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ORIGINAL INVESTIGATION

Delta-9-tetrahydrocannabinol (THC) serum concentrations and pharmacological effects in males after smoking a combination of tobacco and cannabis containing up to 69 mg THC

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Abstract
Rationale Δ^9-Tetrahydrocannabinol (THC) is the main active constituent of cannabis. In recent years, the average THC content of some cannabis cigarettes has increased up to approximately 60 mg per cigarette (20% THC cigarettes). The pharmacokinetics of THC after smoking cannabis cigarettes containing more than approximately 35 mg THC (3.55% THC cigarettes) is unknown. To be able to perform suitable exposure risk analysis, it is important to know if there is a linear relation at higher doses.

Objectives The present study aimed to characterise the pharmacokinetics of THC, the active metabolite 11-OH-THC and the inactive metabolite THC-COOH after smoking a combination of tobacco and cannabis containing high THC doses.
Materials and methods This double-blind, placebo-controlled, four-way, cross-over study included 24 male non-daily cannabis users (two to nine joints per month). Participants were randomly assigned to smoke cannabis cigarettes containing 29.3, 49.1 and 69.4 mg THC and a placebo. Serial serum samples collected over a period of 0–8 h were analysed by liquid chromatography electrospray tandem mass spectrometry. Effects on heart rate, blood pressure and 'high' feeling were also measured.
Results Mean maximal concentrations (C_{max}) were 135.1, 202.9 and 231.0 μg/L for THC and 9.2, 16.4 and 15.8 μg/L for 11-OH-THC after smoking a 29.3-, 49.1- and 69.4-mg THC cigarette, respectively. A large inter-individual variability in C_{max} was observed. Heart rate and 'high' feeling significantly increased with increasing THC dose.
Conclusions This study demonstrates that the known linear association between THC dose and THC serum concentration also applies for high THC doses.

Keywords Cannabis · THC · Pharmacokinetics · Heart rate · High

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Introduction

Cannabis is the most used drug worldwide with about 4% of the world's adult population using cannabis at least once

a year and 0.6% daily (World Drug Report 2006). This drug is prepared from the plant *Cannabis sativa* and contains more than 400 chemicals including Δ^9-tetrahydrocannabinol (THC), its main psychoactive constituent. Cannabis is mainly used for recreational purposes. Several drug types can be produced from the cannabis plant: herbal cannabis that comprises leaves and flowers of the plant, cannabis resin, the pressed secretions of the plant and cannabis oil. European users use herbal cannabis usually mixed with tobacco whereas North America users prefer pure cannabis.

The pharmacokinetics of THC have been repeatedly studied (Lindgren et al. 1981; Barnett et al. 1982; Perez-Reyes et al. 1982; Ohlsson et al. 1982; Huestis et al. 1992a; Ramaekers et al. 2006). When smoking a cannabis cigarette, THC is already detectable in plasma seconds after the first puff. The kinetics of THC in the body are governed by its lipophilicity (Thomas et al. 1990; Grothenhermen 2003) and its strong initial binding to serum proteins (approximately 97%). This explains why THC is distributed to highly vascularised tissues, the most important of which being the liver, heart and brain (Widman et al. 1974; Grothenhermen 2003). THC is metabolised into the active metabolite 11-OH-THC and further into the inactive metabolite THC-COOH (Huestis et al. 1992b), mainly by CYP2C9 liver enzymes (Bornheim et al. 1992). The main physical effects reported in previous reports are an increase in heart rate, a conjunctival injection, a subjective 'high' feeling and an impairment of cognitive and psychomotor functions (Agurell et al. 1986).

Although European cannabis users preferably smoke joints made of a mixture of cannabis and tobacco (including nicotine), few studies have been conducted in humans with administration of cannabis and tobacco together. Most of these studies focused on the irritant effects of smoke upon the respiratory system. Only one previous study has investigated the cognitive and psychomotor effects of a combination of cannabis and tobacco containing nicotine (Ramaekers et al. 2006). The specific behavioural and biochemical consequences of the interaction between THC and nicotine at a receptor level have not been studied in humans, and rarely studied in animals (Valjent et al. 2002). An interaction between cannabis and nicotine at a metabolic level is hardly conceivable since different cytochrome P450 enzymes are involved in the metabolism of cannabis and nicotine. CYP2C9 and CYP3A4 are the enzymes specially involved in the metabolism of THC (Watanabe et al. 2007) whereas CYP2B6 is the main enzyme taking part in nicotine metabolism in humans (Anzenbacher and Anzenbacherová 2001).

THC doses tested in previous kinetic studies were much lower than the THC doses contained in joints currently available in Europe and America. For instance, the average levels of THC in netherweed cannabis sold in The Netherlands rose from 11.3% in 2000/2001 to 20.4% in 2003/2004 and 16% in 2006/2007 (Niesink et al. 2007). The 20.4% and 16% concentrations correspond to THC doses of 61 and 48 mg, respectively, in European joints that are usually made of 300 mg cannabis mixed with 700 mg tobacco. In North America, the average THC concentration in the 2003 illicit cannabis samples was 6.25%, meaning approximately 60 mg THC for a 952-mg American cannabis cigarette (El Sohly 2004). Abuse of cannabis with high THC content has raised concerns over its potential adverse impact on human health. For instance, the number of unexpected adverse effects after cannabis consumption has increased in emergency rooms in the United States (World Drug Report 2006). In previous kinetic studies by other authors, the maximal THC dose administrated was approximately 35 mg per joint (3.55% pure cannabis cigarette or 500 μg/kg cannabis mixed to tobacco in the Huestis and Ramaekers studies, respectively), which is far under the THC dose of approximately 60 mg mentioned above (Huestis et al. 1992a; Ramaekers et al. 2006).

