BMJ Open

What are the characteristics of vitamin D metabolism in opioid dependence? An exploratory longitudinal study in Australian primary care

Albert Stuart Reece,[1] Gary Kenneth Hulse[2,3]

To cite: Reece AS, Hulse GK. What are the characteristics of vitamin D metabolism in opioid dependence? An exploratory longitudinal study in Australian primary care. *BMJ Open* 2018;**8**:e016806. doi:10.1136/bmjopen-2017-016806

► Prepublication history and additional material for this paper are available online. To view these files, please visit the journal online (http://dx.doi.org/10.1136/bmjopen-2017-016806).

Received 12 March 2017
Revised 25 September 2017
Accepted 27 September 2017

[1]Department of Psychiatry and Clinical Neurosciences, University of Western Australia, Brisbane, Queensland, Australia
[2]Department of Psychiatry and Clinical Neurosciences, University of Western Australia, Perth, Queensland, Australia
[3]Psychiatry, Edith Cowan University at Joondalup, Western Australia, Australia

Correspondence to
Dr Albert Stuart Reece;
sreece@bigpond.net.au

ABSTRACT

Objective Compare vitamin D levels in opioid dependence and control population and adjust for relevant confounding effects. Nuclear hormone receptors (including the vitamin D receptor) have been shown to be key transducers and regulators of intracellular metabolism and comprise an important site of pathophysiological immune and metabolic dysregulation potentially contributing towards pro-ageing changes observed in opioid-dependent patients (ODPs).

Design Longitudinal prospective comparing ODPs with general medical controls (GMCs).

Setting Primary care.

Participants Prospective review comparing 1168 ODP (72.5% men) and 415 GMC (51.6% men, p<0.0001). Mean ages were 33.92±0.31 (mean±SEM) and 41.22±1.32 years, respectively (p<0.0001). Opioid use in the ODP has been previously reported and shown to be typical.

Interventions Nil. Observational study only.

Primary and secondary outcomes Serum vitamin D levels and relevant biochemical parameters.

Results Vitamin D levels were higher in the ODP (70.35±1.16 and 57.06±1.81 nmol/L, p<0.0001). The difference in ages between the two groups was handled in an age-matched case–control subanalysis and also by multiple regression. Sexes were analysed separately. The age:status (or age:time:status) was significant in case–control, cross-sectional and longitudinal analyses in both sexes (p<0.05). Modelled vitamin D was 62.71 vs 57.81 nmol/L in the two groups. Time-dependent mixed-effects models quadratic in age outperformed linear-only models (p=0.0377). ODP vitamin D was shown to vary with age and to correlate with alanine aminotransferase establishing it as a biomarker of age in this group. Hepatitis C seronegativity was significant in regression models (from p=0.0015).

Conclusion Vitamin D was higher in ODP in both sexes in bivariate, cross-sectional, case–control and longitudinal analyses and was robust to the inclusion of metabolic and immune biomarkers. That Hepatitis C seronegativity was significant suggests opioid dependence has an effect beyond simply that of its associated hepatitides. This finding may relate to the accelerated ageing process previously described in opioid dependence.

Strengths and limitations of this study

- ► Strengths of this study included its large sample size, prospective and longitudinal design and real-world sampling in primary care.
- ► The involvement of nuclear hormone receptor signalling in coordinating metabolic and immune systems provides an important insight into a major network of pathways which may contribute to the altered pathophysiological experience of drug dependent cohorts.
- ► Study weaknesses included the unavailability of socioeconomic or occupational data which may impact on the amount of sun exposure derived from differing lifestyles and vocational exposure.
- ► Vitamin D receptor assays and assays for the various vitamin D metabolites were also not available to this study.

INTRODUCTION

Vitamin D and its nuclear steroid receptor have been implicated in a wide range of physiological and developmental functions including skeletal, immunological, muscle strength and tendon function, metabolic syndrome and obesity, cardiovascular disease, endocrine disorders including diabetes, neuropsychiatric disorders, oncogenesis of several cancers, development and many cellular function.[1–4] Many of these age-related morbidities are increasingly associated with opiate use.[5–9] However, a literature search of the PubMed, Scopus and Ebsco Host online databases revealed no reports of the impact of opiate dependency on vitamin levels, other than a single report of its association with the adequacy of pain control in a cohort of patients with chronic pain.[10]

This whole area has recently been shown to be of enormous clinical importance by the increasing significance of vitamin D in multiple sclerosis[11–17]although this relationship remains controversial.[18–20]

The subject is of importance for several reasons. There are several reports of elevated rates of osteoporosis/osteopaenia in opioid-dependent cohorts.[5 8 21] Higher levels of calcium, phosphate and their solubility product have

previously been reported in opioid-dependent patients (ODPs).[22]

Increased vascular stiffness has also been reported in both male and female patients.[7 23] It may be that higher levels of vascular stiffness—and vascular age—are causally associated with disruptions of vitamin D physiology. Moreover, a number of tumours have also been noted to be seen at higher incidence in ODPs[24–26] which may relate to either altered nutritional, metabolic or immune factors. Chronic muscle and joint pain is also a common feature of opioid withdrawal which many dependent patients experience on a daily basis and is known not to respond to non-narcotic analgesics.[10 27]

The evidence from numerous sources is remarkably consistent and increasingly strong for a pattern of accelerated ageing in opioid dependence.[7 23] Subcellular oxidative damage particularly arising from immune mechanisms is increasingly emerging as a principal determinant of ageing processes.[28 29] As an important modifier of immune dysregulation,[30–34] it is therefore plausible that vitamin D physiology may impinge on the ageing process in a clinically significant manner and may be of particular relevance to the immune dysregulation well described in opioid dependence[35–39] and the accompanying syndrome of accelerated ageing.

