Heavy cannabis use is associated with low bone mineral density and an increased risk of fractures

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Clinical significance:

• The effects of cannabinoids on bone mass and bone turnover in humans are unknown.

• Using a cross-sectional study design we found that heavy cannabis use is associated with low body mass index, high bone turnover, low bone density and an increased risk of fracture.

• Heavy cannabis use has a detrimental effect on bone health by a direct effect on the skeleton and an indirect effect on body mass index.

Abstract

Purpose: To investigate possible associations between recreational cannabis use and bone health in humans.

Methods: Cross-sectional study of individuals recruited from primary care in the UK between 2011 and 2014. Cases were regular smokers of cannabis divided into moderate (n=56) and heavy user (n=144) subgroups depending on whether they reported fewer or more than 5000 cannabis smoking episodes during their lifetime. Controls comprised 114 cigarette smokers.

Results: Heavy cannabis users had lower total hip bone mineral density (mean ± SD Z-score: -0.20±0.9 vs. +0.2±0.9, $p<0.0005$), lower spine bone mineral density (-0.5±1.2 vs. 0.0±1.2, $p<0.0005$) and lower BMI (26.5±6.0 vs 29.0±7.0, $p=0.01$) than controls. Fracture rate was also increased in heavy users (rate ratio=2.17, 95% confidence interval 1.59 to 2.95; $p<0.001$). When compared with controls, CTX serum concentrations were raised in heavy cannabis users (0.3±0.1 vs. 0.2±0.1 pg/ml, $p=0.045$) as were P1NP concentrations (47.1±19.2 vs. 41.2±17.8 pg/ml, $p=0.01$). Serum 25(OH)D concentrations were reduced in heavy users compared with controls (25.3±16.8 vs. 36.9±26.7 nmol/l, $p=0.002$). Multiple regression analysis revealed that heavy cannabis use was an independent predictor of spine bone mineral density accounting for 5.4% of the variance ($p=0.035$) and total hip bone mineral density accounting for 5.8% of the variance ($p=0.001$) but mediation analysis suggested that the effect on spine bone mineral density was indirect and mediated through low BMI.

Conclusion: Heavy cannabis use is associated with low bone mineral density, low BMI, high bone turnover and an increased risk of fracture. Heavy cannabis use negatively impacts on bone health both directly and indirectly through an effect on BMI.

Introduction

Heavy cannabis use has previously been associated with adverse effects on mental health[1,2] and physical health[3] but also has been found to protect against insulin resistance through an association with reduced body mass index.[4] While preclinical studies have revealed that cannabinoid receptors and their ligands play important roles in regulating bone density, bone turnover and bone cell activity[5,6] the effects of cannabis use on bone health in humans are unknown.

We have previously reported that young adult mice with targeted inactivation of the type 1 cannabinoid receptor (*Cnr1*$^{-/-}$) have increased bone density due to a reduction in osteoclast activity[7] and that cannabinoid receptor agonists stimulate bone resorption and osteoclast activity *in vitro.*[8] However, with increasing age, *Cnr1*$^{-/-}$ mice show increased rates of bone loss due to defects in osteoblast differentiation and accumulation of adipocytes in the bone marrow.[9] Mice with deficiency of the type 2 cannabinoid receptor (*Cnr2*$^{-/-}$) also show enhanced bone loss with age due to reduced bone formation.[5,10,11] In keeping with this, type 2 cannabinoid receptor agonists stimulate osteoblast proliferation and promote bone nodule formation *in vitro.*[12,13] The GPR55 receptor has also been reported to influence osteoclast differentiation *in vitro* and bone mass *in vivo*. Specifically, male mice with inactivation of GPR55 have increase bone mass and increased bone resorption whereas the GPR55 antagonist cannabidiol inhibits osteoclast formation induced by the GPR55 agonist lipophosphatidylinosotol.[14] The complexity of the situation is emphasised by the fact that the effects of cannabinoid receptor deficiency in mouse models are age and gender dependent[10,15] as well as being dependent on background strain of the mice.[16]

The aim of this study was to determine if heavy cannabis use influences bone metabolism in humans. In order to achieve this aim we investigated the association between recreational use of cannabis and various markers of bone health in a cross-sectional study of 170 regular cannabis users and 114 controls recruited from a single inner city general practice in the United Kingdom.

