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Pupillary effects in habitual cannabis consumers quantified with pupillography

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ABSTRACT

Driving under the influence of alcohol (DUIA) and drugs (DUID) is considered an elevated risk for traffic safety. When assessing a driver's fitness to drive, standardized and objective measurement methods are still required, in order to clarify the question whether an individual is under the influence of substances acting on the central nervous system (CNS). We exposed healthy test subjects (n = 41) as well as persons who were under the influence of cannabis after repeated inhalation to multiple light stimuli using infrared technology and measured the pupillary light reflex (PLR).

Toxicological tests of blood samples taken from every subject followed. The aims of this study were to assess the differences in pupillography response between cannabis consumers after a washout period and no cannabis consumers as well as the dose related effects on pupillography parameters of cannabis in cannabis consumers. All four pupillary parameters changed according to a weakened pupil function after acute administration of cannabis in all test subjects. Furthermore, it could be observed that habitual cannabis consumers showed an altered pupillary function just before the first dose was taken, suggesting that the long-term effects and addiction also have to be taken into account, when effects of the CNS are discussed. The results of the present study show that almost all pupil parameters could be reliable indicators for the detection of subjects under the acute effect of cannabis.

1. Introduction

Since 2006 an US/European international study about the onsite oral fluid (saliva) drug testing devices has shown that no device is reliable enough in order to be recommended for roadside screening of drivers [1]. Standard neurological tests for gross motor coordination such as the observation of gait or one-leg stand, the ability to walk in a straight line or finger-to-nose or the examination of the pupillary light reflex using a halogen penlight are not reliable to determine if a driver is under the influence of alcohol and/or drugs because too much subjective. In fact, driving under the influence of alcohol (DUIA) and drugs (DUID) is worldwide considered an elevated risk for traffic safety and a crime [2]. In Germany for example, like in other European countries, the legal requirements for assessing a driver's fitness to drive are so far set out in national Road Traffic Regulations [3]. Therefore, during a routine traffic control as well as a medical examination assessing fitness to drive it is necessary to establish a method that helps to clarify the question whether an individual is under the influence of substances acting on the central nervous system (CNS).

Several studies have already demonstrated the applicability of the non-invasive infrared pupillography as an objective measurement method to indicate the possible influence of drugs on CNS [4]. Various pupil function parameters (latency time, velocity of contraction, relative amplitude, reaction time) can change significantly between healthy individuals and subjects under the influence of drugs or medications. Based on previous results [5,6], the pupil examination by infrared pupillography can assess the neurological status of such individuals who warrant further clinical examination, including blood testing. The effects on pupil function by several illicit and prescription drugs have been already studied among which opiates (like methadone, morphine, codeine), benzodiazepines (like diazepam, flunitrazepam, oxazepam),

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cocaine and cannabinoids (THC) [5,6]. However, the correct interpretation of parameters is not an easy task due to a partially large range of variation of corresponding parameters from individual to individual also according to an un-satisfactory definition of a "normal" pupil. The discriminatory power of pupil parameters also needs to be better investigated in order to improve the reliability of the method.

The present study focuses on cannabis consumers. In fact, after alcohol, tetrahydrocannabinol (THC) is the most used substance among injured drivers [7]. Cannabis can produce psychoactive effects and impair psychomotor performance in a wide of operative tasks, such as motor coordination, individual's equilibrium, perception, and attention as long as 24 h after smoking [8]. Chronic health effects of cannabis are well-known [9] including dependence syndrome, which could affect daily life functions and impairment of cognitive functions (such as learning, memory, problem solving, etc.). Acute subjective effects can reduce the speed of visual or auditory stimuli (often referred to as reaction time) increasing the risk of motor vehicle accidents [10,11]. However, previous studies on cannabis-related effects on simulated driving performances did regularly not show relevant impairments of motor behavior, and it is only an assumption that documented deficits are related to attention or perception [12,13].