Moreover, complex interactions are increasingly being documented between nutritional, immune, gastrointestinal and metabolic biomarkers[40–42] making multiway interactions both analytically feasible and physiologically meaningful.[43]

The following study was therefore conducted prospectively to ascertain (i) the comparative levels of vitamin D in opiate-dependent and non-dependent clinical populations including sex differentials and comparative levels of hypovitaminosis D; (ii) to document the relationship of chronological age with changes in vitamin D status in dependent and non-dependent groups and therefore its role as a potential biomarker of ageing and (iii) to document significant associated changes in metabolic and innate and adaptive immune function with vitamin D levels. As our clinic sees significant numbers of both ODPs and non-dependent patients, we are ideally suited to compare opioid-dependent and non-dependent cohorts.

METHODS

Patient selection

All ODPs and general medical controls (GMCs) attended a single metropolitan outpatient clinic, with data collected by retrospective review of patient records. All patients in whom a vitamin D assay was requested were included in the analysis. There was no selection of patients based on age. Hepatitis C serology was routinely only performed on opiate-dependent patients, and was used as a surrogate marker for retrospectively identifying opiate-dependent patients. Hence, patients were considered to be opioid dependent where the hepatitis C test was performed or the hepatitis C virus (HCV) RNA PCR test was positive. In a few cases, data were manually curated to correct anomalies. The two study groups are thus described as being ODPs and GMCs. Other blood tests were taken as clinically required in the process of routine medical care.

Pathology analysis

As a high rate of abnormally low vitamin D levels was quickly noted in all our patients, this test was ordered routinely on all patients who required clinical pathology to be performed. All clinical pathology was undertaken by the Queensland Medical Laboratory (QML) which is accredited by the National Association of Testing Authorities Australia to the Australian Laboratory standard AS-15189. QML is also accredited to the international standard ISO 9001 the international laboratory clinical standard. The form of vitamin D measured was 25-hydroxycholecalciferol. The calcium–phosphate solubility product was defined as is usual in chemistry as the product of the cube of the serum calcium concentration and the square of the phosphate concentration as previously described.[22]

Statistics

The pathology results were downloaded as an Excel spreadsheet from QML for the period 1995–2017. Data are listed as mean±SEM. Categorical data were compared in EpiInfo V.7.2.0.1 from Centres for Disease control in Atlanta, Georgia, USA using the adjusted Mantel-Haenszel statistic. Bivariate statistics were compared by categories in Statistica V.7.1 from Statsoft, Oklahoma, USA. Student's test for t with separate variances was utilised as indicated by the Levene test. This is reported in table 1 as fractional df.

'R' V.3.3.2 was downloaded from the University of Melbourne Central 'R' Archive Network mirror. Multiple regression was performed in 'R' and graphs were drawn in R using the ggplot2 package. Loess (localised polynomial curves) were drawn at a span=0.95. Multiple regression model reduction was performed by the classical method with deletion of the least significant term until only significant terms remained. Deidentified confidential data may be made available to interested readers and researchers on written request to the authors. Continuous data of such as vitamin D, alanine aminotransferase (ALT), serum globulins and C-reactive protein were log transformed in multiple regression analyses as indicated by the results of the Shapiro test. Chronological age was not log transformed in the interests of improving model fit. Linear and polynomial models were compared using analysis of variance (ANOVA) tests in R. Missing data were case-wise deleted. All t-tests were two tailed. p Value <0.05 was considered significant.

Ethics

Strict patient confidentiality was maintained throughout the data analysis phase. The study was conducted in accord with the Declaration of Helsinki.

RESULTS

In the period 1995–2017, 1583 patients had a vitamin D study performed on 2099 occasions. Of these, 1168