Materials and methods

Study subjects

We approached individuals aged $\geq$18 who were attending a general practice surgery for routine appointments between November 2011 and March 2013. Individuals were invited to take part if they had a history of current or previous use of tobacco or cannabis. After providing written informed consent participants completed a questionnaire in which details were collected on demographics, smoking and alcohol intake, cannabis use, use

of other illegal drugs, dietary calcium intake by food frequency questionnaire, fracture history, family history of hip fracture, participation in sports, whether the subjects were weight bearing for more than 4 hours a day and medical history. Participants were also invited to undergo bone densitometry and to provide a blood sample for biochemical analysis.

Ethics

The study was approved by NHS Lothian Research & Development (project number: 2011/W/RH/01) and South East Scotland Research Ethics Committee 01 (reference number: 11/SS/0029).

Bone mineral density measurements

Bone density was measured by dual-energy X-ray absorptiometry (DEXA) using a QDR 4500 densitometer (Hologic, Bedford, MA, USA). The coefficient of variation was 1.4%, 1.3% and 1.2% for femoral neck, lumbar spine and total hip, respectively. The bone mineral density measurements were expressed as g/cm^2 as well as gender specific Z-scores using the manufacturer's reference data.

Biochemical measurements

Serum creatinine was measured using standard techniques. Serum N-terminal propeptide of type 1 procollagen (P1NP) and C-terminal telopeptide of type 1 collagen (CTX) were measured using enzyme chemiluminescent immunoassays on a COBAS 600 analyser (Roche Products Ltd, Penzberg, Germany) with a CV of <4% for P1NP and <5% for CTX across the working range of the assay. Serum total 25-hydroxyvitamin D [25(OH)D] was measured using tandem mass spectrometry. For this assay, $25(OH)D_2$ and D_3 were extracted from serum after zinc sulphate protein precipitation using Isolute solid phase C18 extraction cartridges. Potential interfering substances were removed by initial elution by 50% methanol followed by elution of the vitamins using 10% tetrahydrofuran in acetonitrile. Dried extracts were reconstituted prior to injection into a High Performance Liquid Chromatography Tandem Mass Spectrometer (LC-MS/MS) (Platinum Ultima Waters UK) in multiple reaction mode. The assay has a limit of detection of 2.5 nmol/L for D_3 and 2.5 nmol/L for D_2 and a CV of <10% for both D_3 and D_2 across the working range. The assay was calibrated using NIST aligned calibration material and has achieved certification in the Vitamin D External Quality Assessment Scheme (DEQAS) scheme.

Sample size

The study was powered to provide 90% or greater power to detect a 0.4-0.5 Z-score units difference in bone mineral density between heavy cannabis users and controls, which we felt it would be a clinically significant

difference. Based on this assumption between 80 and 130 participants were required in the heavy cannabis use and control groups.