Concentrations of 7–10 ng/ml THC in serum are thought to evoke comparable impairments to 0.5 g/kg blood alcohol concentration (BAC), which is the legal limit provided by most of the European Road Traffic Regulations. Therefore, THC serum concentrations below 10 ng/mL should not increase the risk of a traffic accident [14], while THC consumption in doses up to 300 μg per kilogram body weight can be considered to cause relevant cognitive and psychomotor impairments comparable to 0.5 g/l BAC [15]. In our opinion these considerations have to be interpreted critically. In a recent study [16] a defined THC concentration that leads to an inability to ride a bicycle could not be determined. In fact, estimates of risk are hard to obtain due to the rapid decline of THC concentrations in blood [16]. Also for this reason, in several European countries, every case needs to be treated individually because of cutoff values for the absolute impairment to drive are not available for most of the common illicit drugs other than alcohol [17].

Acute influence of cannabis on operative tasks of the individual can be assessed by the cannabis influence factor (CIF) and a CIF of 10 was proposed as a threshold for a driving impairment [18]. However, when using the CIF, it must be kept in mind that the formula to calculate the CIF might significantly disadvantage one-time consumers and treat regular consumers preferentially [16].

The aims of this study were to assess: a) the differences in pupillography response between cannabis consumers after a washout period and no cannabis consumers and b) the dose-related effects on pupillography parameters of cannabis in cannabis consumers.

To evaluate the cannabis effects on the ability in driving, infrared pupillography was performed in individuals before and after inhalative consumption of cannabis.

2. Material and methods

2.1. Study population

The individuals included in the study were divided into two groups on the basis of cannabis consumption: habitual cannabis consumer (CC, n = 14) and no cannabis consumers as control group (Ctr, n = 41). Because of the pupil's physiology depends on different factors, the two groups were matched for age and gender. The 55 subjects included in CC and Ctr groups were part of the study population of 115 individuals recruited in previous studies [4,5,16]. In particular, the 14 subjects representing the CC group were part of 74 persons participating in a drug substitution program [4,5,16]. The trial was approved by the ethics committee of the University of Dusseldorf (study number: 4583R), and it was referred to all the study population.

Individuals with a history of regular cannabis use were recruited in the CC group and also asked to answer about their medical and psychiatric history, drug use history, current medications, frequency and extent of cannabis use and the time of their last use of the drug. Inclusion criteria were healthy condition (including health certificate) and a history of regular consumption of cannabis (at least twice per month). Exclusion criteria were: pregnancy, disorders of the eye or the central nervous system, other acute diseases, history of significant cardiovascular, pulmonary, or psychiatric disorders, history of other drug abuse or positive urine screening other than cannabis, intake of psychoactive medication, rare consumption of cannabis use.

Individuals with negative blood screening test for THC and most common drug of abuse were included in the study as Ctr group.

2.2. Toxicological analysis

All toxicological analyses were carried out by using fully validated gas chromatography/mass spectrometry (GC/MS) methods according to the current German forensic guidelines [19]. The proceedings of the toxicological analysis are described in detail by Hartung et al., [16].

In CC and Ctr, no subjects with central nervous system disorders, psychiatric or ophthalmological diseases were included.

The Cannabis Influence Factor (CIF) was calculated as molar blood [THC] + [11−OH-THC] divided by [THCCOOH] according to previous studies [20,21].

2.3. Informed consent

At the admission, all participants were evaluated for age, body mass index (BMI), the time of the last use of cannabis. Informed consent was obtained from all participants. All cannabis consuming test persons gave their written consent to participate in the study on the day of testing after explicit information about the procedure.

2.4. Cannabis consumption

The cannabis consumption was standardized according to methods for clinical research involving cannabis administration [22]. Participants in the CC group were invited to smoke a non-tobacco cigarette. Each joint contained 300 μg of THC per kilogram of body weight. The test persons were instructed to consume the joints in the following way: 4-s inhalation, 10-s holding breath, and 15-s exhalation. A maximum of three joints could be consumed.