Table 1 Bivariate comparisons—initial cross-sectional data

	Valid N		Mean (+SEM)		Statistics		
	Control	ODP	Control	ODP	t	df	P value
All patients—initial values							
Age (years)	176	739	41.22 (1.32)	33.92 (0.31)	−5.38	194.71	<0.0001
Haemoglobin (g/dL)	167	731	142.65 (1.00)	147.23 (0.50)	3.98	896.00	0.0001
Platelets (×10^9/L)	166	728	267.72 (4.98)	267.81 (2.65)	0.01	892.00	0.9882
White cell count (×10^9/L)	167	731	7.95 (0.59)	8.4 (0.09)	0.75	173.80	0.4515
Neutrophil absolute (×10^9/L)	167	731	4.91 (0.60)	4.97 (0.08)	0.10	171.52	0.9169
Lymphocyte absolute (×10^9/L)	167	731	2.82 (0.60)	2.54 (0.03)	−0.47	167.06	0.6401
Monocytes absolute (×10^9/L)	167	731	1.18 (0.60)	0.65 (0.01)	−0.88	166.07	0.3813
ESR (mm/hour)	167	720	12.08 (1.20)	10.88 (0.37)	−0.95	198.43	0.3408
High-sensitivity CRP (mg/dL)	166	739	6.21 (1.75)	6.93 (0.50)	0.54	903.00	0.5921
Total bilirubin (mmol/L)	171	739	10.85 (0.66)	8.1 (0.18)	−3.99	196.36	0.0001
Alkaline phosphatase (IU/L)	171	739	80.87 (3.81)	81.33 (1.05)	0.12	196.52	0.9070
ALT (IU/L)	171	739	29.24 (1.63)	80.59 (9.11)	5.55	782.28	<0.0001
AST (IU/L)	171	739	28.26 (1.03)	60.21 (8.40)	3.78	759.46	0.0002
LDH (IU/L)	171	738	172.84 (2.91)	199.87 (6.00)	2.15	907.00	0.0315
GGT (IU/L)	171	739	33.27 (2.61)	51.25 (2.32)	5.14	477.24	<0.0001
Total protein (g/L)	171	739	73.28 (0.39)	75.03 (0.19)	3.96	908.00	0.0001
Albumin (g/L)	171	739	43.47 (0.40)	42.76 (0.12)	−2.20	908.00	0.0278
Globulin (g/L)	171	739	30.4 (0.49)	32.26 (0.15)	4.75	908.00	<0.0001
Cholesterol (mmol/L)	171	739	5.64 (0.57)	4.55 (0.04)	−1.90	171.53	0.0586
Triglyceride (mmol/L)	171	739	2.15 (0.61)	1.45 (0.04)	−1.15	171.29	0.2500
LDL (mmol/L)	84	717	14.55 (3.49)	2.38 (0.03)	−3.49	83.01	0.0008
HDL (mmol/L)	84	717	13.25 (3.54)	1.24 (0.02)	−3.39	83.00	0.0011
Vitamin D (nmol/L)	176	739	57.06 (1.81)	70.35 (1.16)	6.19	334.41	<0.0001
Case–control series							
Paired t-tests							
Age (years)	331	331	40.08 (0.62)	40.34 (0.64)	5.5716	330.00	<0.0001
Vitamin D (nmol/L)	331	331	59.73 (1.66)	64.24 (1.52)	2.0044	330.00	0.0458
Non-paired t-tests							
ALT (IU/L)	303	331	32.59 (1.47)	61.74 (7.20)	6.8776	593.15	<0.0001
AST (IU/L)	303	331	28.41 (0.83)	49.22 (3.17)	8.9718	547.13	<0.0001
Globulin (g/L)	303	331	30.31 (0.21)	33.18 (0.25)	8.7564	629.68	<0.0001
High-sensitivity CRP (mg/dL)	272	329	5.26 (0.73)	6.34 (0.76)	2.4738	578.35	0.0137

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; ODP, opioid-dependent patient.

patients were treated for opioid dependence and 415 patients were treated for general medical conditions. It is noteworthy that 98% of this study was sampled prior to the introduction of universal coverage for hepatitis C treatment in Australia in March 2016.

Overall, there were 1061 men and 522 women. Out of 1168 ODPs, 847 (72.52%) were men and out of 415 GMCs, 214 (51.57%) were men (Mantel-Haenszel χ^2=60.77, p<0.0001). In the longitudinal sample including repeat tests, 1031/1437 (71.75%) of opioid-dependent samples were from men and 350/662 (52.87%) of the general medical samples were taken from men (Mantel-Haenszel χ^2=71.72, p<0.0001).

Patients were considered to be hepatitis C positive if either the HCV antibody or their serum PCR study was positive for hepatitis C RNA. Among the ODPs, 301 were hepatitis C antibody seronegative and 867 were antibody positive. Among the medical controls, 406 were untested, 1 was seronegative and 8 were positive. Hence, 74.23% of the ODPs were hepatitis

C seropositive, compared with only 1.93% (8/415) of the GMCs (Mantel-Haenszel extended χ^2 test for trend=12300.10, p<0.0001).

Table 1 gives the initial bivariate cross-sectional comparative data for selected laboratory parameters. In particular, one notes a significant difference in chronological age between the opioid-dependent and general medical groups 33.92±0.31 versus 41.22±1.32 years (mean±SEM, t=5.38, sep. var. df=194.71, p<0.0001). Several of the liver tests are higher in the opioid-dependent group. Some of the lipid parameters are lower likely reflecting a lower body weight as has previously been reported in patients from this cohort.[7] Drug use data in this cohort have been previously reported.[7 23 44–46]

One notes that the vitamin D level is much higher in the ODPs 70.35±1.16 vs 57.06±1.81 mmol/L than in the GMCs (t=6.19, sep. var. df=334.41, p<0.0001). Low levels of vitamin D (<50 nmol/L) were less frequent in the ODP group than in the GMC group (27.48% vs 45.3%, OR=0.46, 95% CI 0.36 to 0.58, Mantel-Haenszel χ^2=44.53, df=1, p<0.0001).

