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ORIGINAL ARTICLE

Early endothelial dysfunction as a marker of vasculogenic erectile dysfunction in young habitual cannabis users

A Aversa[1], F Rossi[1], D Francomano, R Bruzziches, C Bertone, V Santiemma and G Spera

Chair of Internal Medicine, Department of Medical Pathophysiology, Sapienza University of Rome, Rome, Italy

Aim of the study was to evaluate whether endothelial dysfunction is a marker of erectile dysfunction (ED) in recreational drug abuse. Sixty-four non-consecutive men complaining of ED from at least 3 months were included. All patients underwent detailed history about recreational drug abuse and were then submitted to dynamic penile duplex ultrasound (PDU). According to pharmaco-stimulated peak systolic velocity (PSV) cutoff at 35 cm s^{-1}, patients were divided into two groups: organic (O; $n = 30$) and non-organic (NO; $n = 34$) ED. All subjects and 7 healthy age-matched subjects as controls, underwent veno-occlusive plethysmography (VOP) for the evaluation of endothelium-dependent dilatation of brachial arteries. Blood pressure, total and free testosterone, prolactin, estradiol, low-density lipoprotein and high-density lipoprotein cholesterol were also evaluated; patients were classified with regard to insulin resistance through the HOMA-IR index. Cannabis smoking was more frequent in O-ED vs NO-ED (78% vs 3%, $P < 0.001$) in the absence of any concomitant risk factor or comorbidity for ED. VOP studies revealed impaired endothelium-dependent vasodilatation in O-ED but not in NO-ED and controls (12 ± 6 vs 32 ± 4 and 34 ± 5 ml min^{-1}, respectively; $P = 0.003$). Overall patients showed a direct relationship between HOMA-IR and PSV ($r^2 = 0.47$, $P < 0.0001$), which was maintained in men with organic ED ($r^2 = 0.62$, $P < 0.0001$). In cannabis consumers, a direct relationship between HOMA-IR and VOP was also found ($r^2 = 0.74$, $P < 0.0001$). Receiver-operating characteristic (ROC) curve analysis revealed that VOP values below 17.22 ml min^{-1} were suggestive for vasculogenic ED. We conclude that early endothelial damage may be induced by chronic cannabis use (and endocannabinoid system activation); insulin resistance may be the hallmark of early endothelial dysfunction and may concur to determine vascular ED in the absence of obesity. Further studies are warranted to establish a direct relationship between cannabis abuse, onset of insulin resistance and development of vascular ED.

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Introduction

Present epidemiological studies suggest that the incidence of severe erectile dysfunction (ED) in men below 40 years is very low (2.3%).[1] In the same age range, such incidence increases notably (27%) in the presence of cigarette smoking.[2] Nevertheless, there are few vascular penile blood flow studies showing an organic etiology of ED in such an age range. It has been suggested that in young men below 30 years of age, the vascular origin of ED can be a false positive of duplex ultrasound procedure mainly because of the presence of an adrenergic overtone during the office setting,[3] but such data have been disregarded.[4] More notably, in this age range, cannabis use is highly prevalent (up to 80%) and even if the majority of drug users report a stimulation of sexual desire and improvement in sexual performance,[5] the real effects of cannabis use on vascular penile function appears to be poorly investigated. Animal studies have not underlined any significant modification in sexual activity, which would have led to the exclusion of any 'aphrodisiac' effect of this recreational drug.[6]

The endothelium regulates vascular tone and the interaction between vessel walls, blood and