The use of cannabis with high THC content may have consequences in terms of behavioural toxicity. For this reason, it is important to study the pharmacokinetics of cannabis with high THC in order to better understand the onset, extent and duration of its pharmacodynamic effects. The present study has been performed to assess the potential risk of cannabis joints with high THC content. This article focuses on the pharmacokinetics and acute physical effects of cannabis with THC doses up to 69.4 mg (23% THC). The cognitive and psychomotor effects after smoking cannabis cigarettes with high THC doses will be reported in a second article.

Materials and methods

Subjects

Twenty-four recreational cannabis users between 18 and 33 years of age were recruited through newspaper advertisements. Inclusion criteria were previous cannabis use (between two and nine joints per month) and no chronic use of medication. Only male volunteers were included. Exclusion criteria were history of psychiatric illness, liver disease, respiratory or cardiovascular system diseases or severe or chronic illnesses; use of other illicit drugs or evidence of excessive alcohol abuse. The screening of the participants included a questionnaire on medical history, a medical examination and an electrocardiogram. Blood and urine samples were also collected in order to conduct standard blood chemistry, haematology and drug screen tests. All subjects provided written informed consent prior to their inclusion in the study and were paid for their participation. The study protocol was approved by the

Ethics Committee of the University Medical Centre Utrecht. The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and amended in 2000.

Design, doses and administration

The study was conducted according to a four-way, double-blind, placebo-controlled, cross-over design. Each participant smoked four joints on four exposure days with a minimum washout period of 7 days between two treatments. Participants were asked to refrain from any drugs (except alcohol) 15 days before and during the study period. Participants arrived the evening prior to each test day and stayed overnight in our unit to ensure drug and alcohol abstinence of at least 10 h before the experiment. Urine drugs screens were performed upon arrival of the participants using the DrugControl® tests to assess for the presence of amphetamines, barbiturates, benzodiazepines, cocaine metabolites, methaqualone, opiates, MDMA (ecstasy), 3,4-methylenedioxyamphetamin (MDA) and THC (cutoff level 50 ng/ml THC-COOH).

The joints were prepared according to a standardised protocol and consisted of a mixture of 300 mg cannabis and 700 mg tobacco. The cannabis contained various concentrations of THC: 0.003% for the placebo, 9.8% for the low dose (29.3 mg per joint), 16.4% (49.1 mg per joint) for the middle dose and 23.1% for the high dose (69.4 mg per joint). The cannabis batches for the active joints were obtained from the Office for Medicinal Cannabis (Dutch Ministry of Health) and the placebo batch was supplied by the National Institute on Drug Abuse (NIDA, USA). Subjects were instructed to smoke the cigarettes according to a computer-controlled paced procedure, i.e. 3 s for getting ready, 2 s for inhalation, 3 s for breath holding and 32 s for normal breathing and relaxation. This sequence was repeated until the whole joint was smoked, this usually took about 22 min.

Analytical methodology

Venous blood was sampled from a catheter in the participant's forearm and transferred to Vacutainer® serum separator tubes (BD, USA) at baseline (between 2 h and 30 min before the onset of smoking) and at 5, 10, 15, 20, 25, 30, 42 and 55 min and 1 1/2, 2, 3, 5 and 8 h after the onset of smoking. The tubes were allowed to clot 0.5–2 h, then they were centrifuged for 10 min at 1,300×*g* and stored at −20°C until analysis. Serum concentrations of THC, 11-OH-THC and THC-COOH were determined by DeltaLab (The Netherlands) using solid phase extraction and liquid chromatography electrospray tandem mass spectrometry detection (Kintz and Cirimele 1997; Gustafson et al. 2003; Maralikova and Weinmann 2004). More details on the analytical methodology are given in the Appendix. The limits of quantification (LOQ) were 0.5, 0.5 and 1.0 μg/L for THC, 11-OH-THC and THC-COOH, respectively. To reduce variation, all samples from each participant were analysed in the same batch.

Outcome measures

Serum concentrations of THC and its main metabolites (11-OH-THC and THC-COOH) were the primary outcome measures. The blood pressure and the heart rate were also monitored with a Passport 2® monitor model (Datascope, USA). Because cannabis can induce sudden hypotension, each participant was seated on an emergency stretcher during the smoking procedure. In order to limit the health risks for participants, an upper limit for the heart rate was set at 170 bpm and a lower limit for the mean arterial blood pressure was set at 55 mmHg. Participants were asked to estimate their 'high' feeling on a 100-mm long visual scale (anchored by '0—not at all' and '100—tremendous high'). Drowsiness was recorded in a similar way. Participants were allowed to look at the high and drowsiness ratings that they had already scored before in order to rate the next score.

Pharmacokinetics

The serum pharmacokinetics of THC and its metabolites were analysed by conventional non-compartmental approaches using a pharmacokinetic software (TopFit, v2.0) (Tanswell and Koup 1993). Peak concentration (C_{max}), time to reach C_{max} (t_{max}) and area under the curve (AUC) were determined from the individual serum concentrations. AUC (up to the last concentration equal or above the LOQ) determination was based on the logarithmic trapezoidal rule. Participants with a THC concentration higher than the LOQ at baseline were excluded from the analyses because this indicated that they had smoked a cannabis cigarette outside the frame of the study. Participants with a positive urine drug test for cannabinoids were included as cannabinoids can be tested positive up to 15 days after exposure to cannabis.