Vitamin D levels in the cohort is charted by age in figure 1A,B and by time in figure 2 (see the online supplementary figure 1) provides a similar plot to figure 1A but is drawn with loess curves which closely approximate straight lines, confirming the utility of linear modelling in this analysis. The effect of time is shown in the online supplementary figure 2, where it is shown that linear modelling approximates the effect of passing time less well.

ALT is a well-recognised clinical biomarker of metabolic ageing.[39 47] (Log) vitamin D level was shown to correlate with the (log) serum ALT in opioid dependence both in the cross-sectional initial group (Spearman R=0.0951, t=3.26, df=1166, p=0.0011) and the longitudinal group (Spearman R=0.0984, t=3.75, df=1435, p=0.0002). This correlation was not significant among GMCs. Another biomarker, serum globulin, was not significantly correlated with vitamin D in any group.

These figures show a clear age effect. Hence, the very different ages in our two major groups may have had an effect on the parameter of interest. This age disparity was addressed in two ways. First by using a case–control comparison group from within the larger group, and second by using multiple regression and correcting for the effects of chronological age.

Case–control series

In the case–control study, it was possible to pair 331 GMCs with 331 ODPs by age. The age of the ODPs was 40.07±0.62 years and that of the GMCs was 40.34±0.64 years (Student's paired t-test t=7.13, df=330, p<0.0001). The mean difference in ages was 0.26 years. However, the ages were not significantly different if a non-paired t-test was used (t=0.29, df=660, p=0.76). In 20 of the 331 pairs (7.5%) the age difference was >|1|; and in 49/331 pairs (14.8%) the age difference was greater than an absolute difference of |0.5|. The comparative age distributions are shown in superimposed histogram and contour plots (see the online supplementary figure 3).

While the two groups had a similar sex ratio, being 50.76% and 51.36% men, respectively (Mantel-Haenszel χ^2=0.2, p=0.87) they were not sex-matched at the single pair level (Wilcoxson W=53 464, p=0.82).

Selected bivariate parametric comparison from the case–control cohort are shown in the lower part of table 1.

The mean vitamin D in these two groups was 64.24±1.52 and 59.73±1.66, respectively (Student's paired t-test t=5.57, df=330, p<0.0001). These values are charted for the initial cross-sectional group in figure 1C.

When the (log) vitamin D status was regressed against age in a linear regression model in each case the age: addictive status interaction was significant in all patients, and in women (top half of table 2). The age: status interaction approached significance in men (p=0.066). In a linear model quadratic in age, both the addictive status and the age squared:status interaction were significant (table 2). The model quadratic in age was superior to the model linear in age (F=3.422, df=3, p=0.0178).

When the (log) vitamin D level was regressed against age and time in a mixed-effects model using restricted maximum likelihood modelling techniques, the addictive status was significant both in its own right (p=0.0009) and in interaction with age (p=0.0180), as show in the top of table 3. In a model quadratic in age, the addictive status was significant (p=0.0004) and it was also significant in interactions with age and time (from p=0.0021). The model quadratic in age was superior to the model linear in age (log ratio=14.80, df=5, p=0.0112).

Multiple regression in the whole dataset

We then move on to the whole dataset first looking at the initial cross-sectional values. In these studies, the (log) vitamin D level is regressed against age and addictive status. These results are summarised in the lower half of table 2 which found significant differences by group and by sex.

The effect size between the two groups can be calculated from this model. When one enters the median age (35.83 years) for the cross-sectional dataset in the regression model, the modelled levels of vitamin D are 62.71 and 57.81 nmol/L in the ODP and GMC groups, respectively. This represents a 4.90 nmol/L difference or 8.47% advancement on the level in the control group.

A model quadratic in age was also calculated for the initial cross-sectional data with results shown in the lower half of table 2. However, on formal testing, it was not superior to the strictly linear model (F=1.7085, df=2, p=0.1815).

When all the data (n=2099) are considered in a time-dependent mixed-effects model, the results shown in the middle portion of table 3 are obtained. In these models, the (log) serum vitamin D levels is regressed against age, time and drug dependence status with the case number as the random effect. A similar model was calculated quadratic in age with results shown at the top