Cannabis was imported for the trial (import authorization no. E5304/2014) with the allowance from the German Federal Opium Agency, an authorized import agency of the Dutch medical cannabis flos (22 % dronabinol, <1 % cannabidiol) from Bedrocan, Netherlands (Cannabis flos: Bedrocan, 22 % dronabinol, <1.0 % cannabidiol; supplier: Dutch Ministry of Health,Welfare and Sport, Office of Medicinal Cannabis, P.O. Box 16114, NL-2500 BC The Hague).

Acute adverse effects of cannabis administration such as the tachycardia, orthostatic hypotension, pulmonary irritation, motor incoordination, cognitive impairment, anxiety, paranoia, psychosis, have been evaluated.

2.5. Pupillography

The infrared pupillography was applied in the study as described in previous articles [4–6]. The measurements were taken using a developed version of the previous described puillograph, the F2D2 (AMTech/Dossenheim, Germany) - a portable device, which is more suitable to be used during routine police check-points.

The device measures the horizontal pupil diameter using a two dimensional camera and determines the diameter of the pupil with a resolution of 0.02 mm at a measuring frequency of 25 Hz over a period of 2 s on the basis of the different reflexivity of the iris and pupil.

The eye is continuously and diffusely illuminated with two infrared diodes in order to not induce pupillary light reflex (PLR) and to magnify the pupil, The duration of the stimulus can be freely selected between 0.1 and 0.2 s and it was set up at 200 ms. The brightness of the stimulus can also be freely selected between 254 and 1. The luminous intensity values have no unit of measurement and range between ~ 0.22 and 56 lx measured at the vertex of the cornea.

For our investigation we chose 60 and 254 PLR values on the basis of the previous research studies. It can be seen that the major differences between the study groups are more apparent when the parameters are detected at these luminance values [5,6]. Once the measurement has been taken, pupil movement is shown in graphic form on the connected laptop. At the same time, special software breaks down the pupil reaction into various parameters according to predetermined measurement points and intervals. On the monitor, the tester gains a first impression of the pupil and its ability to react and can also read off the parameters calculated in the respective window.

The research study for the CC group included a baseline (T1) recording consisting of pupillographic analysis and drug blood test with evaluation of the main active THC metabolites along with 11-OH-THC and the main secondary metabolite THC-COOH, repeated four times at 2 (T2), 4 (T3), 6 (T4) and 8 (T5) hours from baseline. The CC group assumed cannabis at T2, T3, and T4. The drug blood test and the pupillographic analysis were performed 20 min after the assumption. At T5 no cannabis was administrated but only drug blood test and pupillographic analysis were performed.

The Ctr group underwent to drug blood test and pupillographic analysis only at baseline.

2.6. Statistical analysis

At each step, data were compared between and within groups by using Student's *t*-test for continuous variables, and χ^2 for categorical variables. All data were analysed by Statistical Package for Social Science (SPSS) version 23.0 (SPSS, Inc, Chicago, IL, USA). Statistical significance after Bonferroni correction was accepted at p value <0.05. A multivariate analysis was performed when appropriate. The results were visualized using categorized whisker plots with a confidence interval of 95 %.

3. Results

3.1. Study population

Twelve men (85.7 %), and 2 women (14.3 %), were included in the CC group with a mean age of 26.21 ± 3.93 years (range 21–33). The Ctrl was represented by 41 healthy individuals (35 men, 85.4 %; and 6 women, 14.6 %), with a mean age of 28.12 ± 2.98 years (range 22–30). No differences were found in age and gender distribution between the groups (p = 0.063 and p = 0.975, respectively).

In the CC group the regularly consumed amount of cannabis varied between approximately 1 g per week and 1 g per day.