Statistics

Multivariate mixed models ANOVA were employed to analyse all outcome measures with repeated measures across THC dose (four doses) (Proc GLM in SAS v9.1). Polynomial contrast specification was used to test the hypothesis that the relationship between THC exposure dose and effects was linear. Tukey's HSD post hoc tests were performed to document whether pharmacokinetic parameters significantly differed between the low, medium and high THC doses. A Huynh–Feldt epsilon correction

was applied to counter sphericity violations, when necessary. Additionally, a linear mixed model analysis (Proc MIXED in SAS v9.1) was conducted to test whether inter-individual differences in THC C_{max} could be explained by differences in previous cannabis use, body mass index (BMI, defined as weight/length^2), time required to smoke the cigarette and rate of metabolism of THC. Previous cannabis use was measured by the self-reported average number of joints smoked during the last 12 months, and rate of metabolism of THC by the 11-OH-THC serum concentration measured 10 min after the THC peak. A P value less than or equal to 0.05 was considered significant.

Results

Flow of participants and sample characteristics

Twenty-four people were randomly assigned, and 12 people were kept as reserve. Six of the 24 allocated persons did not complete the intervention due to illnesses unrelated to the intervention (two persons) or due to smoking problems after one experiment (four persons, two with the low dose, one with the middle dose and one with the high dose) and were, therefore, replaced by six persons from the reserve group. The four persons with smoking problems were not able to finish an entire joint within approximately 22 min, mainly because they normally only smoke part of the joint and do not smoke tobacco otherwise. Furthermore, participants with a baseline THC serum concentration higher than the LOQ were excluded from the analyses since this indicates they had smoked a cannabis cigarette aside from the study. One participant with eight out of 14 blood samples missing was also excluded from the analyses. The analyses include finally 20, 18, 20 and 20 participants for the placebo, 29.3, 49.1 and 69.4 mg THC cigarettes, respectively. Participants' demographics are detailed in Table 1.

Smoking duration and temporary stops

Despite the use of a paced smoking procedure, the time used to smoke the cannabis cigarette was dose-dependent [$F(3,36)=9.80$, $P<0.001$]. The actual smoking duration was computed by excluding the duration of the stops necessary for safety reasons (i.e. when the MAP or the heart rate were approaching the limits defined in the protocol). It increased from 19.0 min on average (SD=3.4) with the placebo cigarette to 21.6 min (SD=5.0), 23.1 min (SD=4.6) and 24.4 min (SD=4.4) with the 29.3, 49.1 and 69.4 mg THC cigarettes, respectively. The number of people obliged to stop smoking temporarily because they reached the maximum heart rate limit or the minimum blood pressure limit was larger with the middle and high THC doses: with the placebo, no one had to stop; one person was stopped with the low dose; four with the middle dose and three with the high dose. These people re-started smoking after the physician present during the experiment gave his/her agreement. The median duration of these stops was 9 min (range 1–24 min). During these stops, the cannabis cigarette usually stopped burning and had to be lit up again when the participant re-started to smoke. It is important to specify that all participants who stopped smoking transitorily, stopped after the THC peak had occurred. The median time elapsed when they stopped smoking, since the onset of smoking, was 14.5 min with a range of 4–29 min.

Δ^9-Tetrahydrocannabinol concentrations in serum

Serum THC concentrations after smoking a 29.3-, 49.1- and 69.4-mg THC cigarette are shown in Fig. 1. Mean THC concentrations were 102, 112 and 173 μg/L 5 min after onset of smoking for the 29.3-, 49.1- and 69.4-mg THC doses, respectively. Observed mean THC peaks were 123.8 μg/L (SD=65.5), 168.0 μg/L (SD=100.3) and 190.4 μg/L (SD=106.8), respectively. The maximum THC C_{max} observed was 462 μg/L with the highest THC dose, but wide inter-individual differences in THC C_{max} were observed. The range of THC C_{max} was 38–276, 59–421 and 24–462 μg/L for the lowest, middle and highest THC doses, respectively. THC C_{max} did not increase proportionally: the increase in C_{max} was 1.5 times when the dose increased from 29.3 to 49.1 mg THC, but only 1.1 times when the dose increased from 49.1 to 69.4 mg.

The time to reach the maximal serum THC concentration did not differ significantly across THC dose. The disappearance of THC from serum appeared to occur in two phases. In the initial phase, after exposure to the 69.4-mg THC cigarette, THC serum levels dropped rapidly to an average value of 39.0 μg/L. In the second phase, from 57 min after onset of smoking onwards (point of inflection), serum THC levels decreased much more slowly. Similar patterns were observed with the 29.3- and 49.1-mg THC doses. At the last data point, 8 h after onset of smoking, the mean THC levels were still above the LOQ (low dose: mean=1.44 μg/L, SD=1.5; middle dose: mean=1.72 μg/L, SD=2.0; high dose: mean=2.0 μg/L, SD=1.3). Differences in mean THC serum concentration were significant across doses [$F(2,26)=13.0$, $P<0.001$].

11-OH-THC and THC-COOH levels in serum

Figure 2 shows the mean serum concentrations of THC, 11-OH-THC and THC-COOH over time for the 29.3-, 49.1-, and 69.4-mg THC dose on a semi-logarithmic scale. The active metabolite 11-OH-THC was directly measurable in

Table 1 Participants' characteristics

Subject	Age (years)	Height (cm)	Weight (kg)	Age at first cannabis use (years)	Average use (monthly)[a]	Drug use previous year
1[b,c,d]	23	180,00	76.0	17	4	N, E
2	23	183,00	67.0	18	4	E
3	26	183,00	72.0	17	8	N, E
4	20	180,00	76.0	17	2	E
5	24	185,00	79.0	17	12	N, E
6[b]	22	189,00	80.0	14	8	E
7	24	185,00	86.0	14	10	N, E, C
8	33	184,00	76.0	15	12	N, E
9	20	183,00	74.0	15	8	E
10	21	192,00	75.0	15	4	N, E
11[b,e]	22	185,00	81.0	18	5	N, E
12[c]	24	190,00	68.0	12	10	N
13	27	179,00	65.0	15	15	N, E
14	24	190,00	78.0	16	4	N, E
15	27	190,00	71.0	19	4	N, E
16[d]	20	178,00	75.0	17	1	N, E, C
17	31	181,00	82.0	19	8	N, E
18	24	182,00	69.0	19	2	N, E, C
19	25	179,00	76.0	15	8	N, E
20[c]	24	175,00	72.0	18	8	N, E
21[b,c,d]	18	184,00	68.0	14	12	N, E
22	18	183,00	69.0	16	2	E
23[b]	20	176,00	71.0	16	6	N, E, C, MDMA
24[b]	33	185,00	71.0	17	4	N, E
Mean±SD	23.9±4.1	183.4±4.5	74.0±5.3	16.2±1.8	7.9±3.7	