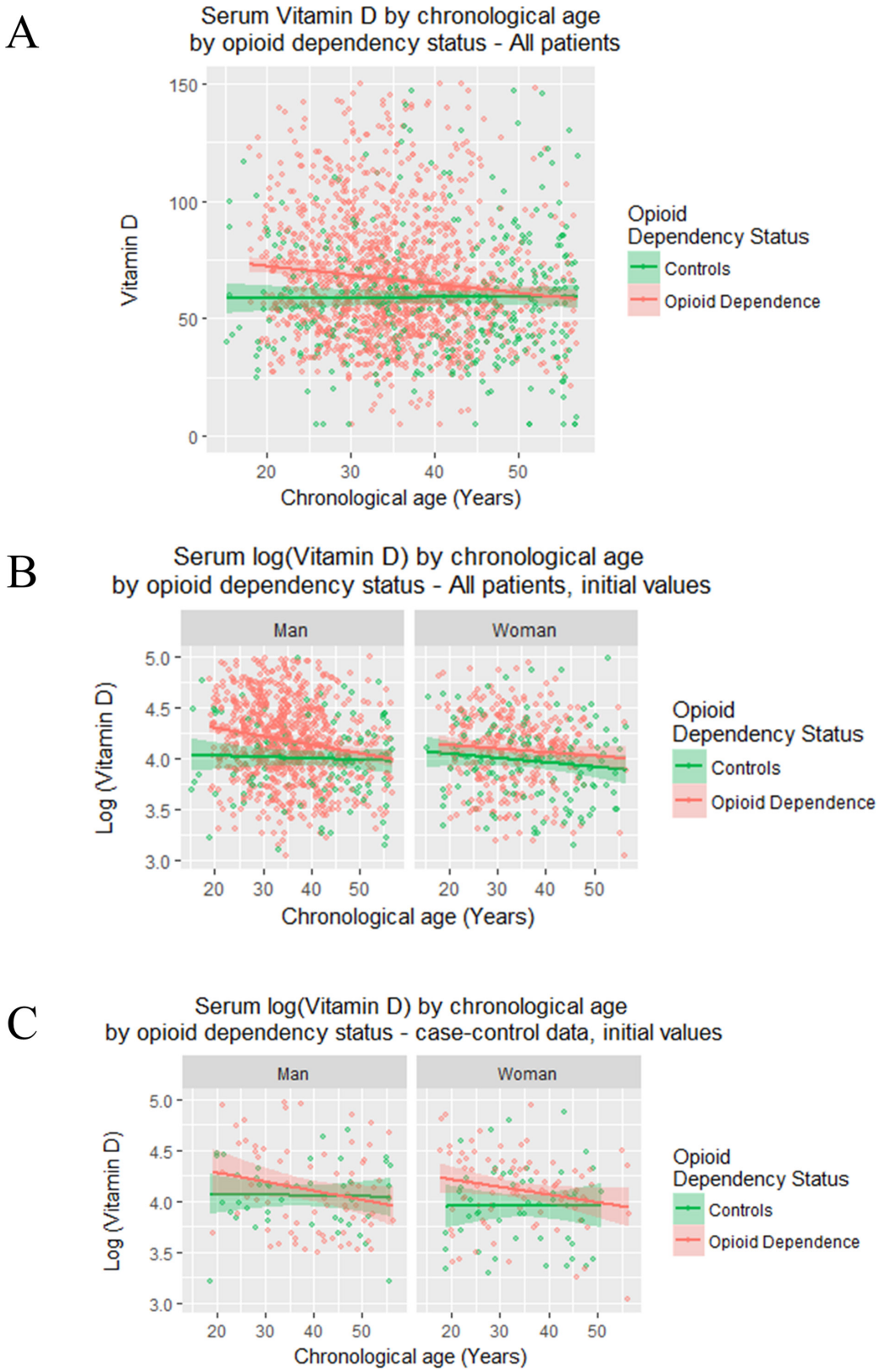

Figure 1 Vitamin D and log vitamin D by chronological age, opioid dependency and sex in (A) whole cohort, (B) initial values and in (C) case–control study.

of table 3. It was found to be superior to the linear model (log ratio=8.445, df=3, p=0.0377).

One notes that when sex is included in the model the age:time:status interaction is significant. Sex is included in one term in the final model. The age:status interaction is significant when all patients are considered together and in men and women separately. In women, this appears as an interaction between time, age and

