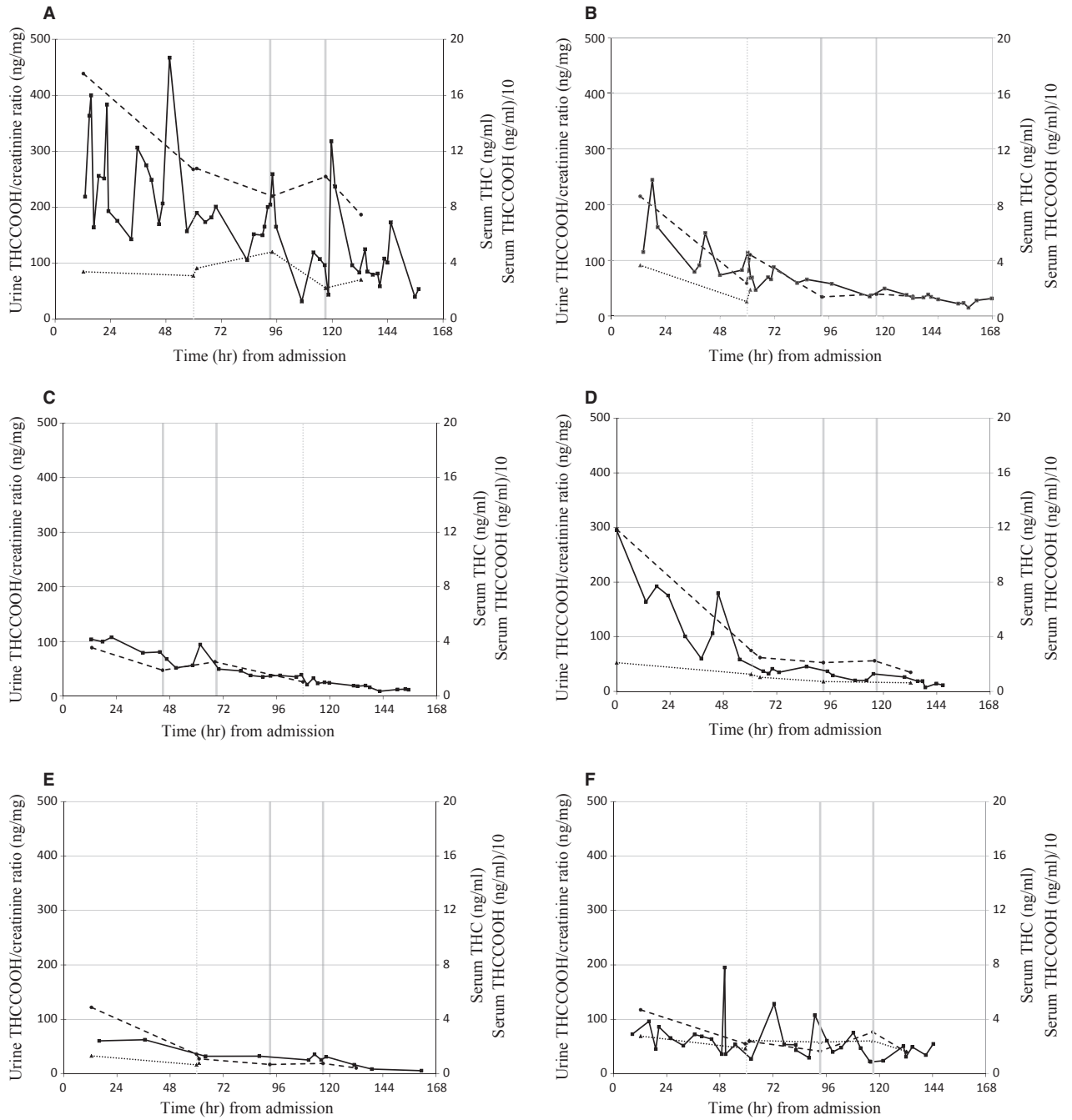

Fig. 1. Serum concentrations of Δ9-tetrahydrocannabinol (THC)(▲) and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THCCOOH) (●), and urine THCCOOH to creatinine ratio (■) for all participants (A–F). The exercise intervention is shown with the vertical grey dotted lines, and the food deprivation period is shown by the shaded area.

users who have recently stopped taking cannabis [5,14]. No subjects had THC levels exceeding 3.6 ng/ml at any time during the study. Thus, our study supports that the rise in serum cannabinoid levels during fasting or exercise is probably modest.

In urine, the CC ratios declined in five of six subjects during exercise and in all six subjects during food deprivation (tables 2–3). The urinary CC ratios fluctuated during decline as they are known to do [4], but any discernible peaks in this fluctuating pattern did not correspond to the times of exercise and food deprivation (fig. 1). Thus, we conclude that exercise within moderate limits (such as jogging) and short-term fasting (such as skipping meals for 1 day) are unlikely to cause interpretational difficulties for urinary drug testing.