Statistical methods

Statistical analyses were performed using IBM SPSS Statistics, version 19 (IBM, Armonk, NY). Bootstrap analysis was conducted to account for missing and/or non-normally distributed data.[17,18] Data was missing completely at random (Little's missing completely at random [MCAR] test = 0.61) and accounted only for 1.15% of the total data. Differences between cannabis use groups were assessed by General Linear Model (GLM) using bootstrapped values for continuous variables and by Chi-square test for categorical variables. Multiple linear regression analysis with bootstrapping was employed to determine whether heavy cannabis use was a significant independent predictor of bone mineral density at the lumbar spine, femoral neck and total hip among other possible predictors including: age, gender, BMI, menopausal status, 25[OH]D, P1NP, CTX, tobacco smoking, alcohol intake, dietary calcium intake, participation in sports, weight bearing for >4 hours daily and other illegal drugs use. All these variables were previously shown to contribute to the variance of bone mineral density.[19-28] The R^2 indicates the proportion of the variance of the dependant variable that is explained collectively by all variables. Relative weight analysis was performed as previously described[29] in order to determine the relative importance of each of the predictor variables and hence how much variance is explained by each one of them. The raw resulting weights range from 0 to 1 and their sum equals to R^2. PROCESS macro for SPSS, (v. 2.15) was used to investigate A) the potential indirect effect between heavy cannabis use and bone mineral density via theoretically specified mediators (here BMI and dietary calcium intake; model 4) and B) possible interactions with moderator variables (here other drug use; model 5) (Figure 1). For both models the PROCESS macro implements a Bootstrap inference and outputs a 95% confidence interval. Other drug use was inserted in the model as a moderator rather than mediator since proposed mediators should not be categorical variables.[30] To compare the number of fractures between groups, the incidence rate ratio was calculated by counting the number of fractures that had occurred since the age of starting cannabis use (or the age of starting smoking for the controls) and the difference between groups was calculated by Revman 5.3 software. The significance level was set at p=0.05 with the Bonferroni correction applied for multiple comparisons.

Results

Participant characteristics

Relevant clinical and demographic details of the study population are shown in Table 1 in which cannabis users were divided into moderate and heavy subgroups. Moderate cannabis use was defined as taking cannabis on more than 5 and less than 5000 times and heavy cannabis use defined as taking cannabis on more than 5000 occasions as suggested by Gruber et al.[31] Participants with 5 or fewer exposures were classified as controls. Heavy cannabis users were younger than controls and they reported taking other illegal drugs more frequently. Details of the illegal drugs taken other than cannabis in each group are shown in Table 2. There was a predominance of males in the heavy cannabis user group and fewer of the females were postmenopausal as compared with the controls and moderate users. Dietary calcium intake was higher in heavy cannabis users than in controls but body mass index (BMI) was significantly lower. There was no difference between the groups in alcohol intake, the proportion of individuals with a family history of hip fracture or the proportion who reported taking part in sports regularly. However, the proportion of heavy cannabis users who reported standing for more than 4 hours a day was lower than the control group. Moderate cannabis users were younger than controls, and of the females, fewer were postmenopausal. Moderate cannabis users smoked fewer cigarettes and took more illegal drugs than controls but otherwise had similar characteristics to the controls.

Bone mineral density and biochemical markers

Associations between cannabis use and markers of bone health are summarised in Table 3. Bone mineral density, expressed as the gender specific Z-score was significantly lower in heavy cannabis users when compared with controls at the lumbar spine, femoral neck and total hip. Serum concentrations of the c-terminal telopeptide of type 1 collagen (CTX) and the amino terminal propeptide fragment of type I collagen (PINP) were significantly higher in heavy cannabis users as compared with controls. Conversely, heavy cannabis users had significantly lower total 25(OH)D than controls. There was no significant correlation between 25(OH)D levels and either CTX or PINP concentrations (data not shown)

Fractures were also more common in heavy cannabis users.

Multiple linear regression analysis

To evaluate the relative contribution of cannabis exposure to the reduction in bone mineral density as opposed to other variables we employed multiple linear regression analysis using bone mineral density at each skeletal site as the dependent variable, and age, gender, BMI, menopausal status (yes/no), serum 25(OH)D (nmol/L), serum PINP (μg/L), serum CTX (μg/L), tobacco smoking (pack years), alcohol (units/week), dietary

calcium intake (mg/day), participation in sports (yes/no), weight bearing for >4 hours daily (yes/no), and use of other illegal drugs (yes/no) as explanatory variables. All these variables were previously shown to contribute to the variance of bone mineral density.[19-24,26-28] Heavy cannabis use was introduced as a final possible predictor in the model, since it was the main variable of interest. The results, summarised in Table 4, showed that heavy cannabis use was an independent predictor of low bone mineral density at all three skeletal sites. Relative weight analysis[29] showed that the heavy cannabis use explained 5.4% (p=0.035), 3.9% (p=0.010) and 5.8% (p=0.001) of the variance in bone mineral density at the lumbar spine, femoral neck and total hip, respectively. Body mass index explained a large part the variance at the lumbar spine (38.7%, p=0.002), femoral neck (32.3%, p=0.001) and total hip (55.2%, p=0.001).