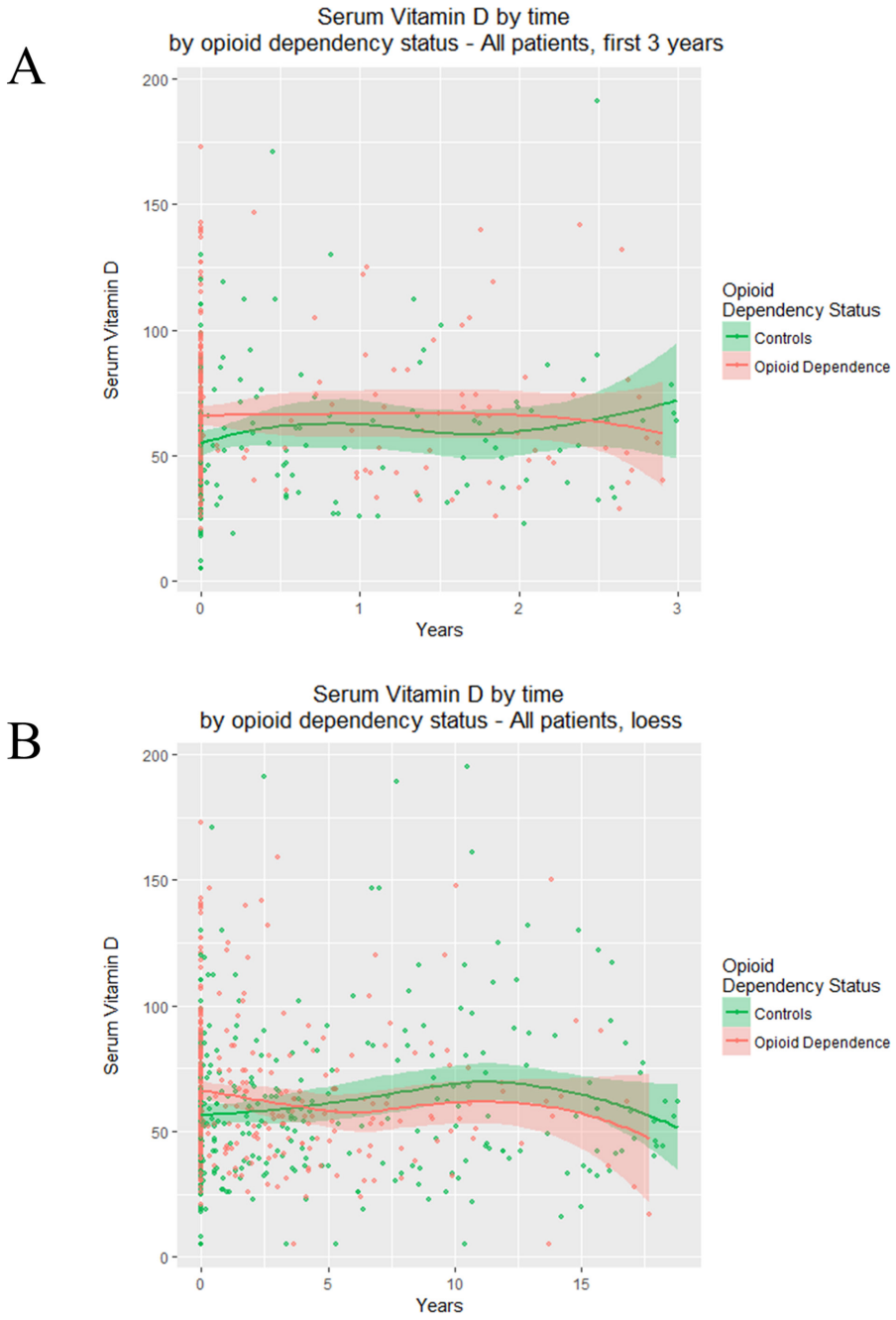

Figure 2 Serum vitamin D status by time in (A) first 3 years and (B) across whole period.

status. These results imply that vitamin D is a biomarker of age.

No correlation between vitamin D status and the calcium–phosphate solubility product in the whole sample was demonstrated (Pearson R=0.019, t=0.86, df=2044, p=0.39).

In previous studies, the serum globulins and ALT have been the most discriminative clinical pathological variables and these parameters also show marked age-dependent effects.[39 48 49] Therefore, these results were included in time-dependent mixed-effects linear regression models for all patients and for each sex separately. The outcomes of these analyses are shown in the lower part of table 3. In each case, the addictive status is significant.

The demonstration that ALT and serum globulins are close correlates of the elevated vitamin D in opioid dependence raises the question of the possible relationship of liver disease with this observation. Preliminary analyses showed that the hepatitis B serostatus was less discriminatory than hepatitis C serostatus, so the analysis in this section focused on hepatitis C. Note that in this study, technical factors may blur the definitions of the opioid dependence status and hepatitis C serostatus as in the present work, as elsewhere,[37–39 49–53] the opioid dependence status was defined in terms of the hepatitis serological results.

The results are as shown in figure 3 where the majority of the effect seems to reside with the hepatitis C-positive

Table 2 Age-dependent linear regression cross-sectional analyses

	Parameter estimates				Model			
	β-Estimate	SE	t Value	P value	F	Adj. R^2	df	P value
Case–control series								
Linear model								
All patients								
Age:status addicted	−0.4778	0.1768	−2.702	0.0073	6.83	0.0613	3, 265	0.0002
Status addicted	24.8561	11.0893	2.241	0.0258				
Quadratic model								
Status addicted	0.2634	0.0609	4.326	0.0000	0.0961	6.7000	5, 263	<0.0001
$(Age)^2$:status addicted	3.1664	1.0301	3.074	0.0023				
$(Age)^2$	−2.5316	0.8370	−3.025	0.0027				
Men								
Age:status control	−0.4232	0.2288	−1.85	0.0668	2.6300	0.02524	2, 124	0.0760
Women								
Age	−0.0145	0.0038	−3.788	0.0002	9.6000	0.0664	2, 139	0.0001
Age:status addicted	0.0071	0.0021	3.331	0.0011				
All patients								
Linear model								
Status addicted	0.4547	0.0852	5.337	<0.0001	0.0585	33.75	3, 1579	<0.0001
Age:status addicted	−0.0071	0.0016	−4.505	<0.0001				
Quadratic model								
Status addicted	0.2112	0.0321	6.572	<0.0001	0.0593	20.95	5, 1577	<0.0001
Age:status addicted	−4.1647	1.3631	−3.055	0.00229				
Men								
Status addicted	0.5628	0.1195	4.709	<0.0001	0.0599	23.52	31, 057	<0.0001
Age:status addicted	−0.0083	0.0019	−4.423	<0.0001				
Women								
Status addicted	0.1723	0.0400	4.308	<0.0001	0.03261	18.56	1, 520	<0.0001

