CLINICAL RESEARCH STUDY

Heavy Cannabis Use Is Associated With Low Bone Mineral Density and an Increased Risk of Fractures

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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 2013. 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, $P < .0005$), lower spine bone mineral density (−0.5 ± 1.2 vs 0.0 ± 1.2, $P < .0005$), and lower body mass index (BMI; 26.5 ± 6.0 vs 29.0 ± 7.0, $P = .01$) than controls. Fracture rate was also increased in heavy users (rate ratio = 2.17; 95% confidence interval, 1.59-2.95; $P < .001$). When compared with controls, serum cross-linked C-telopeptide of type 1 collagen (CTX) concentrations were raised in heavy cannabis users (0.3 ± 0.1 vs 0.2 ± 0.1 pg/mL, $P = .045$), as were serum N-terminal propeptide of type 1 procollagen (P1NP) concentrations (47.1 ± 19.2 vs 41.2 ± 17.8 pg/mL, $P = .01$). Serum total 25-hydroxyvitamin D concentrations were reduced in heavy users compared with controls (25.3 ± 16.8 vs 36.9 ± 26.7 nmol/L, $P = .002$). Multiple regression analysis revealed that heavy cannabis use was an independent predictor of spine bone mineral density, accounting for 5.4% of the variance ($P = .035$), and total hip bone mineral density, accounting for 5.8% of the variance ($P = .001$), but mediation analysis suggested that the effect on spine bone mineral density was indirect and mediated through low body mass index.

CONCLUSIONS: 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.

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KEYWORDS: Bone mineral density; Cannabinoids; Cannabis; Fracture

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 (BMI).[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 increased 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 emphasized by the fact that the effects of cannabinoid receptor deficiency in mouse models are age and sex 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 years 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.

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 mediated by low body mass index.

Ethics

The study was approved by National Health Service 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 using a QDR 4500 densitometer (Hologic, Bedford, Mass). 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 sex-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 serum cross-linked C-telopeptide of type 1 collagen (CTX) were measured using enzyme chemiluminescent immunoassays on a COBAS 600 analyzer (Roche Products Ltd, Penzberg, Germany) with a coefficient of variation 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 sulfate protein precipitation using ISOLUTE solid phase C18 extraction cartridges (Biotage AB, Uppsala, Sweden). 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 (Platinum Ultima; Waters Corporation, Elstree, Hertfordshire, 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 coefficient of variation of < 10% for both D_3 and D_2 across the working range. The assay was calibrated using

National Institute of Standards and Technology-aligned calibration material and has achieved certification in the Vitamin D External Quality Assessment 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 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 or non-normally distributed data.[17,18] Data were missing completely at random (Little's Missing Completely at Random test = 0.61) and accounted for only 1.15% of the total data. Differences between cannabis use groups were assessed by general linear model using bootstrapped values for continuous variables and by chi-squared 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, sex, 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 have previously been reported to contribute to the variance of bone mineral density.[19-28] The R^2 indicates the proportion of the variance of the dependent variable that is explained collectively by all variables. Relative weight analysis was performed as previously described[29] to determine the proportion of the variance explained by each of the predictor variables. The raw resulting weights range from 0 to 1 and their sum equals R^2. The PROCESS macro for SPSS (v. 2.15) was used to investigate the possibility that the association between heavy cannabis use and bone mineral density was indirect and mediated by BMI and dietary calcium intake; model 4) and to investigate 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 because 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, for the controls, the age of starting smoking) and the difference between groups was calculated by Revman 5.3 software (Cochrane, London, UK). The significance level was set at $P = .05$, with the Bonferroni correction applied for multiple comparisons.
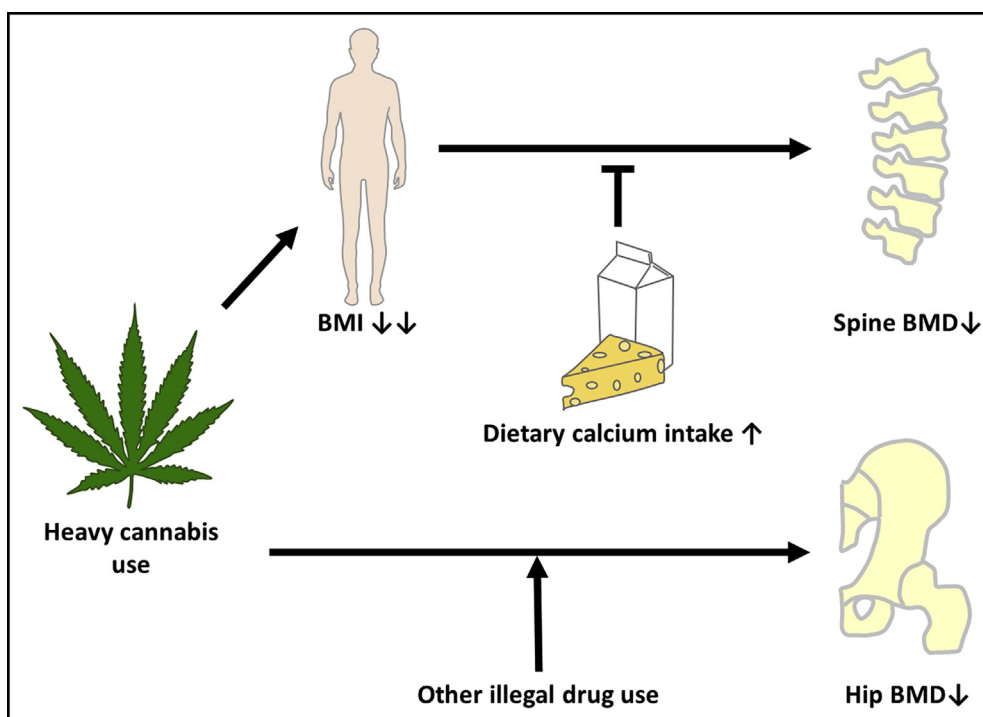