N nicotine, *E* ethanol, *C* cocaine, *MDMA* 3,4-methylenedioxy-*N*-methylamphetamine (ecstasy)
[a] Average number of cannabis cigarettes smoked during the last 12 months prior to the study
[b] Participant with a baseline THC>LOQ during the low-dose experiment
[c] Participant with a baseline THC>LOQ during the middle-dose experiment
[d] Participant with a baseline THC>LOQ during the high-dose experiment
[e] Participant with eight out 14 blood samples missing during the high-dose experiment

the first serum sampling after the onset of smoking (at 5 min). With the 69.4-mg THC dose, 11-OH-THC was present in serum in concentrations varying from approximately 10% of the THC levels at 30 min to approximately 30% of these levels at 1 h, approximately 49% at 2 h and 63% at 3 h. Mean peak 11-OH-THC concentrations occurred 31 min after the start of smoking and were 7.7 μg/L (range 2–29), 13.3 μg/L (range 3–42) and 13.8 μg/L (range 3–41) after one 29.3-, 49.1- and 69.4-mg THC cigarette, respectively (Fig. 2).

The inactive metabolite THC-COOH was present in higher serum concentrations than 11-OH-THC. On average, it was detected 7.6 min after the start of smoking. THC-COOH concentrations increased slowly to peak at 26.1 (range 4–64), 45.9 (range 9–162) and 42.4 (range 11–128) after one 29.3-, 49.1- and 69.4-mg THC cigarette, respectively (Fig. 2). Its concentration in serum equaled the THC serum concentration at 43, 46 and 55 min after onset of smoking for the low, middle and high THC doses.

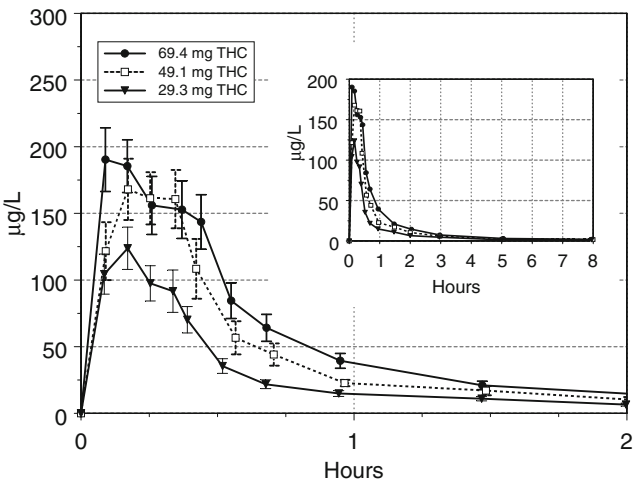

Fig. 1 Mean (±SEM) THC serum concentrations after smoking a single cannabis cigarette containing 29.3, 49.1 and 69.4 mg THC, respectively. Data represent the mean responses of 18, 20 and 20 participants, respectively. Drug was not detectable after smoking of placebo cigarettes

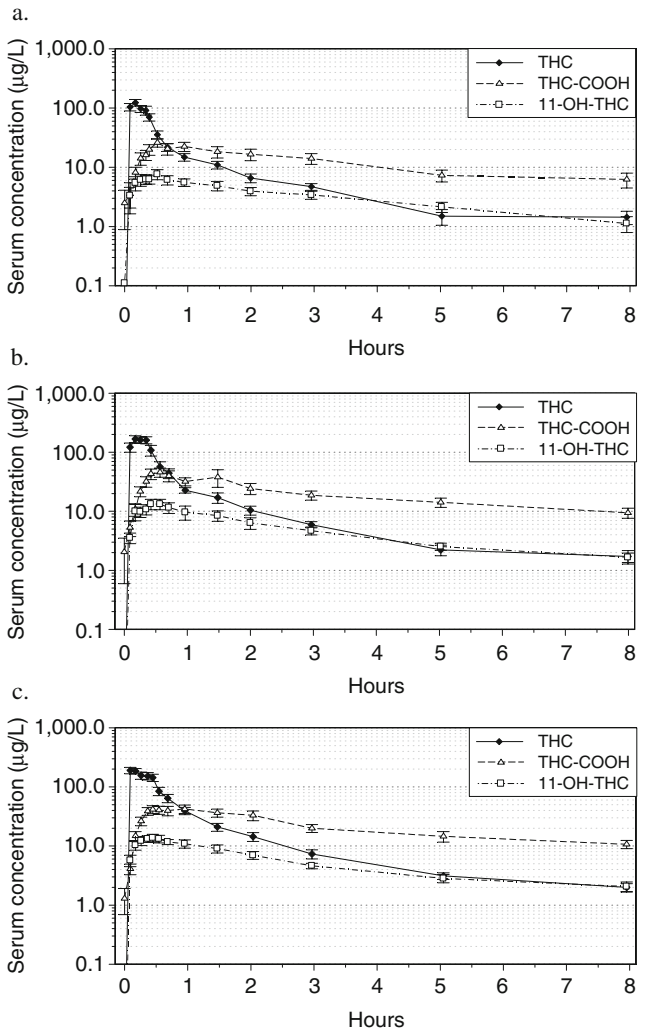

Fig. 2 Mean (±SEM) THC, 11-OH-THC and THC-COOH levels during smoking of a cannabis cigarette containing: **a** 29.3, **b** 49.1 and **c** 69.4 mg of THC, respectively. The curves represent the mean concentrations of 18, 20 and 20 participants for the 29.3-, 49.1- and 69.4-mg THC doses, respectively