group. This is formally confirmed by regression analysis results shown in the first segment of table 4. However, as shown in the lower panels of figure 3, some of the data for the hepatitis C serostatus is apparently non-linear. Therefore, low-order models polynomial in age were compared with the linear model. The results of the model quadratic in age are shown in the lower section of table 4. Formal model comparison using ANOVA testing confirmed that the model quadratic in age was superior to the linear model (log ratio 19.83 on 5 df, p=0.0013). A similar model quadratic in time failed to converge. A model quadratic in both age and time also failed to converge.

A complete mixed-effects model of the multiple regression correlates of serum vitamin D was therefore constructed including age, time, ALT, globulins and hepatitis serostatus were included in the model and reduced by the classical model reduction procedure. Because of the superiority of the quadratic model in the more streamlined analysis described in the preceding paragraph, this model included a term quadratic in age. Sex was not included in the final model as it was not significant in the applicable preceding model as noted above. The final model derived by this procedure is shown in the third section of table 4. In this final model, the only parameter which was independently correlated with serum vitamin D was the serum ALT level. No quadratic term in age was significant in the final model. Interestingly, notwithstanding the inclusion of both globulins and ALT, the hepatitis C serostatus was significantly correlative and was included in 8 of the 12 terms in the final model. Terms including hepatitis C seropositivity were significant from p<0.0001 and terms including hepatitis C seronegativity were significant from p=0.0475.

This final model was shown to be superior to the preceding quadratic model without biochemical parameters included by ANOVA testing (log ratio=19.53 on df=9, p=0.0021).

DISCUSSION

The main results of this prospective longitudinal study were that the serum vitamin D levels in ODPs were higher than that in the general medical controls both as a mean level overall and in multiple regression

Table 3 Mixed effects final longitudinal models

	Parameter					Model		
Variable	**Value**	**SE**	**df**	**t Value**	**p Value**	**AIC**	**BIC**	**logLik**
Case–control series								
Linear model								
Status addicted	0.3814	0.1121	118	3.4025	0.0009	1046.232	1077.646	−516.1161
Age:status addicted	−0.0062	0.0026	118	−2.3991	0.0180			
Days	0.0001	0.0000	118	2.3023	0.0231			
Age:days	0.0000	0.0000	118	−2.2675	0.0252			
Quadratic model								
Status addicted	0.1798	0.0497	113	3.6176	0.0004	1077.582	1131.343	−526.7911
$(Age)^2$:days:status addicted	−0.0017	0.0005	113	−3.1549	0.0021			
Age:days:status addicted	0.0026	0.0009	113	2.8283	0.0055			
Days:status addicted	−0.0001	0.0000	113	−2.7344	0.0073			
Age:status addicted	−2.3528	0.8716	113	−2.6995	0.0080			
Age:days	−0.0009	0.0004	113	−2.5102	0.0135			
Days	0.0000	0.0000	113	2.1706	0.0321			
All patients								
Status addicted	0.4253	0.0588	514	7.2353	0.0000	2737.466	2776.991	−1361.733
Age:status addicted	−0.0072	0.0015	514	−4.8060	0.0000			
Days:status addicted	−0.0003	0.0001	514	−4.7578	0.0000			
Age:days	0.0000	0.0000	514	3.7269	0.0002			
All patients including sex								
Status addicted	0.3496	0.0637	512	5.4913	0.0000	2745.271	2796.080	−1363.636
Age:status addicted	−0.0072	0.0015	512	−4.7819	0.0000			
Age:days:status addicted	0.0000	0.0000	512	−4.4102	0.0000			
Sex man:status addicted	0.1127	0.0289	512	3.9009	0.0001			
Age:days	0.0000	0.0000	512	3.6454	0.0003			
Men								
Age:status addicted	−0.0084	0.0018	317	−4.6726	0.0000	1809.922	1851.731	−896.9608
Status addicted	0.4560	0.1124	317	4.0554	0.0001			
Days:status control	0.0002	0.0001	317	2.7606	0.0061			
Days:status addicted	−0.0001	0.0001	317	−2.7125	0.0070			
Women								
Age:days:status addicted	0.0000	0.0000	193	−3.3598	0.0009	984.101	1011.518	−486.0503
Status addicted	0.1027	0.0381	193	2.6984	0.0076			
Age:days	0.0000	0.0000	193	2.5055	0.0131			
All patients including biochemistry								
ALT:status addicted	0.1022	0.0191	442	5.3506	0.0000	2641.788	2703.304	−1309.894
Age:ALT:status addicted	−0.0018	0.0004	442	−4.4320	0.0000			
Age:days:ALT:globulin:status addicted	0.0000	0.0000	442	−3.3350	0.0009			
Age:days:ALT:globulin:Status control	0.0000	0.0000	442	2.8871	0.0041			
ALT:globulin:status control	−0.0107	0.0050	442	−2.1345	0.0334			
Males with biochemistry								
ALT:status addicted	0.1022	0.0209	281	4.8779	0.0000	1862.039	1924.291	−919.0196
Age:ALT:status addicted	−0.0020	0.0005	281	−4.3398	0.0000			
Days:ALT:status addicted	−0.0001	0.0000	281	−3.9903	0.0001			
Days:ALT	0.0001	0.0000	281	2.8297	0.0050			
Age:days:ALT:globulin	0.0000	0.0000	281	−2.3787	0.0180			