Correspondence: Dr A Aversa, Chair of Internal Medicine, Department of Medical Pathophysiology, Sapienza University of Rome, Viale Policlinico 155, 00161 Rome, Italy.
E-mail: antonio.aversa@uniroma1.it
[1]These authors equally contributed to this work
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the circulating substances. These functions are regulated by the production of endothelial vasoactive substances that, generally, remain under equilibrium in physiological conditions. Nitric oxide promotes vasodilatation of the vessels by stimulating the production of 3′,5′ cyclic guanosine monophosphate from vascular smooth muscle cells and by inhibiting their growth and migration, platelet aggregation, thrombosis, adhesion of monocytes, inflammation and oxidative processes.[7] On the contrary, substances such as angiotensin-II and endothelin-1 may determine vascular damage as they act as promoters of inflammation, that is, stimulation of leukocyte migration, stimulation of platelet aggregation and thrombogenesis. Under physiological conditions, insulin is believed to act as an important endothelium-protecting substance and the occurrence of insulin resistance, which is linked to visceral adiposity extent, is associated with the presence of higher number of components of the metabolic syndrome and higher degrees of endothelial dysfunction through the following mechanisms: increase of free fatty acid; increase of proinflammatory adipokines—tumor necrosis factor-α, leptin; increase of oxidative stress; increase of oxidized low-density lipoprotein cholesterol; reduction of high-density lipoprotein cholesterol; hypertension; hyperuricemia; hyperglycemia.[8] Clinical studies have shown that insulin-resistance is associated with the presence of hypertension,[9] obesity and type II diabetes whereby endothelial dysfunction is very common.[10]

The aim of this study was to evaluate the role of recreational drug abuse as a risk factor for developing organic ED in young men without risk factors for organic ED and to identify possible biochemical markers for this condition.

Materials and methods

In this retrospective study, 64 men were selected from overall 300 penile recordings. The study received the approval of the Internal Review Board of our Institution. All patients had undergone a detailed medical and sexual history, which included the question: 'Are you currently using cannabinoids or other drugs for recreational use?' A 'yes' response was followed by a rapid immunochromatographic urinalysis testing to determine positivity for marijuana use (iScreen Drug test, Instant Technologies INC, Norfolk, VA, USA). The Δ-9 tetrahydrocannabinol (THC) drug test for marijuana abuse is based on the principle of the highly specific immunochemical reactions between antigens and antibodies, which are used for the analysis of specific substances in biological fluids. The cutoff of the test is $50\,ng\,ml^{-1}$ of THC. Inclusion criteria were: presence of ED from at least 3 months as assessed by the administration of the International Index of Erectile Function-5 (IIEF5)[11] and age <40 years. Exclusion criteria were: diabetes, hypertension, psychiatric, neurological or hormonal alterations and continuative use of any pharmacological therapy.

In all patients, penile arteries duplex ultrasound (PDU) was evaluated after a maximal challenge with vasoactive agents. According to their peak systolic velocity (PSV) cutoff at $35\,cm\,s^{-1}$, patients were divided into two groups: organic (O; $n=30$) and non-organic (NO; $n=34$) ED. Basal and endothelium-dependent flow of brachial arteries were also evaluated in each subject by performing veno-occlusive plethysmography (VOP). Healthy age-matched subjects without ED were enroled as controls for VOP ($n=7$). Blood pressure, total and free testosterone, prolactin (PRL), estradiol, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol, fasting blood glucose and insulin were also evaluated according to earlier published procedures.[12] The Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index was expressed as follows in the fasting state: glucose $(nmol\,l^{-1}) \times$ insulin $(mUI\,ml^{-1})/22.5$.

Penile blood flow studies

Briefly, dynamic PDU for the evaluation of the cavernous arteries was carried out by a dedicated machine (PHILIPS HDI 5000, Philips, Germany) using broadband linear array transducer and colour-power Doppler software according to a procedure published earlier.[13] Flow parameters included PSVs, end diastolic velocities and the resistance index (RI) after a pharmacostimulation test with 10 mcg PGE1 and subsequent audiovisual sexual stimulation were evaluated. Re-dosing of 10 mcg PGE1 plus 1 mg of the non-selective α-blocker phentolamine was performed when necessary to maximize the erectile response and to induce 'best quality erection', as described earlier.[4] Arteriogenic ED was defined by the presence of an altered inflow at the level of cavernous arteries (PSV $<35\,cm\,s^{-1}$) by duplex ultrasound after 20 min after a maximal pharmacostimulation.[14]