Pharmacokinetic parameters

Pharmacokinetic parameter estimates are summarised in Table 2. THC C_{max} and AUC differed significantly between dose levels, whereas t_{max}, $t_{½,\alpha}$, and $t_{½,\beta}$ did not. The linear trends for THC C_{max} and AUC were also significant [$F(1,14)=10.24$, $P=0.006$ and $F(1,14)=23.5$, $P<0.001$, respectively], indicating that THC C_{max} and AUC increased with increasing THC exposure dose. Tukey's post hoc tests ($\alpha=0.05$) showed the C_{max} and AUC at both medium and high THC doses to be greater than at low THC dose [$F(1,14)=6.18$, $P=0.03$ and $F(1,14)=10.2$, $P=0.006$ for medium versus low dose and high versus low dose for THC C_{max}, respectively; $F(1,14)=2.88$, $P=0.002$ and $F(1,14)=7.0$, $P<0.001$ for medium versus low dose and high versus low dose for THC AUC, respectively]. Similar results were obtained for the analysed THC metabolites. None of the pharmacokinetic parameter estimates differed significantly between the subjects who stopped smoking transitorily and the other subjects (data not shown). Parameter estimates for the placebo exposure were not computable since all serum concentrations were at or near the LOQ.

In the multivariate mixed model analysis, no difference in BMI or previous cannabis use could explain the inter-individual difference in THC C_{max} [$F(1,19)=1.32$, $P=0.26$; $F(1,19)=0.35$, $P=0.56$, respectively]. The THC dose, time used to smoke the cigarette and 11-OH-THC serum concentration observed 10 min after the THC peak were significant predictors of THC C_{max} [$F(2,19)=5.05$, $P=0.02$; $F(1,19)=4.59$, $P=0.045$ and $F(1,19)=8.45$, $P=0.01$, respectively].

Heart rate

Heart rate increased significantly with increasing THC dose [$F(3,36)=25.9$, $P<0.001$] (Table 3). The dose–effect relationship was linear [$F(1,12)=57.7$, $P<0.001$]. In 26 out of 58 non-placebo exposures (45%), the participant's heart rate exceeded 140 bpm, and in three out of 58 non-placebo exposures (5%), subjects had to stop smoking temporarily because they reached the maximum heart rate limit of 170 bpm (subject 12 with the low dose, subjects 3 and 18 with the high dose). Individual heart rates over time after smoking a cannabis cigarette containing 69.4 mg THC are illustrated in Fig. 3a. With the 69.4-mg THC cigarette, accelerations up to 121% above baseline (from 76 to 168 bpm) were observed. Subjects 12, 3 and 5 showed the greatest increase in pulse rate whilst their THC serum concentration was 277, 348, and 154 µl/L, respectively. After the initial increase within the first 12 min, the heart rate decreased slowly until 8 h post-smoking. At this time, 14 of the 20 participants included in the analyses had still an increased heart rate relative to the baseline.

Blood pressure

There were no significant effects of dose on any measure of systolic or diastolic or mean arterial blood pressure (Table 3). However, in 13 out of 58 non-placebo exposures (22%), the participant was placed in supine position (during or after the smoking period) because of symptoms of hypotension, and in two out of 58 non-placebo exposures (3%), subjects had to stop smoking temporarily because they nearly reached the lower limit of 55 mmHg set in the study protocol (subjects 21 and 23 after exposure to the high dose). In seven out of 58 non-placebo exposures (12%), the participant had nausea or vomited. The general trend was a decrease in blood pressure within the first 43 min after onset of smoking, but an initial increase in

Table 2 Pharmacokinetics of THC, 11-OH-THC and THC-COOH for the three non-placebo doses

Parameters	Mean (SD)			Inter-dose difference[a]		Linear trend[a]	
	29.3-mg THC (9.8%) n=18	49.1-mg THC (16.4%) n=20	69.4-mg THC (23.1%) n=20	F (2,28)	P value	F (1,14)	P value
THC							
C_{max} (μg/L)	135.1 (68.5)	202.9 (112.4)	231.0 (108.5)	4.54	0.02	10.24	0.006
t_{max} (min)	9.8 (4.7)	14.1 (6.1)	12.3 (8.8)	2.06	0.15	0.03	0.86
$AUC_{0–8}$ (μg h/L)	76.4 (38.2)	113.1 (53.1)	150.4 (72.8)	13.65	<0.001	23.52	<0.001
11-OH-THC							
C_{max} (μg/L)	9.2 (7.6)	16.4 (15.1)	15.8 (8.8)	9.01	0.001	25.64	<0.001
t_{max} (min)	25.4 (11.3)	21.9 (7.5)	25.0 (15.5)	1.24	0.31	0.95	0.35
$AUC_{0–8}$ (μg h/L)	24.2 (15.9)	36.7 (24.4)	40.4 (19.4)	10.90	<0.001	18.80	0.001
THC-COOH							
C_{max} (μg/L)	30.4 (20.0)	59.7 (56.9)	54.5 (29.9)	12.11	<0.001	15.00	0.002
t_{max} (min)	46.2 (27.6)	42.2 (20.7)	38.8 (18.5)	0.53	0.59	0.76	0.40
$AUC_{0–8}$ (μg h/L)	90.2 (78.4)	149.7 (119.4)	160.9 (99.8)	11.28	<0.001	19.10	0.001