Figure 1   Models of mediation and moderation analyses. Models of used for PROCESS to theoretically assess mediation and moderation of the effects of heavy cannabis use on bone mineral density outcomes.

Table 1 Demographic and Lifestyle Characteristics of Study Population

	Controls (n = 114)	Moderate Cannabis Users (n = 56)	Heavy Cannabis Users (n = 114)
Age (y)	49.5 ± 9.8	43.3 ± 12.3***	40.5 ± 9.3***
Females	79 (69.3%)	36 (64.3%)	51 (44.7%)***
(of which postmenopausal)	50 (63.3%)	13 (36.1%)**	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 (0.52-1.37)	2.17 (1.59-2.95)***
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 P1NP 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 > 4 h/d	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 general linear model on bootstrapped values or chi-squared test.

25(OH)D = total 25-hydroxyvitamin D; BMI = body mass index; CTX = cross-linked C-telopeptide of type 1 collagen; P1NP = amino terminal propeptide fragment of type I collagen.

*$P < .05$, **$P < .01$, ***$P < .005$ from controls.

†Among alcohol users.

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 more than 5 and < 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, other than cannabis, taken 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 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.

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 the numbers 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
Sex	−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
Sex	−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 that 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-coefficient = standardized beta-coefficient; BMI = body mass index; % relative weight = relative contribution to R^2; SE = standard error of the beta-coefficient.

Significance is indicated by *$P < .05$; **$P < .01$; ***$P < .005$.

Bone Mineral Density and Biochemical Markers

Associations between cannabis use and markers of bone health are summarized in Table 1. Bone mineral density, expressed as the sex-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 CTX and the amino terminal propeptide fragment of type I collagen (P1NP) 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 P1NP 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, sex, BMI, menopausal status (yes/no), serum 25(OH)D (nmol/L), serum P1NP (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. With the exception of drug use, all of these variables have previously been reported 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 because it was the main variable of interest. The results, summarized in Table 3, showed that heavy cannabis use was an independent predictor of low bone mineral density at all 3 skeletal sites. Relative weight analysis[29] showed that the heavy cannabis use explained 5.4% ($P = .035$), 3.9% ($P = .010$), and 5.8% ($P = .001$) of the variance in bone mineral density at the lumbar spine, femoral neck, and total hip, respectively. BMI explained a large part of the variance at the lumbar spine (38.7%, $P = .002$), femoral neck (32.3%, $P = .001$), and total hip (55.2%, $P = .001$).

Mediation and Moderation Analyses

Mediation and moderation analyses were performed 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 influenced by a moderator variable.[30] The results of the analysis are summarized in Table 4 and Figure 2. For lumbar spine, the mediation model included heavy cannabis intake, BMI, and dietary calcium intake because 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 low BMI but was partially offset 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, sex, BMI, and other illegal 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 sex 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).

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 to 0.028)	0.016
Indirect effect	BMI		−0.012 (−0.024 to −0.005)	0.005
Indirect effect	Dietary calcium intake		0.007 (0.002 to 0.015)	0.003
Femoral neck bone mineral density*				
Direct effect		Other drugs = No	−0.036 (−0.076 to 0.004)	0.020
Direct effect		Other drugs = Yes	−0.065 (−0.128 to −0.003)	0.032
Indirect effect	BMI		−0.008 (−0.021 to 0.002)	0.006
Total hip bone mineral density*				
Direct effect		Other drugs = No	−0.045 (−0.085 to −0.006)	0.020
Direct effect		Other drugs = Yes	−0.077 (−0.139 to −0.015)	0.032
Indirect effect	BMI		−0.013 (−0.029 to 0.003)	0.008

The standardized 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 body mass index (BMI) and dietary calcium intake for the spine; and BMI, other illegal drug use, age, and sex at the hip sites.

Significant effects (whether direct or indirect) are indicated when the 95% confidence intervals do not cross zero.

*Age and sex used as covariates.