Veno-occlusive plethysmography studies

The plethysmographic study was carried out on a separate day and began at 0900 h, after the subjects fasted for at least 1200 h. The subjects were kept in a supine position in a quiet, dark, air-conditioned room (constant temperature of 22 °C). After 30 min in the supine position, the basal forearm blood flow (FBF) was measured in both arms simultaneously by strain gauge plethysmography, as already reported.[15] The strain gauges were placed at the widest part of the forearms, held above the right atrium, and

connected to a plethysmographic device (EC6R, DE Hokanson, Inc., WA, USA). Wrist cuffs were inflated to a pressure of 200 mm Hg, 1 min before each measurement and throughout the measurement of FBF, to exclude circulation of the hands from the measurement. The upper arm cuffs were inflated to 40 mm Hg for 7 s in each 15 s cycle to occlude venous outflow from the arms using a rapid cuff inflator (EC20, DE Hokanson Inc.). The FBF output signal was transmitted to a recorder and it was expressed as ml min^{-1} 100 ml^{-1} of forearm tissue. Having assessed the basal FBF, the endothelium-dependent vasodilatation was evaluated as a response to ischemia. In brief, cuff was inflated at 80 mm Hg exceeding the systolic blood pressure for 30, 60, 180 and 300 s (twice for each ischemic time) around the non-dominant arm, and maximum FBF (post-ischemic vasodilator response) was estimated as mean of the two measurements for each time. FBF response to ischemia was expressed as FBF-delta (ΔFBF) computed as mean difference between maximum FBF in the experimental arm and FBF in the contralateral (control) arm.[16]

Statistical evaluation

Values are expressed as means ± s.d. Differences in baseline characteristics of the two groups were investigated using the unpaired *t*-test. Pearson's correlation test was performed to evaluate statistical correlation between PSVs, VOP and HOMA-IR scores. To investigate the efficacy of VOP to correctly identify endothelial dysfunction in O-ED, an iterative receiver-operating characteristic (ROC) curve analysis was used to determine the most proper threshold for the screening of endothelial dysfunction, and sensitivity and specificity at that threshold were calculated. Results were considered statistically significant if the two-tailed *P*-value was <0.05.

Results

Clinical characteristics of the study population are showed in Table 1. The two groups of patients with ED were age-matched and did not differ in body mass index, mean systolic blood pressure, serum total testosterone and plasma glucose and IIEF scores.

As shown by the presence of 11-nor-Δ9-THC-9 COOH in the urine, cannabis smoking resulted as the only recreational drug used by more than one third of overall patients ($n=24$; 37%). When considering those patients with O-ED, 23 out of 30 (77%) were cannabis habitual consumers whereas the remaining patients had congenital arterial anatomical variations as a cause of vascular ED, as reported elsewhere.[17] On the contrary, only one patient (3%) in NO-ED group was a cannabis consumer. The incidence of cigarette smoking was similar in both groups of patients and the number of packs per day was 0.8 ± 0.2 for a period of Time less than 15 years (mean 12.4 ± 1.2 years s.d.).

Table 1 Clinical characteristics of patients at baseline

	Organic ED (n = 30)	*Non-organic ED (n = 34)*
Mean age (years)	30 ± 2	30 ± 3
BMI (kg/m^2)	21 ± 0.4	20 ± 0.6
Systolic BP average (mm Hg)	120 ± 3	121 ± 3
IIEF5 score	18 ± 2	19 ± 2
Cannabis use (first use before 20 years)	77% (70%)	3% (0%)
Smokers	24%	26%
Fasting blood glucose (mg per 100 ml)	90 ± 6	92 ± 4
Total testosterone (ng ml^{-1})	5.8 ± 0.7	6.1 ± 0.9
Testosterone/estradiol	18 ± 4	20 ± 4

BMI, body mass index; BP, blood pressure; ED, erectile dysfunction; IIEF5, International Index of Erectile Function-5.

As expected, mean PSV of cavernous arteries was higher in NO-ED vs O-ED (48.5 ± 9.7 vs 24.6 ± 7.8 cm s^{-1}, $P<0.0001$) (Figure 1a) with normal veno-occlusive function in both groups (data not shown). VOP studies revealed normal endothelium-dependent vasodilatation in NO-ED and age-matched controls but not in O-ED; in these latter patients, the ΔFBFs were lower (12 ± 6 vs 32 ± 4 and 34 ± 5 ml min^{-1}, respectively; $P<0.005$) (Figure 1b). Both these techniques showed the presence of early vascular damage in the O-ED group of patients either for endothelium-dependent (VOP) or endothelium-independent (PDU) vasodilatatory responses.