C_{max}, maximal serum concentration, t_{max}, time to reach the maximal serum concentration, $AUC_{0–8}$ area under the serum concentration–time curve (up to 8 h)

[a] For the analyses, the parameters were normalised by logarithmic transformation

blood pressure was observed among some participants. Concerning individual mean arterial blood pressure, the largest decreases in mean arterial blood pressure were observed with the high THC dose with drops up to 41% below baseline (from 121 to 71 mmHg). Subjects 2, 23 and 12 showed the greatest decreases in mean arterial blood pressure whilst their THC serum concentration was 34, 213, and 137 μl/L, respectively, at 43, 17 and 7 min after onset of smoking. Subjects 10, 22 and 19 showed limited initial increase in mean arterial blood pressure (up to 37% above baseline, from 87 to 119 mmHg). Mean arterial blood pressure was still below baseline levels 8 h post-smoking for a majority of the participants.

‘High’ feeling and drowsiness

The drug high increased significantly with increasing doses with a significant linear trend (Table 3). Figure 3b illustrates the individual high scores from baseline after smoking a cannabis cigarette containing 69.4 mg THC. The high feeling was tremendous for a majority of participants and was often felt as an unpleasant “heavy body” or “stoned” feeling. The high feeling disappeared for the majority of the participants within 3.5 and 5 h post-smoking. Subject 24, who felt a high until 7 h post-smoking, was also the person with the highest observed THC C_{max} (462 μg/L). No significant differences in

Table 3 Physical and subjective effects

Outcome[a]	Mean (SD)				Inter-dose difference		Linear trend	
	Placebo THC (0%) (n=20)	29.3-mg THC (9.8%) (n=18)	49.1-mg THC (16.4%) (n=20)	69.4-mg THC (23.1%) (n=20)	F (3,36)	P value	F (1,12)	P value
Cardiovascular effects								
ΔHeart rate (bpm)	26.1 (11.8)	57.1 (16.2)	59.3 (15.0)	68.7 (14.7)	25.9	<0.001	57.7	<0.001
ΔSystolic BP (mmHg)	−14.8 (12.1)	−16.1 (14.4)	−15.7 (14.6)	−20.6 (18.1)	0.27	0.85	0.02	0.89
ΔDiastolic BP (mmHg)	−5.6 (7.6)	−9.4 (11.3)	−11.7 (12.5)	−14.8 (13.9)	0.64	0.59	2.90	0.12
ΔMean arterial BP (mmHg)	−9.9 (6.2)	−13.4 (13.6)	−12.9 (13.3)	−18.1 (15.1)	0.48	0.70	0.72	0.41
Subjective effects								
ΔHigh score (mm)	8.1 (13.1)	52.7 (27.9)	61.8 (29.3)	79.9 (18.7)	36.4	<0.001	172.7	<0.001
ΔDrowsiness (mm)	5.9 (32.2)	20.8 (25.1)	23.8 (25.1)	31.1 (36.3)	1.2	0.34	2.6	0.14

BP blood pressure

[a] Δ values represent maximum change scores (after onset of smoking−before onset of smoking; same day)

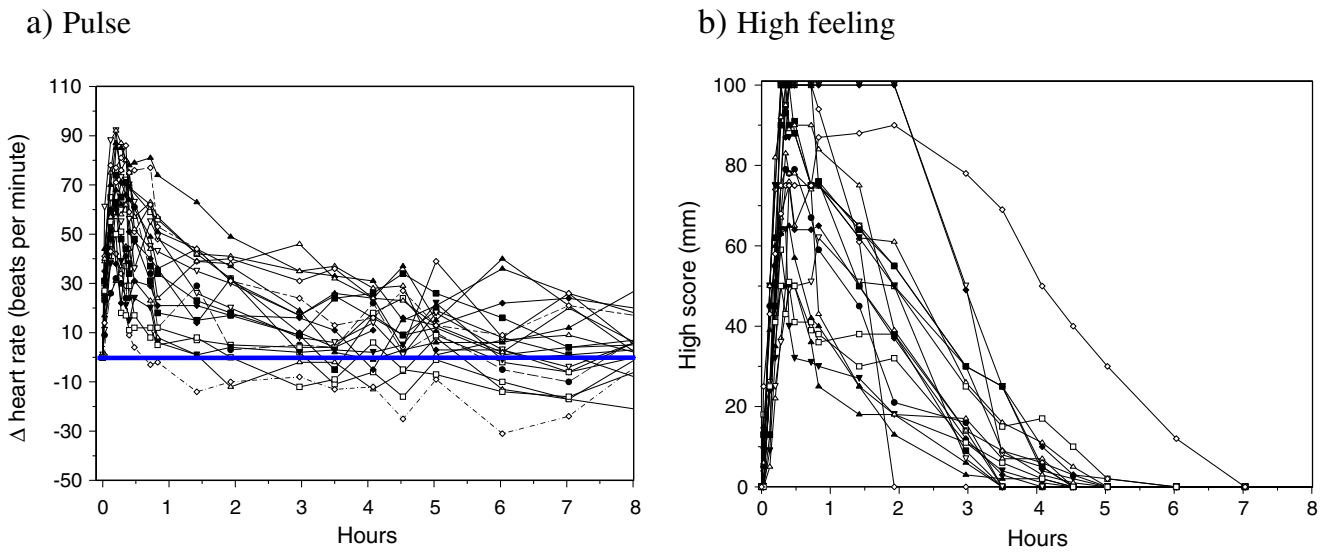

Fig. 3 Cardiovascular effects high feeling and drowsiness after smoking of a single cannabis cigarette containing 61.4 mg. **a** Individual changes from baseline in pulse rate (in bpm). **b** Individual "high" feeling scores (in mm)

drowsiness were observed between the different THC doses (Table 3).