Mediation and moderation analyses

Mediation and moderation analyses were performed in order to examine the potential mechanisms by which heavy cannabis use affects bone mineral density at each skeletal site, taking other predictive factors into account. Mediation analysis provides an insight into whether the effects of a variable of interest on an endpoint of interest is mediated directly or indirectly and moderation analysis predicts whether the effects of this variable on the outcome of interest are influence by a moderator variable .[30] The results of the analysis are summarised in Table 4 and Figure 2. For lumbar spine the mediation model included heavy cannabis intake, BMI and dietary calcium intake since these variables were found to be independent predictors of bone mineral density at this site by regression analysis. The model showed that the effects of heavy cannabis use on spine bone mineral density were indirect and mediated through BMI and paradoxically by dietary calcium intake which was increased in heavy cannabis users (Figure 2). However, the β coefficient of BMI mediation (negative effect) was larger than the β coefficient of mediation through dietary calcium intake (positive effect) resulting in an overall negative effect on spine bone mineral density (Table 4). Similar analyses for femoral neck and total hip bone mineral density included heavy cannabis use, age, gender, BMI and other illicit drug use which were found to be independent predictors of bone mineral density at these sites by regression analysis. BMI was considered as a possible mediator, other drug use was added as a moderator and age and gender were added as covariates. Mediation and moderation analyses showed that heavy cannabis use had a direct negative effect on femoral neck and total hip bone mineral density, with other drugs influencing this relationship. However, among those that did not take other drugs, the relationship between heavy cannabis use and total hip bone mineral density was also negative and statistically significant (Table 4).

Discussion

Cannabinoid receptors and their ligands play key roles in several physiological and pathological processes.[1,32,33] The type 1 (CNR1) and type 2 (CNR2) cannabinoid receptors[5,9,12] as well as the orphan cannabinoid receptor GPR55[14] and their ligands have previously been reported to influence bone cell function, bone turnover and bone mass in mice with complex effects that are dependent on the ligand tested and the experimental system used.[5,10] With this in mind, the aim of the present study was to investigate the effects of recreational cannabis use on bone health in humans, given that cannabis is one of the most widely used illegal drugs. The *Cannabis sativa* plant contains more than 100 cannabinoids but the most important are ∆9- tetrahydrocannabinol (THC) a partial agonist at the CNR1 and CNR2 receptors and cannabidiol (CBD) an antagonist at the GPR55 receptor.[32] Based on previous research, both of these cannabinoid receptor ligands might be expected to influence bone metabolism in man,[5] but it is difficult to predict what the overall effects on bone health might be given the fact that the effects of cannabinoid receptor ligands on the skeleton in preclinical studies are influenced by age, gender and genetic background.

The main findings to emerge from the present study were that heavy cannabis users had reduced bone density at all sites measured as compared with controls as well as a reduced body mass index, high bone turnover and an increased rate of fractures. The heavy cannabis user group also differed from the controls in age and gender distribution, serum 25(OH)D concentrations and dietary calcium intake as well as the proportion of time spent weight bearing each day. In view of the complexity of the situation we performed multiple regression analysis to determine if heavy cannabis use was an independent predictor of bone mineral density. The regression analysis identified heavy cannabis use as an independent predictor of bone mineral density at all three skeletal sites with a relative contribution to the total amount of variance explained of 5.8% at the spine, 3.9% at the femoral neck and 5.4% at the total hip. Other significant predictors were body mass index and dietary calcium for spine bone mineral density; and age, gender, body mass index and use of other illegal drugs for hip bone mineral density.