Surprisingly, significant differences in fasting insulin levels between O-ED vs NO-ED were found (11.7 ± 3.3 vs 8.6 ± 4.4, $P=0.006$) (Figure 2a) leading to higher HOMA-IR when comparing the two groups (2.5 ± 0.7 vs 1.9 ± 1.0, $P=0.02$) (Figure 2b). Also, controls showed lower HOMA-IR when compared with O-ED (2.5 ± 0.7 vs 1.8 ± 0.2, $P<0.05$). More important, overall patients showed a direct correlation between HOMA-IR and PSV ($r^2=0.47$, $P<0.0001$) that was maintained in subjects ($n=30$) with O-ED only ($r^2=0.62$, $P<0.0001$) (Figure 3). In cannabis consumers, a direct relationship between HOMA-IR and VOP was also found ($r^2=0.74$, $P<0.0001$) (Figure 4a) and HOMA-IR was higher in consumers when compared with non-consumers (2.7 ± 0.4 vs 1.6 ± 0.3, $P<0.0001$) (Figure 4b). Comparison of endothelial function and PSV among patients with organic ED who were cannabis consumers vs non-consumers showed significant differences, suggesting vascular endothelium damage by cannabis use (6.5 ± 1.2 vs 14.9 ± 1.8 ml min^{-1}, $P<0.0001$ and 27.3 ± 6.9 vs 15.6 ± 1.5 cm s^{-1}, $P<0.0001$, respectively) (Figures 5a and b). When a VOP threshold of <17.22 ml min^{-1} was chosen,

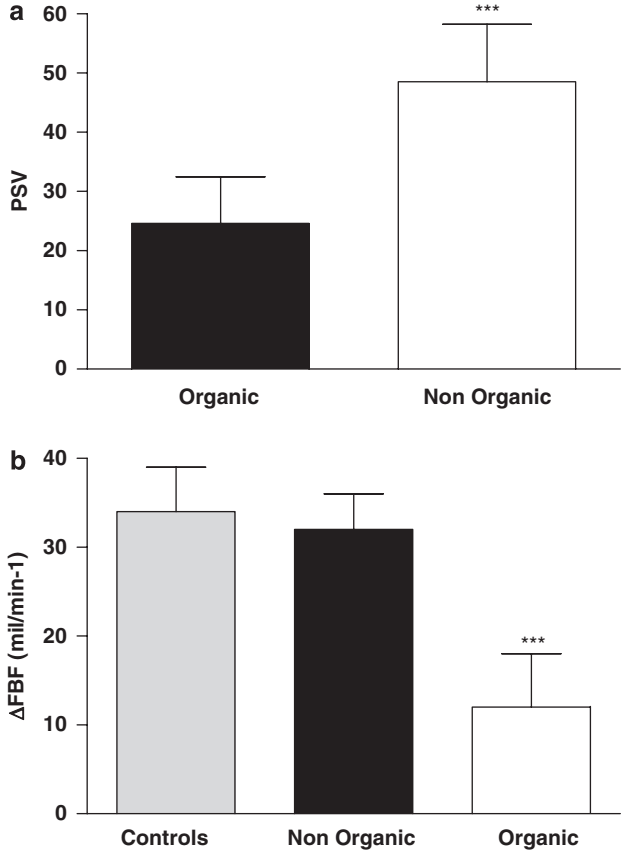

Figure 1 (**a**) Differences between peak systolic velocity (PSV) values in different group of subjects (***$P<0.0001$); (**b**) Forearm blood flow (FBF) studies as performed by veno-occlusive plethysmography (VOP) in different group of subjects as compared with controls (***$P<0.005$).