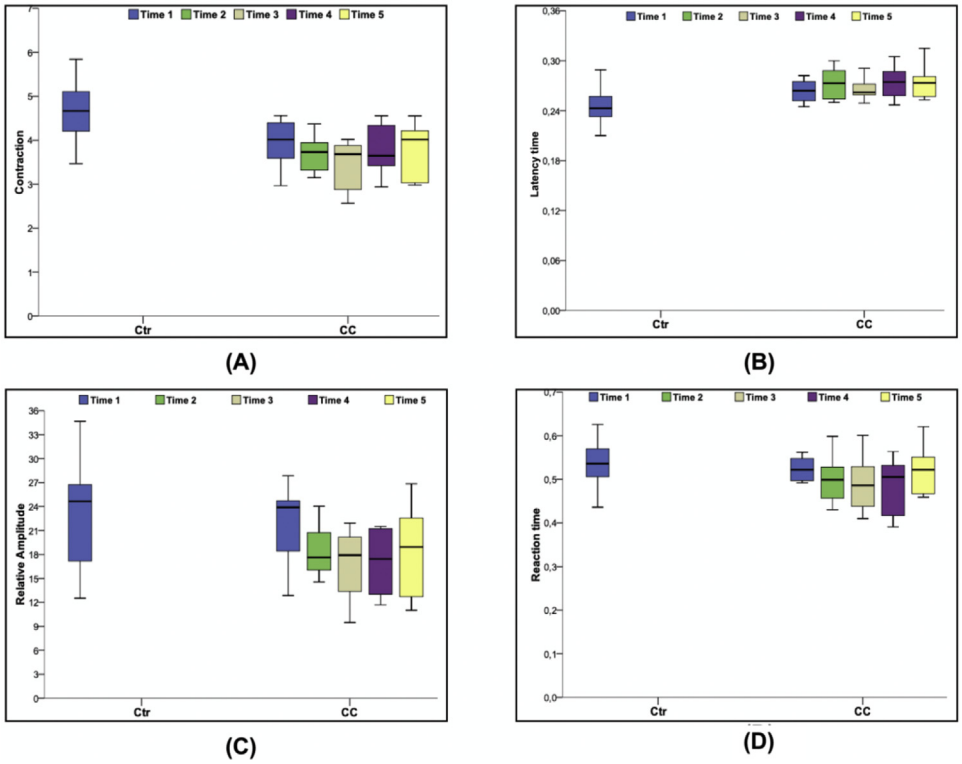

Fig. 1. Infrared pupillographic parameters: comparison between cannabis consumer (CC) and control group (Ctr) at baseline. A) the velocity of contraction; B) the latency time; C) the relative amplitude; D) the reaction time.

3.2. Chemical toxicological results

All the blood samples from the Ctr were negative (i.e. no substances were detected that affect the CNS) and all breath alcohol testing and urine samples from the CC were negative (except THC and metabolites) at the beginning of the trail.

Serum concentrations of THC, 11-OH-THC, and THCCOOH of fourteen test persons and related CIF are reported in Hartung et al., [16]. For the chemical toxicological analysis the following calibration ranges were applied: THC = 0,3–50 ng/ml, 11-OH-THC = 0,3–50 ng/ml, THC-COOH = 1–200 ng/ml. Values above the calibration ranges were obtained by diluted serum analyses; asterisks (*) indicate cannabis consumption immediately before; Time (min) means the period of time between the start of the cannabis consumption and the draw of blood.

The test persons' initial THC concentrations in serum (before smoking cannabis) varied between < LOD and 9.4 ng/mL. The initial THC-COOH concentrations ranged from 3.6 to 279 ng/mL. After the consumption of the joints, THC concentrations of up to 117 ng/mL were measured. Several test persons arrived with THC concentrations that indicated an acute or subacute influence of cannabis, e.g., test persons 1, 12 and 13. Immediately after smoking the cannabis cigarettes, the CIF regularly rose above 30.

Despite negative urine screening tests during the initial examinations, the follow-up examinations revealed the intake of amphetamines and/or MDMA of four test subjects.