In order to evaluate to what extent the effects of heavy cannabis use on bone density were direct or indirect we performed mediation analysis. This showed that while heavy cannabis use was an independent predictor of bone mineral density at the spine, the effects were indirect and mediated by reduced BMI but offset to an extent by high dietary calcium intake. At the femoral neck and total hip there was evidence of a direct effect heavy cannabis use on bone mineral density but that other illegal drug use was a moderator of the effect.

However, heavy cannabis use negatively influenced total hip bone mineral density even among those who did not use other illegal drugs.

The lower BMI that was observed in heavy cannabis users at first sight seems counterintuitive since agonists at the CNR1 receptor stimulate appetite.[34] However several investigators have found that cannabis users have lower BMI values when compared with controls.[4,35] The underlying mechanisms are unclear but it has been speculated that with long term use, THC might act as an antagonist at CNR1 and CNR2 receptors and reduce food intake through central and peripheral mechanisms.[35]

So far as we are aware this is the first study of bone health in cannabis users. A notable strength is that we investigated a well characterised population of cannabis users who were matched with controls from the same geographical location. However the participants were derived from an inner city general practice in the UK and about two thirds of the heavy cannabis users had also taken other illicit drugs. While we cannot infer a cause and effect relationship between cannabis use and low bone mineral density because of the observational nature of the study, the participants underwent detailed phenotyping and we were able to correct for potential confounding factors through regression and mediation analysis which suggested that the association with low bone mineral density that we observed was due in part to the effects of cannabis. While further research will be required to investigate the mechanisms underlying the association we observed the findings reported here have important clinical implications in identifying heavy cannabis use as potential cause of low bone mineral density, increased bone turnover and predisposition to fractures.

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Figure 1. Results of mediation analysis

Mediation analysis showed that the effects of heavy cannabis use on spine bone mineral density were indirect and mediated by a reduced BMI whereas there was evidence for a direct effect on bone mineral density at the hip. See Table 2 and text for more details.

Table 1. Demographic and lifestyle characteristics of study population

	Controls (n=114)	Moderate cannabis users (n=56)	Heavy cannabis users (n=114)
Age (years)	49.5 ± 9.8	43.3 ± 12.3***	40.5 ± 9.3***
Females (of which post-menopausal)	79 (69.3%) 50 (63.3%)	36 (64.3%) 13 (36.1%)**	51 (44.7%)*** 14 (27.5%)***
Spine Z-score	0.0 ± 1.2	0.0 ± 1.5	-0.5 ± 1.2***
Femoral neck Z-score	0.1 ± 1.0	0.3 ± 1.3	-0.2 ± 0.8*
Total hip Z-score	0.3 ± 0.9	0.3 ± 1.2	-0.2 ± 0.9***
Previous fracture	44 (38.6%)	17 (30.4%)	52 (45.6%)
Number of previous fractures	66	22	102
Clinical fracture rate ratio	1	0.84 (0.52 to 1.37)	2.17 (1.59 to 2.95)***
BMI (kg/m^2)	29.0 ± 7.0	27.7 ± 7.3	26.5 ± 6.0**
Serum 25(OH)D (nmol/L)	36.9 ± 26.7	33.9 ± 23.1	25.3 ± 16.8***
Serum PINP μg/L	41.2 ± 17.8	39.8 ± 18.4	47.1 ± 19.2*
Serum CTX μg/L	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1*
Dietary Calcium (mg/day)	880 ± 453	1047 ± 891	1243 ± 841***
Alcohol users	47 (41.2%)	23 (41.1%)	37 (32.5%)
Alcohol (units/week)[+]	21.2 ± 22.3	14.2 ± 9.2	32.8 ± 37.9
Tobacco (pack years)	32.4 ± 23.7	21.3 ± 18.0*	26.9 ± 46.6
Cannabis exposures (n)	0.3 ± 1.0	1031.3 ± 1238.4***	47491.6 ± 37225.8***
Other illegal drug use	3 (2.6%)	12 (21.4%)***	73 (64.0%)***
Standing >4hr/day	92 (80.7%)	48 (85.7%)	74 (64.9%)**
Parental hip fracture	8 (7.0%)	3 (5.4%)	4 (3.5%)
Taking part in sports regularly	52 (45.6%)	30 (53.6%)	44 (38.6%)