There are some limitations to our study. Firstly, practical issues limited the number of participants to six individuals. However, even though the low number of partaking subjects precludes firm conclusions, our study still provides valuable information on a previously unexplored issue, notably urine elimination of cannabinoids during exercise and fasting.

Secondly, we trusted the participants to collect urine specimen themselves. Thus, we cannot exclude specimen manipulation. However, as analysis results were anonymous and not used for sanctionary purposes, participants would have nothing to benefit from manipulation.

Thirdly, all study participants were in the lower range of BMI and hence did not have much excess body fat. Future studies should attempt to include obese cannabis users, who in theory should be more sensitive to redistribution phenomena. It may be of interest to assess effects of other types of physical exercise, such as interval training, contact sports or long-distance running. It would also be interesting to measure the effect of rapid weight loss, or decreased food intake for more than 24 hr.

To summarize, neither exercise at moderate intensity for 45 min. nor 24-hr food deprivation caused significant elevations in blood or urine cannabinoid levels in our six human subjects. Our results are in accordance with data from a similar study [13], where only slight and transient THC plasma elevations were noted during exercise, and none during fasting. We conclude that exercise and fasting in regular cannabis users are unlikely to cause sufficient concentration changes to hamper interpretation in drug testing programmes.

References

1 Westin AA, Huestis MA, Aarstad K, Spigset O. Short communication: urinary excretion of 11-nor-9-carboxy-Delta(9)-tetrahydrocannabinol in a pregnant woman following heavy, chronic cannabis use. J Anal Toxicol 2009;**33**:610–4.

2 Smith ML, Barnes AJ, Huestis MA. Identifying new cannabis use with urine creatinine-normalized THCCOOH concentrations and time intervals between specimen collections. J Anal Toxicol 2009;**33**:185–9.

3 Huestis MA, Henningfield JE, Cone EJ. Blood cannabinoids. I. Absorption of THC and formation of 11-OH-THC and THCCOOH during and after smoking marijuana. J Anal Toxicol 1992;**16**: 276–82.

4 Grotenhermen F. Pharmacokinetics and pharmacodynamics of cannabinoids. Clin Pharmacokinet 2003;**42**:327–60.

5 Karschner EL, Schwilke EW, Lowe RH, Darwin WD, Pope HG, Herning R *et al.* Do Delta9-tetrahydrocannabinol concentrations indicate recent use in chronic cannabis users? Addiction 2009;**104**:2041–8.

6 Johansson E, Agurell S, Hollister LE, Halldin MM. Prolonged apparent half-life of delta 1-tetrahydrocannabinol in plasma of chronic marijuana users. J Pharm Pharmacol 1988;**40**:374–5.

7 Bergamaschi MM, Karschner EL, Goodwin RS, Scheidweiler KB, Hirvonen J, Queiroz RH *et al.* Impact of prolonged cannabinoid excretion in chronic daily cannabis smokers' blood on *per se* drugged driving laws. Clin Chem 2013;**59**:519–26.

8 Lafolie P, Beck O, Blennow G, Boréus L, Borg S, Elwin CE *et al.* Importance of creatinine analyses of urine when screening for abused drugs. Clin Chem 1991;**37**:1927–31.

9 Lowe RH, Abraham TT, Darwin WD, Herning R, Cadet JL, Huestis MA. Extended urinary Delta9-tetrahydrocannabinol excretion in chronic cannabis users precludes use as a biomarker of new drug exposure. Drug Alcohol Depend 2009;**105**:24–32.

10 Huestis MA. Pharmacokinetics and metabolism of the plant cannabinoids, delta9-tetrahydrocannabinol, cannabidiol and cannabinol. Handb Exp Pharmacol 2005;**168**:657–90.

11 Hunt CA, Jones RT. Tolerance and disposition of tetrahydrocannabinol in man. J Pharmacol Exp Ther 1980;**215**:35–44.

12 Gunasekaran N, Long LE, Dawson BL, Hansen GH, Richardson DP, Li KM *et al.* Reintoxication: the release of fat-stored delta (9)-tetrahydrocannabinol (THC) into blood is enhanced by food deprivation or ACTH exposure. Br J Pharmacol 2009;**158**: 1330–7.

13 Wong A, Montebello ME, Norberg MM, Rooney K, Lintzeris N, Bruno R *et al.* Exercise increases plasma THC concentrations in regular cannabis users. Drug Alcohol Depend 2013;**133**:763–7.

14 Skopp G, Potsch L. Cannabinoid concentrations in spot serum samples 24–48 hours after discontinuation of cannabis smoking. J Anal Toxicol 2008;**32**:160–4.