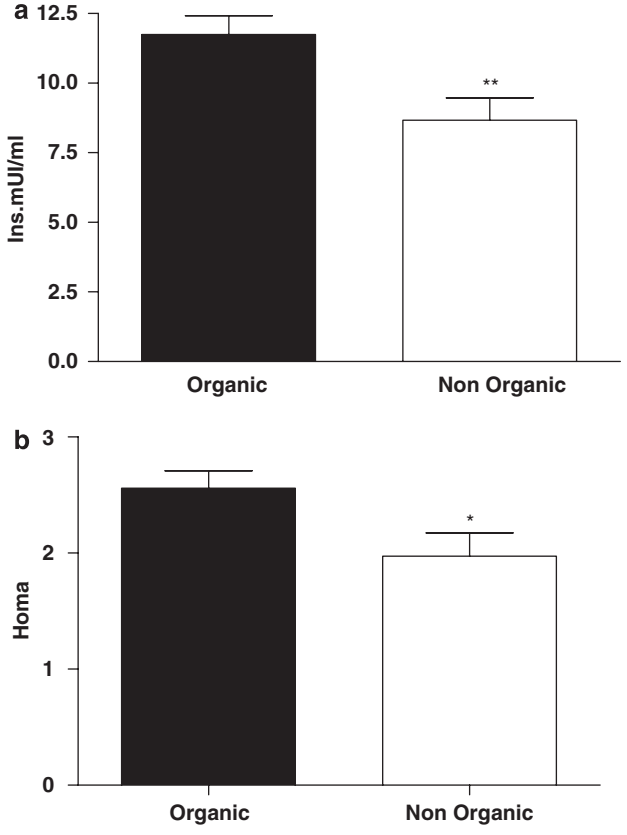

Figure 2 (**a**) Differences between serum insulin levels in different group of subjects (**$P=0.006$). (**b**) Differences between HOMA-index in different group of subjects (*$P=0.02$).

the sensitivity and specificity for identifying arteriogenic ED were 92% and 89% respectively, with an area under the ROC curve of 0.92 (95% confidence interval: 0.74–0.99) (Figure 6).

Discussion

Our results show for the first time that, in young subjects, a relationship between the use of cannabis and the presence of some degree of vascular ED exists, and that the latter can be disclosed by the presence of early endothelial dysfunction, as shown by non-invasive VOP. The biochemical marker of this dysfunctional state was reflected by an increased HOMA-IR, a validated index of insulin sensitivity.[18] To our knowledge, no data on the relationship between cannabis use and ED with documented vascular (penile) and endothelial (brachial) arterial damage have been reported earlier. The limitation of this study stands in the impossibility to measure the quantity of inhaled cannabis by each subject over time and by the fact that dynamic

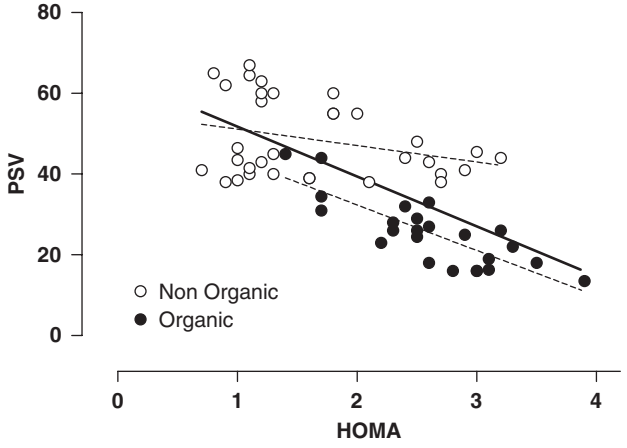

Figure 3 Linear regression between HOMA-index and PSV ($r^2=0.47$, $P<0.0001$). Higher PSVs correlate with lower values of HOMA-index ($n=64$). Dotted lines represent the linear regression of different subgroup of patients. Open circles represent non-organic patients ($n=34$); close circles represent organic patients ($n=30$).

PDU could not be performed in our control group, but VOP results confirmed that the vascular damage was more prominent in those patients reporting their first drug use before the age of 20 years.