Values are number (%) or mean ± standard deviation, except incidence fracture rate ratio which is presented as the risk ratio with 95% confidence intervals. Differences between groups as assessed by GLM on bootstrapped values or Chi-square test. *p<0.05, **p<0.01, ***p<0.005 from controls. [+]Amongst alcohol users.

Table 2. Use of other illegal drugs in study population

Drug class	Drug name	Controls (n=114)	Moderate users (n=56)	Heavy users (n=114)
Opioids	Heroin	2	6	28
	Dihydrocodeine	1	2	24
	Buprenorphine	0	0	7
	Methadone	1	1	36
Benzodiazepines	Diazepam	2	1	42
	Temazepam	1	0	12
	Triazolam	0	0	1
Substituted Amphetamines	Amphetamine	1	4	21
	Ecstasy	0	3	15
Other	Cocaine	0	1	16
	Lysergic acid diethylamide	0	0	12
	Barbiturates	0	0	2

The values shown are number of participants that reported use of the drug in question

Table 3. Independent predictors of bone mineral density identified by multiple regression analysis.

	β–coefficient	SE	% Relative weight
Lumbar Spine bone mineral density			
BMI	0.261***	0.001	38.7
Dietary calcium intake	0.109**	7.680E-6	5.0
Heavy cannabis use	-0.148*	0.019	5.4
Femoral Neck bone mineral density			
Age	-0.447***	0.001	39.4
Gender	-0.178*	0.019	5.1
BMI	0.313***	0.001	32.3
Other illegal drugs use	0.122*	0.017	4.8
Heavy cannabis use	-0.172*	0.017	3.9
Total Hip bone mineral density			
Age	-0.247***	0.001	7.8
Gender	-0.250***	0.020	11.8
BMI	0.453***	0.001	55.2
Other illegal drugs use	0.128*	0.017	2.3
Heavy cannabis use	-0.205***	0.016	5.8

Only the independent predictors of bone mineral density at each site which reached statistical significance are shown. The variables that were entered into the model are described in the results section of the text. At the lumbar spine the variables entered into the model explained 15.5% of the variance in bone mineral density. Corresponding values for the femoral neck and total hip were 24.8% and 33.3% respectively. β, standardized beta coefficient; SE, standard error of the beta coefficient; % relative weight, relative contribution to R^2. Significance is indicated by *p<0.05, **p<0.01, ***p<0.005.

Table 4. Mediation and moderation analyses reveal direct and indirect effects of cannabis use on bone density at different skeletal sites and interactions with other variables

	Mediator	Moderator	β coefficient (95% CI)	SE
Lumbar Spine bone mineral density				
Direct effect			-0.005 (-0.037, 0.028)	0.016
Indirect effect	BMI		-0.012 (-0.024,-0.005)	0.005
Indirect effect	Dietary calcium intake		0.007 (0.002, 0.015)	0.003
Femoral Neck bone mineral density[+]				
Direct effect		Other drugs = No	-0.036 (-0.076, 0.004)	0.020
Direct effect		Other drugs = Yes	-0.065 (-0.128, -0.003)	0.032
Indirect effect	BMI		-0.008 (-0.021, 0.002)	0.006
Total Hip bone mineral density[+]				
Direct effect		Other drugs = No	-0.045 (-0.085, -0.006)	0.020
Direct effect		Other drugs = Yes	-0.077 (-0.139, -0.015)	0.032
Indirect effect	BMI		-0.013 (-0.029, 0.003)	0.008

The standardised beta coefficient (β), 95% confidence intervals (95% CI); and standard error (SE) are shown taking into account the independent predictors of bone density at each skeletal site, which were BMI and dietary calcium intake for the spine; and BMI, other illegal drug use, age and gender at the hip sites. Significant effects (whether direct or indirect) are indicated when the 95% confidence intervals do not cross zero. [+]Age and gender used as covariates.