Unpleasant effects, such as hypotension requiring a supine position, and/or vomiting, and/or tachycardia >140 bpm, were observed in 34 out of the 58 non-placebo experiments (59%, eight in low-dose, 10 in middle-dose and 16 in high-dose experiments) by 17 of the 24 participants (71%).

Discussion

The present study provides data on smoking cannabis cigarettes containing up to 69 mg THC (23%), i.e. doses twice as high compared to previous pharmacokinetic studies on this subject (Lindgren et al. 1981; Barnett et al. 1982; Perez-Reyes et al. 1982; Ohlsson et al. 1982; Huestis et al. 1992a; Ramaekers et al. 2006). These high doses have never been studied before whereas they correspond to the mean THC content of joints currently available on the market (Niesink et al. 2007; El Sohly 2004). The increase in potency of the cannabis herb in recent years is related to advances in growing techniques and selection of the seeds and to the domestic indoor production of 'netherweed'. The domestic production of cannabis with high THC content is a frequently occurring phenomenon since half of European countries reported some domestic cannabis production in 2005 (EMCDDA 2007).

The high THC doses produced higher individual THC serum concentrations compared to previous studies. We found that the relationship between THC exposure dose and THC serum concentration continued to be linear into this high dose range. With the 49.1- and 69.4-mg THC doses, unpleasant effects including nausea, vomiting, 'stoned' feeling, hypotension and tachycardia >140 bpm were observed in the majority of the participants even though the participants were already used to cannabis. Also, we found that C_{max}, AUC of THC, effects on heart rate and high feeling increased with increasing THC dose, but this increase was not proportional. There were differences in C_{max}, AUC, heart rate and high feeling between the middle and high doses and the placebo dose, but not between the middle and high doses with respect to each other. This is consistent with previous results (Lane et al. 2005).

This study was by no means straightforward to conduct. The unpleasant effects of the experiment were the explanation that some subjects of the initial group had to be replaced by other subjects from a reserve pool. Vomiting, hypotension and 'stoned' feeling caused six subjects to stop even though no extreme changes in their blood pressure and heart rate were observed. Participants who completed the present study were less experienced than most subjects included in previous studies in which daily cannabis users (Lindgren et al. 1981; Hart et al. 2001; Moeller et al. 1992) or subjects smoking between eight and 12 joints per month for 9 years or more (Barnett et al. 1982; Huestis et al. 1992a) were included. However, most of the participants in our study had experience with smoking 'netherweed', i.e. cannabis with high THC content. This probably partially explained that our recreational cannabis users could handle 23% THC cigarettes. Another reason may be the smoking time which was longer in our study than in most previous studies with an average of 22 min as opposed to 10–12 min in most studies (Barnett et al. 1982; Huestis et al. 1992a;

Ramaekers et al. 2006). When cannabis users smoke at their own pace, the smoking time results in an average of 30 puffs over 15 to 19 min (Perez-Reyes et al. 1982). Our study reflects, therefore, the common practice of cannabis users better than most previous reports. The extended smoking time in our study probably resulted in more sidestream smoke loss and more pyrolysis of THC and, therefore, limited the absorption of THC.

Still, the THC serum concentration was higher than 200 ng/mL in 25 out of the 68 non-placebo experiments (35%) and individual THC peaks up to 462 ng/mL were observed. At the group level, the THC C_{max} observed after smoking the high dose (173 ng/mL) was higher than the THC plasma concentration of 162 ng/mL previously reported in two studies using 3.55% and 2.54% pure cannabis cigarettes smoked in about 10 and 17 min, respectively (Huestis et al. 1992a; Perez-Reyes et al. 1982). A third study in which participants had to smoke a mix of tobacco and cannabis, at a 500 μg/kg dose (so about 37 mg for a 74-kg subject) in about 10 min, reported a much lower mean THC C_{max}, that is 79 ng/mL (Ramaekers et al. 2006). The variability of THC C_{max} was nearly six times larger in our study than in previous studies (SD= 108.5 versus 18.7 with the 2.54% dose in Perez-Reyes' study) and with a broader range of THC C_{max} values (24–462 μg/L in our study with the 69.4-mg cigarette versus 76–267 μg/L in Huestis' study with a 3.55% cigarette, about 30 mg THC).

This large inter-individual variability in THC C_{max} could be related to the differences in drug absorption, distribution and metabolism. Puff duration and breath holding were controlled in our experiments, but not the depth of inhalation which is highly correlated to the inhalation volume. Previous studies have emphasised the difficulty of controlling this factor which certainly influences THC absorption. Also, lung function may have differed between participants, but we had no data to investigate this hypothesis. Because of the strong lipophilicity of THC, we tested whether differences in BMI could also partially explain the differences in THC C_{max} between participants, but no significant relation was found. However, the range of BMI was narrow in our study (18.8–25 kg/m^2) and BMI may not be a good measure for correlating THC concentrations as a parameter for storage in lipid tissue depots. Percent body fat might have been a better variable to look at for this relationship but this parameter was not recorded in our study. The 11-OH-THC serum concentration observed 10 min after the THC peak differed significantly between participants, suggesting inter-individual differences in time necessary to metabolise THC. This could be related to the induction of enzymes involved in the metabolisation of cannabis (CYP2C9 and CYP3A) with regular cannabis or drugs use or to polymorphism, i.e. a difference in DNA sequences encoding proteins involved in THC metabolism. We found no significant relation between THC C_{max} and participants' cannabis use in the past, but this variable was a recalled parameter and, therefore, not totally reliable.