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 Δ9-tetrahydrocannabinol, a partial agonist at the CNR1 and CNR2 receptors, and cannabidiol, 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 humans,[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, sex, 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 sex 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 3 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 BMI and dietary calcium for spine bone mineral density; and age, sex, BMI, and use of other illegal drugs for hip bone mineral density.

We performed mediation analysis to evaluate to what extent the effects of heavy cannabis use on bone density were direct or indirect. 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 of 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 because 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, Δ9-tetrahydrocannabinol 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-characterized 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
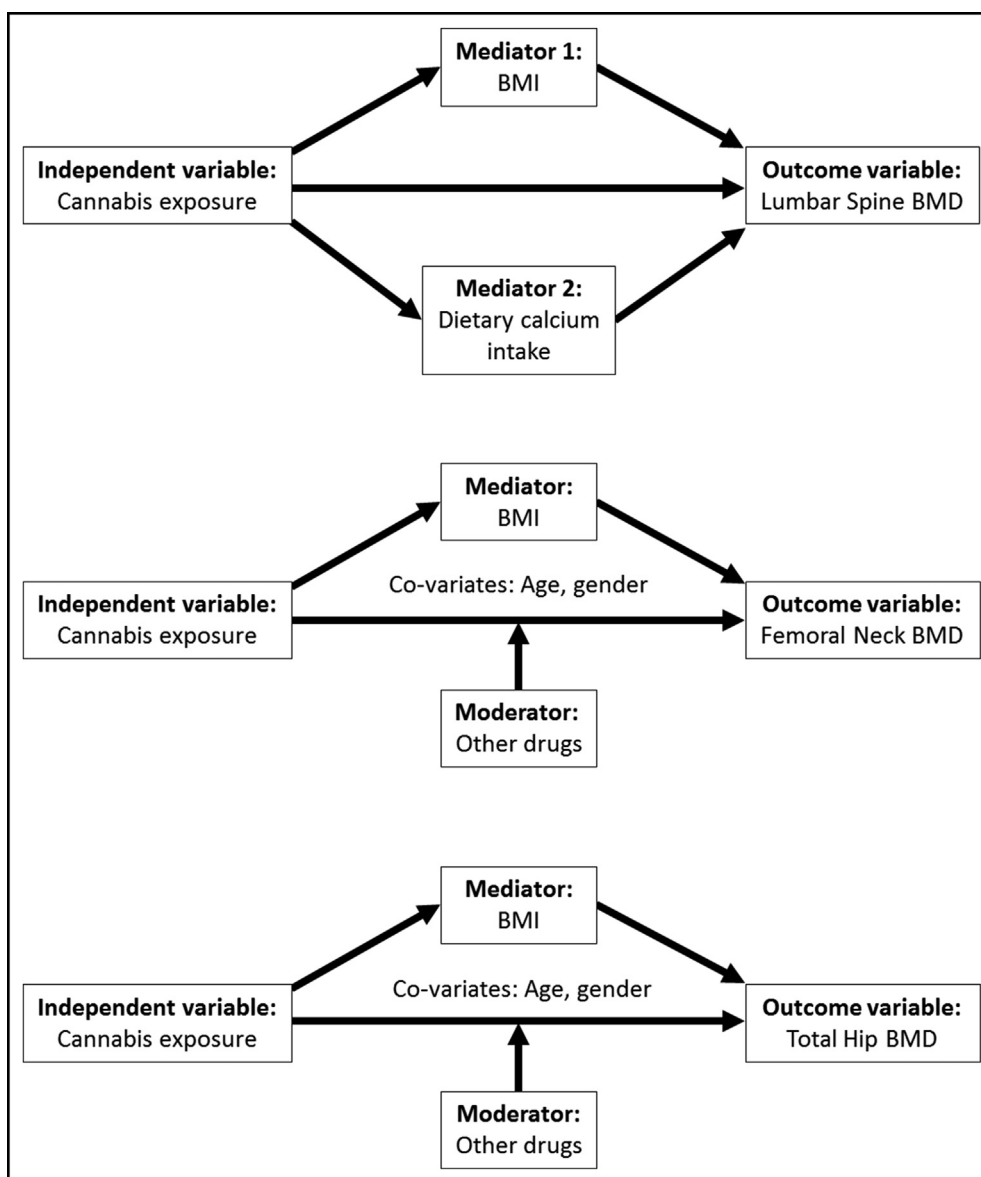
Figure 2   Results of mediation analysis. Mediation analysis showed that the effects of heavy cannabis use on spine bone mineral density were indirect and mediated by a reduced body mass index (BMI), but partially offset by a high dietary calcium intake whereas there was evidence for a direct effect on bone mineral density (BMD) at the femoral neck and total hip as well as an indirect effect mediated by low BMI. See Table 4 and text for more details.

heavy cannabis users had also taken other illegal drugs. While we cannot infer a cause-and-effect relationship between cannabis use and low bone mineral density due to 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 a potential cause of low bone mineral density, increased bone turnover, and predisposition to fractures.

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