Figure 1. Models of mediation and moderation analyses.

Models of used for PROCESS to theoretically assess mediation and moderation of the effects of heavy cannabis use on bone mineral density outcomes.

Figure 2. Results of mediation analysis

Mediation analysis showed that the effects of heavy cannabis use on spine bone mineral density were indirect and mediated by a reduced BMI whereas there was evidence for a direct effect on bone mineral density at the hip. See Table 4 and text for more details

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Reference List

1. Freeman TP. Potent questions about cannabis and mental health. *Lancet Psychiatry.* 2015;2:195-196.

2. Volkow ND, Baler RD, Compton WM et al. Adverse health effects of marijuana use. *N Engl J Med.* 2014;370:2219-2227.

3. Badiani A, Boden JM, De PS et al. Tobacco smoking and cannabis use in a longitudinal birth cohort: evidence of reciprocal causal relationships. *Drug Alcohol Depend.* 2015;150:69-76.

4. Ngueta G, Belanger RE, Laouan-Sidi EA et al. Cannabis use in relation to obesity and insulin resistance in the inuit population. *Obesity (Silver Spring).* 2015;23:290-295.

5. Idris AI, Ralston SH. Cannabinoids and bone: friend or foe? *Calcif Tissue Int.* 2010;87:285-297.

6. Gowran A, McKayed K, Campbell VA. The cannabinoid receptor type 1 is essential for mesenchymal stem cell survival and differentiation: implications for bone health. *Stem Cells Int.* 2013;2013:796715.

7. Idris AI, 't Hof RJ, Greig IR et al. Regulation of bone mass, bone loss and osteoclast activity by cannabinoid receptors. *Nat Med.* 2005;11:774-779.

8. Idris AI, Sophocleous A, Landao-Bassonga E et al. Regulation of bone mass, osteoclast function, and ovariectomy-induced bone loss by the type 2 cannabinoid receptor. *Endocrinology.* 2008;149:5619-5626.

9. Idris AI, Sophocleous A, Landao-Bassonga E et al. Cannabinoid receptor type 1 protects against age-related osteoporosis by regulating osteoblast and adipocyte differentiation in marrow stromal cells. *Cell Metab.* 2009;10:139-147.

10. Sophocleous A, Idris AI, Ralston SH. Genetic background modifies the effects of type 2 cannabinoid receptor deficiency on bone mass and bone turnover. *Calcif Tissue Int.* 2014;94:259-268.

11. Ofek O, Karsak M, Leclerc N et al. Peripheral cannabinoid receptor, CB2, regulates bone mass. *Proc Natl Acad Sci U S A.* 2006;103:696-701.

12. Sophocleous A, Landao-Bassonga E, van't Hof RJ et al. The type 2 cannabinoid receptor regulates bone mass and ovariectomy-induced bone loss by affecting osteoblast differentiation and bone formation. *Endocrinology.* 2011;152:2141-2149.

13. Ofek O, Attar-Namdar M, Kram V et al. CB2 cannabinoid receptor targets mitogenic Gi protein-cyclin D1 axis in osteoblasts. *J Bone Miner Res.* 2011;26:308-316.

14. Whyte LS, Ryberg E, Sims NA et al. The putative cannabinoid receptor GPR55 affects osteoclast function in vitro and bone mass in vivo. *Proc Natl Acad Sci U S A.* 2009;106:16511-16516.