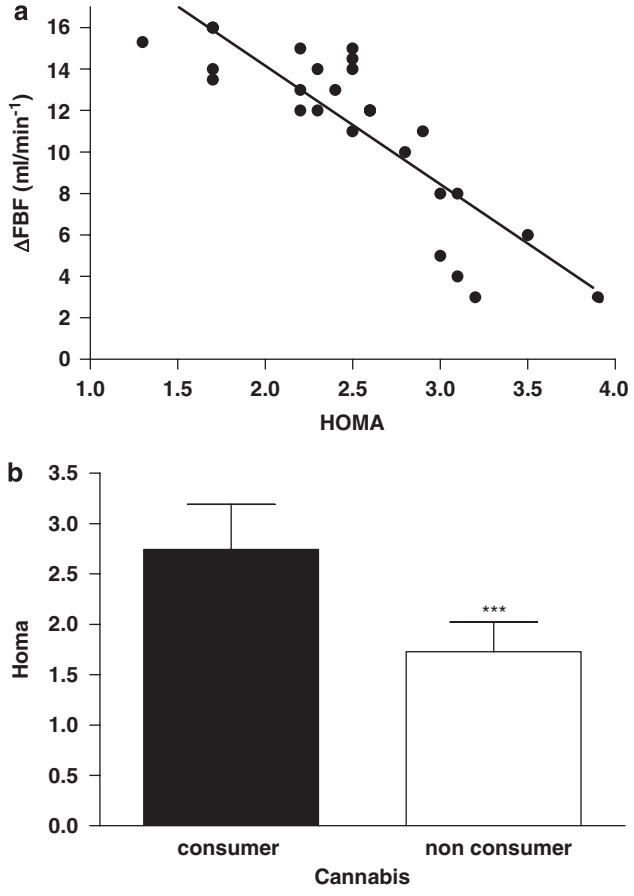

Figure 4 (**a**) Linear regression between HOMA-index and ΔFBF in cannabis consumers ($n=23$) ($r^2=0.74$, $P<0.0001$). (**b**) Differences between Homa-index in cannabis consumer vs non-consumer (***$P<0.0001$).

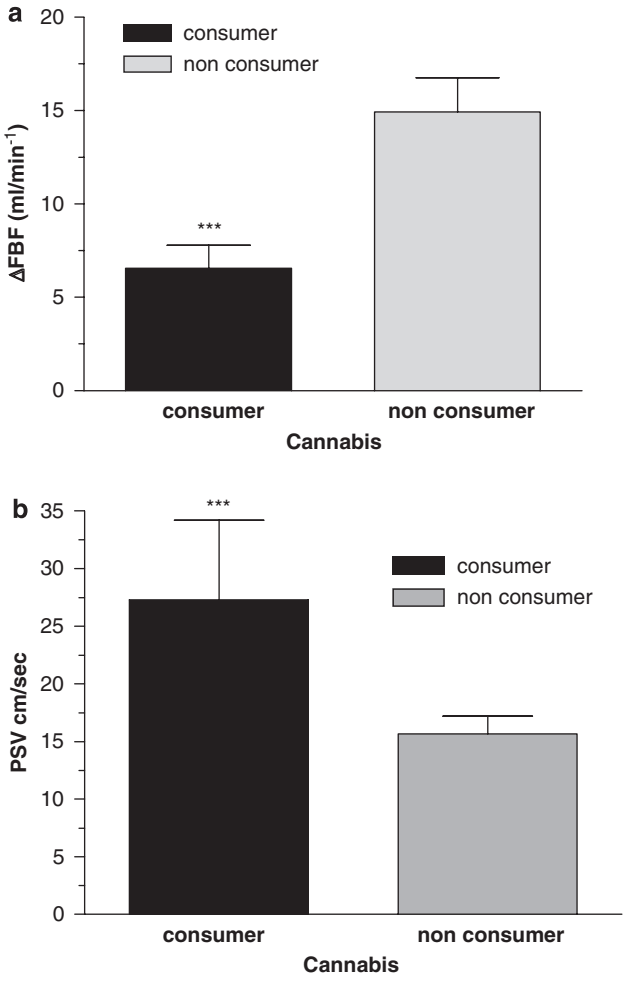

Figure 5 Differences in ΔFBF (**a**) and PSV (**b**) between cannabis consumer and non-consumer groups (***$P<0.0001$).