The test subjects did not show signs of an acute influence of these substances at the beginning of the trials. But only two out of the four subjects with amphetamine/MDMA addiction had amphetamine concentrations that were considered to be no longer effective.

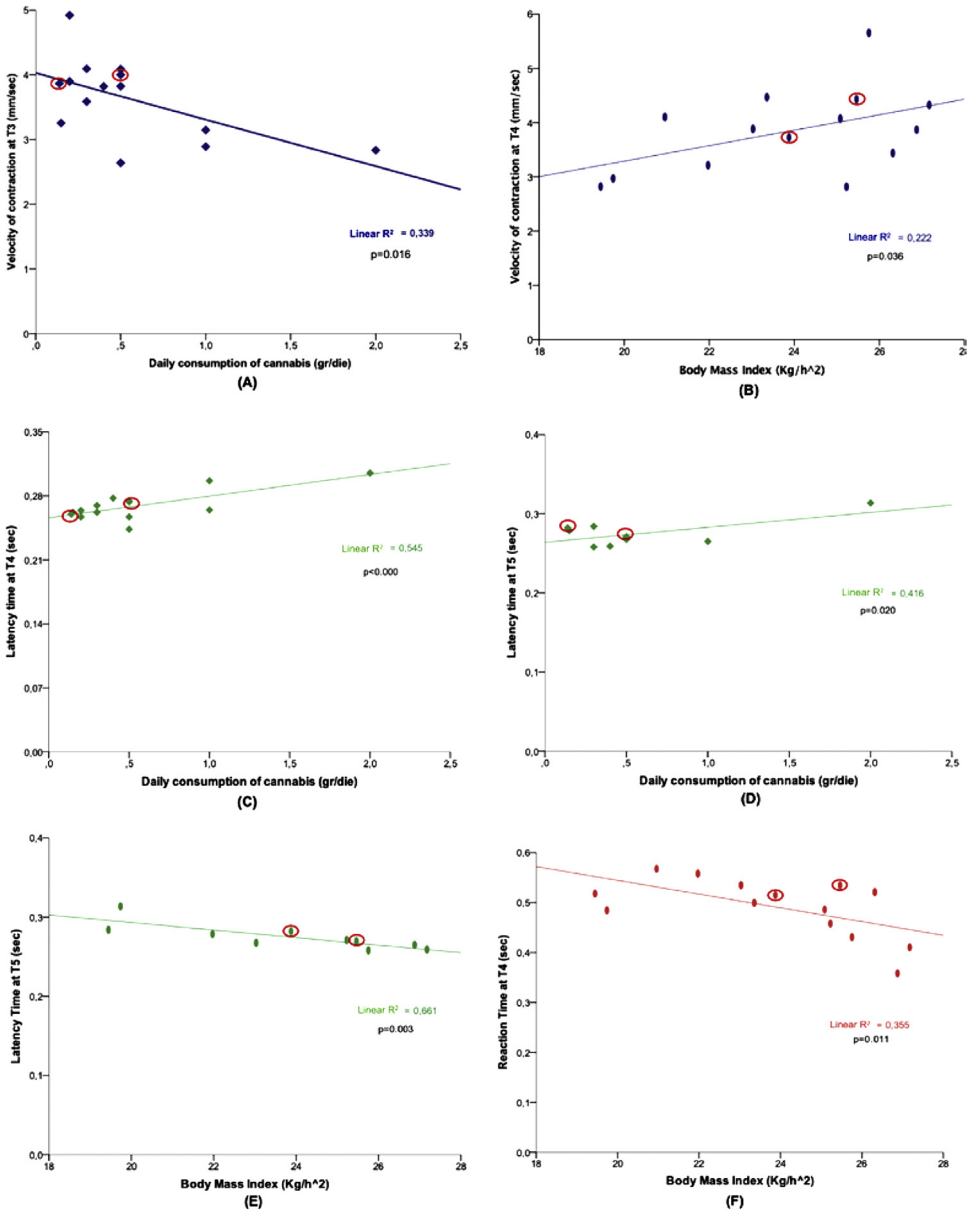

Fig. 2. Correlations between the pupillographic results and the individual characteristics statistically reliable: A) correlation between velocity of contraction at T3 and daily consumption of cannabis (p = 0.016); B) correlation between velocity of contraction at T4 and BMI (p = 0.036); C) and D) correlations between daily consumption of cannabis and latency time at T4 (p < 0.0001) and latency time at T5 (p = 0.020); E) and F) correlations between BMI and latency time at T5 (p = 0.003) and reaction time at T4 (p = 0.011). The *red circles* indicate the two test persons who were under the influence of amphetamine/MDMA (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

3.3. Pupillographic results

All examined parameters at baseline showed significant differences between CC and Ctr, as shown in Fig. 1.

The *velocity of contraction* after light stimulus at baseline in the CC group (3.96 ± 0.50 mm/s) was significantly lower ($p = 0.0001$) than the values recorded for the Ctr (4.70 ± 0.92 mm/s). At T2 the velocity was 3.79 ± 0.48 mm/s, at T3 the velocity value was 3.63 ± 0.62 mm /s, at T4 the speed was 3.84 ± 0.77 mm/s and at T5 it was 3.90 ± 0.63 mm /s (Fig. 1A).

The *latency time* at baseline in the CC group was higher (0.26 ± 0.01 s) than the values recorded for the Ctr (0.24 ± 0.02 s; $p = 0.001$). At T2 latency time was 0.27 ± 0.01 s, at T3 it was 0.27 ± 0.02 s, at T4 latency time was 0.26 ± 0.02 s and at T5 there was an increase to 0.27 ± 0.02 s (Fig. 1B).