15. Tam J, Ofek O, Fride E et al. Involvement of neuronal cannabinoid receptor CB1 in regulation of bone mass and bone remodeling. *Mol Pharmacol.* 2006;70:786-792.

16. Sophocleous A, Sims AH, Idris AI et al. Modulation of strain-specific differences in gene expression by cannabinoid type 2 receptor deficiency. *Calcif Tissue Int.* 2014;94:423-432.

17. Jamshidian M. Strategies for the analysis of incomplete data. In: Hardy M & Bryman A (eds). Handbook of data analysis. London: Sage; 2004. 113-130.

18. Field A. Discovering statistics using IBM SPSS statistics. 4th Edition ed. London: Sage; 2014.

19. Salamat MR, Salamat AH, Abedi I et al. Relationship between Weight, Body Mass Index, and Bone Mineral Density in Men Referred for Dual-Energy X-Ray Absorptiometry Scan in Isfahan, Iran. *J Osteoporos.* 2013;2013:205963.

20. Kota S, Jammula S, Kota S et al. Correlation of vitamin D, bone mineral density and parathyroid hormone levels in adults with low bone density. *Indian J Orthop.* 2013;47:402-407.

21. Sowers MR, Jannausch M, McConnell D et al. Hormone predictors of bone mineral density changes during the menopausal transition. *J Clin Endocrinol Metab.* 2006;91:1261-1267.

22. Van LL, Lefevre J, Claessens AL et al. Influence of participation in high-impact sports during adolescence and adulthood on bone mineral density in middle-aged men: a 27-year follow-up study. *Am J Epidemiol.* 2003;158:525-533.

23. Rector RS, Rogers R, Ruebel M et al. Lean body mass and weight-bearing activity in the prediction of bone mineral density in physically active men. *J Strength Cond Res.* 2009;23:427-435.

24. Ward KD, Klesges RC. A meta-analysis of the effects of cigarette smoking on bone mineral density. *Calcif Tissue Int.* 2001;68:259-270.

25. Deng HW, Chen WM, Conway T et al. Determination of bone mineral density of the hip and spine in human pedigrees by genetic and life-style factors. *Genet Epidemiol.* 2000;19:160-177.

26. Gozashti MH, Shahesmaeili A, Amini ZN. Is opium addiction a risk factor for bone loss? *Iran Red Crescent Med J.* 2011;13:464-468.

27. Chaki O, Yoshikata I, Kikuchi R et al. The predictive value of biochemical markers of bone turnover for bone mineral density in postmenopausal Japanese women. *J Bone Miner Res.* 2000;15:1537-1544.

28. Nguyen TV, Center JR, Eisman JA. Osteoporosis in elderly men and women: effects of dietary calcium, physical activity, and body mass index. *J Bone Miner Res.* 2000;15:322-331.

29. Lorenzo-Seva U, Ferrando PJ, Chico E. Two SPSS programs for interpreting multiple regression results. *Behav Res Methods.* 2010;42:29-35.

30. Hayes AF. Introduction to Mediation, Moderation, and Conditional Process Analysis: A Regression-Based Approach. New York: The Guilford Press; 2013.

31. Gruber AJ, Pope HG, Hudson JI et al. Attributes of long-term heavy cannabis users: a case-control study. *Psychol Med.* 2003;33:1415-1422.

32. Bolognini D, Ross RA. Medical cannabis vs. synthetic cannabinoids: What does the future hold? *Clin Pharmacol Ther.* 2015;97:568-570.

33. Macleod J, Robertson R, Copeland L et al. Cannabis, tobacco smoking, and lung function: a cross-sectional observational study in a general practice population. *Br J Gen Pract.* 2015;65:e89-e95.

34. Kirkham TC. Cannabinoids and appetite: food craving and food pleasure. *Int Rev Psychiatry.* 2009;21:163-171.

35. Le FB, Trigo JM, Sharkey KA et al. Cannabis and Delta9-tetrahydrocannabinol (THC) for weight loss? *Med Hypotheses.* 2013;80:564-567.