11-OH-THC plasma concentrations have not often been measured in previous studies whereas this information is important since this metabolite is thought to contribute to the overall psychoactive effects of THC (Grothenhermen 2003). Huestis et al. (1992b) measured 11-OH-THC peak concentrations in plasma equal to 6.7 and 7.5 ng/ml in their study, after smoking one 1.75% cigarette and one 3.55% cigarette, respectively (about 16 mg and 30 mg). In the present study, the 11-OH-THC serum concentrations were twice as high after smoking a 49.1- and 69.4-mg cigarette, but the 11-OH-THC peak occurred later, about 25 min after the onset of smoking as opposed to 13.5 min in Huestis' study, probably because of the longer smoking time in our study.

Knowledge about the THC-COOH concentration is interesting since concentrations of this metabolite can be used to predict the time of cannabis exposure (Huestis et al. 1992c). Mean THC-COOH C_{max} in our study, ranging between 30 and 60 ng/mL, were comparable to concentrations reported in previous studies. Huestis reported a mean peak THC-COOH concentration of 54 ng/mL at 1.35 h after smoking a 3.55% THC cigarette in 10 min (Huestis et al. 1992b) and Perez-Reyes reported a mean peak THC-COOH concentration of 50 ng/mL at 28 min after smoking a 2.54% THC cigarette in 17 min (Perez-Reyes et al. 1982).

Smokers tended to breathe deeper during the early puffs that resulted in quick effects on heart rate and blood pressure. A decrease in blood pressure was observed but the blood pressure changes did not reach statistical significance. An increase in blood pressure has been observed in a previous study (Huestis et al. 1992a). How do we explain these apparently contradictory results? It has been shown that THC activates cannabis receptors on nerves of arterial blood vessel walls and that, subsequently, dilatation of the blood vessel may occur. If the human body does not counteract, then decrease of blood pressure and relative insufficient blood volume is inevitable. To correct the hypotension, the heart rate usually accelerates as we have observed. Blood pressure drops can be observed when compensation mechanisms fail.

The high score increased significantly with increasing doses. The average high feeling peak observed in our study with the 69.4-mg THC cigarette (80%) was higher than the highest mean peaks reported in previous studies on smoking cannabis, i.e. 74.5, 69 and 66 after smoking a 2.54%, 3.6% and 1.97% cigarette, respectively (Perez-Reyes et al. 1982; Fant et al. 1998). Subjects tended to

become drowsier with increasing doses of cannabis until 5 h post-smoking, but the change in drowsiness did not reach statistical significance, maybe because of the large inter-individual variability observed.

In the present study, several limitations should be mentioned. The inclusion of males limits the generalisability of the results. Only male subjects were investigated because THC is a highly lipophilic substance and males and females differ in their constitution with respect to adipose tissue. The monitoring period (8 h after onset of smoking) was a limitation since it was too short to determine half-lives. Another limitation is that, for ethical reasons, we were obliged to limit the risks the participants were exposed to. Therefore, the participants were asked to smoke the cannabis cigarette in a reclining posture, which is also their customary position at home. Also, participants were requested to stop smoking transitorily when their mean arterial blood pressure was lower than 55 mmHg or their heart rate was >170 bpm. Furthermore, the smoking period was extended in our study compared to previous studies. All these factors have probably resulted in an underestimation of acute effects. Participants were nevertheless instructed to smoke an entire joint to model high-risk behaviour, which may counterbalance the risk underestimation. In the experimental conditions of the study, participants were required to smoke the entire joints—even if it caused unpleasant effects. Finally, the cigarettes contained a mix of tobacco and cannabis since we were interested in assessing the risks relating to the usual practice of European cannabis users. From a pharmacokinetic point of view, mixing tobacco to cannabis resulted in a possible slower absorption rate of THC since the smoking time was extended compared to previous studies. Nicotine may have strengthened the increase in heart rate and counteracted the decrease in blood pressure induced by cannabis.

In conclusion, the linear relationships between exposure THC dose and serum THC concentration and between exposure THC dose and increase in heart rate and high feeling persist at THC concentrations up to 69 mg (23%). Clear effects were observed even for participants already used to cannabis, so cannabis with high THC concentrations may be a concern for public health. Finally, 8 h post-smoking, THC serum concentration was still 2 ng/mL on average, which has been reported to be the lower range of a THC concentration associated to psychomotor impairment in a previous study (Ramaekers et al. 2006).

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Appendix

Serum was cleaned by solid phase extraction on a Focus SPE cartridge (3 ml, 20 mg; Varian, USA) using 1.0-ml samples. Concentrations of THC and its metabolites 11-OH-Δ^9-THC (11-OH-THC) and 11-nor-carboxy-Δ^9-THC (THC-COOH) were determined with liquid chromatography electrospray tandem mass spectrometry (LC-ESI±MS/MS), using a Finnigan surveyor with quantum discovery mass spectrometer (Thermo Electron, USA). Chromatographic separation (20 µl injection volume) was achieved in 10 min over a Polaris C18-A (100×2.0 mm) 5 µ column (Varian, USA) using gradient elution with an aqueous mobile phase (containing 0.01% trifluoracetic acid and a organic phase using methanol and acetonitrile), at a flow rate of 0.4 mL/min. Standards for the drugs were obtained from Cerilliant, Texas, USA and diluted in drug-free human plasma at a concentration ranging between 0 and 50.0 µg/L. D_3-deuterated standards for THC and THC-COOH were purchased from the same corporation and added as an internal standard at the concentration of 50 µg/L. The mass spectrometer was operated in electrospray ionisation positive ion mode, and quantitation was conducted using selected reaction monitoring. Calibration analyses were completed according to internal standard methods with the Xcalibur software (Thermo Electron) using calibration curves with linear fit identification. The LOQ for THC, 11-OH-THC and THC-COOH were 0.5, 0.5 and 1.0 µg/L, respectively.

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