The *relative amplitude* at baseline in the CC group (23.06 ± 4.83 %) is lower than the values recorded for the Ctr (23.96 ± 6.09 %) without reaching a statistically significant difference. At T2 the relative amplitude was 18.87 ± 3.31 %, at T3 it was 17.31 ± 4.03 % and then relative amplitude increased at T4 (18.53 ± 4.74 %) and at T5 (18.53 ± 28.5 %) (Fig. 1 C).

The *reaction time* at baseline in the CC group (0.58 ± 0.05 s) was longer than the values recorded for the Ctr (0.56 ± 0.07 s), although no significant difference was achieved ($p = 0.263$). At T2 reaction time was 0.49 ± 0.05 s, at T3 it was 0.47 ± 0.06 s, at T4 the value was 0.49 ± 0.06 s and at T5 it was 0.51 ± 0.06 s (Fig. 1 D).

To identify the possible predictors, a multivariate linear regression analysis was performed by using the four pupillography parameters (detected at T1, T2, T3, T4 and T5) as dependent variables and the individual characteristics (age, BMI, amphetamine/MDMA, daily cannabis use, years of habitual use and CIF) as independent variables. All statistical significant results are shown in Fig. 2.

The *velocity of contraction* at T3 was significantly and inversely correlated ($p = 0.016$; β=-0.0650; $R^2 = 0.339$) with the cannabis grams consumed per day (Fig. 2A) and at T4 it was correlated ($p = 0.036$; $R^2 = 0.222$) with BMI (Fig. 2B).

The *latency time* at T4 and at T5 ($p < 0.000$; $R^2 = 0.545$ and $p < 0.020$; $R^2 = 0.416$ respectively) was statistically correlated with the cannabis grams consumed per day (Fig. 2C and D) and at T5 it was significantly and inversely correlated ($p = 0.003$; β=-0.687; $R^2 = 0.661$) with the BMI (Fig. 2E).

The *reaction time* measured at T4 is inversely correlated ($p = 0.011$; β=- 0.768; R2 = 0.355) with the BMI (Fig. 2F).