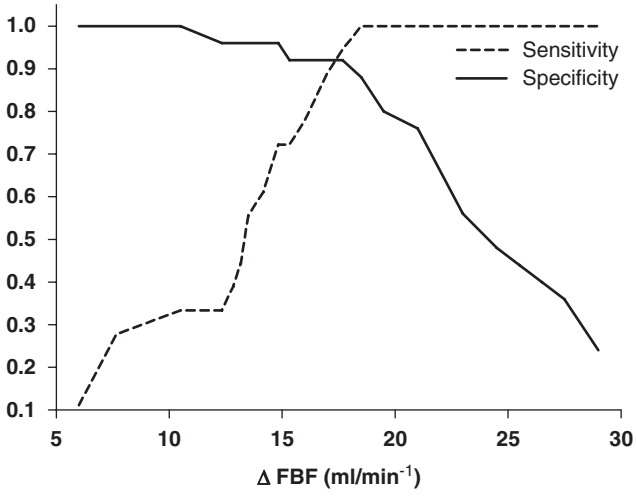

Figure 6 ROC curve for VOP scores in the identification of patients with vascular erectile dysfunction (ED).

Furthermore, in our series of ED patients, the incidence of cigarette smoking habit was similar in both groups (about 25%) (Table 1), thus suggesting that cigarette smoking *per se* does not represent a risk factor of vascular damage necessarily predisposing to ED in young men. However, we underline that diagnostic PDU combined with VOP have shown that almost 50% (30 out of 64) of our study population was surprisingly affected by O-ED. In the latter group, 23 men were proven to be habitual cannabis users and had otherwise unexplained vascular ED, whereas the remaining seven patients had congenital arterial anatomical variations as a cause of vascular ED and were not cannabis users.

Adolescence is at high risk of initial use of drugs. La Pera *et al*.[5] showed that 71% of drug addiction might be because of the presence of one or more sexual dysfunctions (ED, premature ejaculation, low desire, alone or in combination), whereas only 29% of his series did not report any sexual problem. Among those with normal sexual function, only few stated that sexual dysfunctions had influenced their decision; on the contrary, in sexual dysfunction group, one out of three confirmed that the beginning of the assumption of drugs was depending on their sexual disorders, primarily premature ejaculation.

Furthermore, the more severe the sexual dysfunction, the higher the percentage of those who stated that sexual dysfunction influenced their decision to start taking drugs. About 50% of the entire sample admitted they had used drugs to improve sexual performance. Accordingly, in our population, drug abuse often started at a very early age (<20 years of age in about 70% of subjects) and continued use over the time increased the possibility to develop overt ED; however, the presence of sexual dysfunction before first use was not investigated in this study.

Δ-9 tetrahydrocannabinol, the active principle of the cannabis, is a highly lipophilic substance that allows its segregation into the adipose tissues and diffusion beyond hematoencephalic and placental barriers, as well as into the maternal milk. The slow release of THC from the adipose tissue, in which it remains bound for a long time (weeks), may account for the detection of low level of drug in the blood for various days. Therefore, it is possible that during daily consumption, the amount of active principle required to produce the biological effect may be smaller in comparison with the initial ones. Nevertheless, in humans a large number of variables may be confounding for the interpretation of cannabis effects upon its receptors, that is, different concentrations of the active substance, duration and frequency of use.[19] Some authors have observed that chronic activation of cannabinoid (CB) receptor type-1 by cannabis may reduce PRL and luteinizing hormone (LH) levels, probably via interaction with the GABA (gamma-aminobutyric acid) receptor activation.[20,21] Activation of the hypothalamic-pituitary-adrenal axis seems to occur after an acute response to inhalative cannabinoids.[22] However, in chronic consumers a reduction of cortisol response after insulinic stress has been shown, thus suggesting different pathophysiological mechanisms. Similarly, small doses of cannabis do not alter plasmatic levels of testosterone and gonadotrophins[23,24] or the response of testosterone to gonadotroph stimulation, whereas larger doses and prolonged dependence reduce significantly the levels of LH and testosterone, and they can also determine changes in motility and number of the ejaculated sperms.[25,26] Also, it is noteworthy that THC may act directly upon estrogenic receptor (ER) activation[27,28] , thus functioning as an 'endocrine disruptor'. This action is able to stop the proliferation of smooth muscle cells and determine important vascular lesions, as reported in ERα, ERβ or ERα/β knockout mice. This has suggested that the effects of estradiol would be independent from the activity of its own receptors.[29] In our patients, adrenal and pituitary axes were not evaluated; we have not found any significant steroid hormone or prolactin alterations as well as any alteration in testosterone/estradiol ratios, but we cannot rule out an ER activation to explain vascular damage.