4. Discussion

Infrared pupillography can represent a standardized and objective method for measuring the pupil function and assessing the influence of substances acting on the CNS. In fact, the pupillary light reflex can be significantly altered by psychoactive substances, like cannabis. In the present study, all four pupillary parameters seemed to be changed according to a weakened pupil function due to an acute administration of cannabis. Although pupil size as well as the latency depend on age, no relevant age-related bias in pupil diameter and latency were found in age and gender distribution between the CC and Ctr groups. In accordance with previous studies [23], acute cannabis administration may cause pupil constriction, and conjunctival injection (red eye), self-limiting effects, which do no require any particular treatment.

In particular, velocity of contraction and latency time were altered in proportion to the daily use of cannabis. Although the simultaneous influence of amphetamine/MDMA and cannabis has been found to hinder the opthalmological effects caused by cannabis [16], such results cannot be confirmed by the present two test individuals under the influence of amphetamine/MDMA.

According to other studies investigating neurological impairment by drugs [9,24–26] long-term effects can be appreciated especially in THC “heavy users”. Probably cannabis causes long-term effects and addiction because the active substance stimulates the same reward pathways of other drugs [27,28].

The effect of cannabis on the pupil parameters investigated at T4 was probably related to the different metabolization of THC when it accumulates in the body. In fact, high concentrations of cannabinoids are metabolized through ways other than the microsomal system and cytochromes and the resulting products could have different targets with different biological roles [29].

Furthermore, the effects detected during the study could be due to some phenomena of molecular mimicry, or to receptor desensitization phenomena or, again, to the possible effect of cannabinoids active on receptors other than CB1 and CB2 [30].

The acute effect recorded over two hours after the last dose, when this should be terminated, can be explained by the fact that in the usual consumer the kinetics of elimination of the active substance is different [31].

Our findings demonstrate that the *velocity of contraction* was correlated with BMI while the *latency time* and the *reaction time* were significantly and inversely correlated with the BMI.

Although there is still no evidence, it seems that this phenomenon can be related to the lipophilic nature of THC and, consequently, to the possible accumulation at high concentrations in adipose tissue which increases with increasing BMI and lower blood concentration of active molecule and reduced toxicological influence on the pupil.

The results of the study show that almost all pupil parameters are reliable indicators for the detection of subjects under the acute effect of cannabis, at the comparison with a control group after light stimulation of the same intensity. However, to understand the effects of cannabis on the ability to drive, it would be necessary to establish ranges of measured metabolites related to the deficit grade in driving a vehicle, as is the case for alcohol. The demonstration of a correlation between the quantity of the blood metabolites and the pupillary effect should be performed on a larger sample due to the high intra- and inter-individual variability. The method presented seems to be suitable for providing information about a possible and recent consumption of cannabis.

Although the integrity of the pupil function is not always correlated with the ability to drive [32,33] and the examination of the pupil size and function are not sufficient to evaluate the fitness for driving, we can affirm that the pupil function is a useful objective indicator of neurological conditions [5].

Considering the interindividual differences in pupil physiology and the changes during the study, further research studies should be performed possibly by using more precise devices with higher measuring frequency in order to enhance the sensitivity in recording the differences of the pupillographic response. In addition, to validate the pupillography as a medicolegal proof, studies with a larger sample are needed as well as pupillographic analysis in subjects who have taken poli-drugs or drugs and alcohol together. Road traffic injuries are the leading cause of death among people aged between 15 and 29 years and it will rise to become the fifth leading cause of death by 2030. These subjects represent the main prevention target of this method.

CRediT authorship contribution statement

Carlo P. Campobasso: Validation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Formal analysis. **Francesco De Micco:** Validation, Data curation, Visualization. **Graziamaria Corbi:** Validation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Formal analysis. **Thomas Keller:** Investigation. **Benno Hartung:**

Resources, Project administration, Supervision. **Thomas Daldrup:** Investigation. **Fabio Monticelli:** Conceptualization, Methodology, Investigation, Resources, Supervision.

Declaration of Competing Interest

The authors declare that they have no conflict of interests

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