Insulin is the main hormone involved in glucose homeostasis, lipogenesis and weight gain, provoking an anorexigenic action. Also, the CB contributes to the physiological regulation of energy balance, food intake and lipid and glucose metabolisms. Juan-Picó *et al*.[30] have shown the existence of CB1 and CB2 receptors (both are seven domain transmembrane receptors coupled to G-proteins) in endocrine pancreas by PCR and immunocytochemistry studies, and their implication in Ca^{2+} signaling and insulin secretion. Endocannabinoids (EC), 2-acylglycerol and anandamide, regulate the Ca^{2+} channels inhibiting glucose-mediated intracellular calcium rise; these effects are mediated through CB1 and CB2 receptors, or through the TRVP receptors, a separate subgroup of non-selective cationic channels, transient receptor potential (TRP), belonging to the vanilloid receptor family present in the pancreatic islet.[31] An action through TRPV1 is unlikely, as they have an opposite effect to that described here for EC. Activation of TRPV1 in β-cells increases insulin secretion.[32] mRNAs for both types of receptors are present being CB2 predominant in the islets.[33] Nevertheless, CB1 receptor mRNA is present in the pancreatic islet even if it seems to be less involved in the regulation of the β-cellular signal. Laychock SG *et al*.[34] have studied insulin secretion in isolated pancreatic islets in response to glucose or to THC. In this study THC stimulated both basal secretion of insulin and improved the secretory response to glucose. Such results show that the CB2 receptors are involved in the action mediated by 2-acylglycerol on the Ca^{2+} signal and also in insulin secretion in the pancreatic β-cells inside intact Langerhans islets. CB1 receptors are present in adipose tissue and their lipogenic effect seems to be regulated by the EC system. These results suggest that the EC system may play a modulator effect on energy balance, with both central (food intake) and peripheral (adipose cells: lipogenesis) effects.[35] In men, the blockade of CB1 receptors by rimonabant not only produces a significant reduction of weight and waist circumference, but also provokes an improvement of both insulin resistance and biochemical alterations related to the metabolic syndrome.[36] The discovery of a consistent modulation mediated by the EC system (ECS) upon insulin secretion, suggests that substances such as cannabis may have some effects on the physiology of the pancreatic islet. Immunocytochemistry studies showed that CB1 receptors are scarcely expressed in the islet whereas there is a great abundance in glucagon producing cells, whereas CB2 receptors are expressed in both type of cells.

The impact of cannabinoids on endothelial function is still controversial. In animal studies, there is a large evidence supporting a role for ECS in improving endothelial function and nitric oxide production[37] as well as THC has been shown to

improve endothelium-dependent relaxation.[38–40] In addition, the EC anandamide, improves neurogenic relaxation of corpus cavernosum in diabetic rats.[41] On the contrary, occasional myocardial infarction after acute assumption of cannabis has been reported in humans.[42] It is noteworthy that, marijuana smoking by people with either silent or overt cardiovascular disease poses health risks because of the consequences of the resulting increased cardiac work, increased catecholamine levels, carboxyhemoglobin and postural hypotension.[43] Hence, we can hypothesize that in our young healthy subjects with no cardiovascular risk factors, prolonged cannabis use might have acted as a secretagogue on the endocrine pancreas, thus inducing insulin resistance, endothelial dysfunction and different degrees of ED.

In conclusion, we show for the first time that systemic endothelial function and pharmaco-stimulated penile arteries PSV among patients with organic ED who are cannabis consumers vs non-consumers are significantly different. This evidence suggests that chronic cannabis smoking may concur to the alteration of both endothelial-dependent and endothelial-independent vasodilatatory pathways. In such cases, the high sensitivity and accuracy of VOP in detecting early endothelial dysfunction may substitute dynamic PDU performance, as the latter is more invasive with respect to a younger subject. We hypothesize that prolonged activation of ECS is able, somehow, to alter endothelial function and to determine an early damage of the erectile process. Further studies are necessary to determine the direct relationship among abuse of cannabis, plasma THC levels, onset of insulin resistance and the development of ED.

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