Continued

Table 3 Continued

Variable	Parameter					Model		
	Value	SE	df	t Value	p Value	AIC	BIC	logLik
Days:ALT:globulin	0.0003	0.0001	281	2.3190	0.0211			
Age:days:globulin	0.0000	0.0000	281	2.3006	0.0221			
Days:globulin	−0.0010	0.0005	281	−2.2099	0.0279			
Females with biochemistry								
Globulin:status addicted	0.2158	0.0669	150	3.2246	0.0015	979.508	1037.669	−476.754
Age:globulin:status addicted	−0.0076	0.0024	150	−3.1375	0.0021			
Age:dd:ALT:globulin:status addicted	0.0000	0.0000	150	−2.9772	0.0034			
ALT:globulin	−0.0394	0.0139	150	−2.8385	0.0052			
Age:ALT:globulin:status addicted	0.0016	0.0006	150	2.8339	0.0052			
Age:days:ALT	0.0000	0.0000	150	2.5096	0.0132			
Age:days	0.0000	0.0000	150	−2.3426	0.0205			

AIC, Akaike Information Criterion; ALT, alanine aminotransferase; BIC, Bayesian Information Crieterion; DD, days; logLik, Log Likelihood ratio.

models by dependency status and/or by the age: dependency status interaction in both sexes. The advance in the modelled vitamin D level with age was shown to be 8.47%. The vitamin D level did not change with age in the control group, but it did fall significantly with age in the opioid-dependent group, making it a negative biomarker of ageing in this group. It was also shown to be significantly correlated with ALT, a well-recognised biomarker of age. Many differences exist between the ODPs and GMCs, however the effect appeared to be robust to adjustment for many of these features. While hepatitis C was an important predictor of the vitamin D level, it did not account for the effect of opiate dependency, as it was also observed in the HCV seronegative group. On multivariate analysis, vitamin D level was found to be significantly associated with interactive terms including ALT and measures of immune function such as serum globulins. The reasons for the higher levels of vitamin D in the opioid-dependent group are not clear and the present work is not designed as a mechanistic exploration. Unpublished data from this cohort show that the socioeconomic profile of the ODP and GMC groups is very different, with many more tradesmen and welfare-dependents in the ODP group which might be expected to have higher and lower than normal sun exposure, respectively. The primary analyte in this study was 25-hydroxyvitamin D. As the 25-hydroxylation reaction occurs photolytically in the skin, it may be that the higher vitamin D level in the ODP in this study reflects increased occupational exposure. It should be noted that the study was conducted in Queensland, Australia which has such a high incidence of skin malignancy that it is commonly referred to as the 'melanoma capital of the world'.[54] Opioids are well known to have various endocrine[35 50 53 55–57] and immune potentiating actions[35–38 49 51 58] which have been addressed elsewhere and it is possible that indirect effects on vitamin D metabolism may be mediated through such pathways. Moreover, the relationship with vitamin D status and the square of chronological age was fascinating and suggests a positive feed forward process as has been found for arterial stiffness in various drug dependencies.[7 59 60]

Although both elevated levels of vitamin D on the one hand (present report) and calcium and phosphate and their solubility products on the other hand[22] have been noted in our patients in opioid dependency, as no correlation was established between the two sets of parameters, it is unlikely based on the present analysis that a direct relationship exists between them. However, as the vitamin D-binding globulin (VDBG) has not been measured in the present work, it may be that this provides the computational and mechanistic 'missing link' between the two groups of data. Moreover as the vitamin D binding globulin (VDBG, Group Specific Component Globulin, gc component, gc globulin) is generated in the liver[61 62] and liver dysfunction has been well described both in opioid dependency and in HCV infection, this is an important issue for future workers in this area. At the time of writing however, we are not aware that such assays are available in this country. Parallel analyses have found that this situation is of particular relevance to the case of the circulating levels of sex hormones and their binding globulin in men and women in our cohort[53] and it may well be the case therefore in the case of vitamin D physiology. It is important to underscore that the highly significant results obtained in the present analysis for the patients with hepatitis C seronegative opioid dependency imply that hepatic dysfunction alone is not likely to account for the observations reported herein. Biomarkers of ageing have been reported to be derived from any variable which changes with age.[39] Such biomarkers can change in either a positive or negative direction with age. Both ALT and globulins rise dramatically with age and so have been described as positive age-related

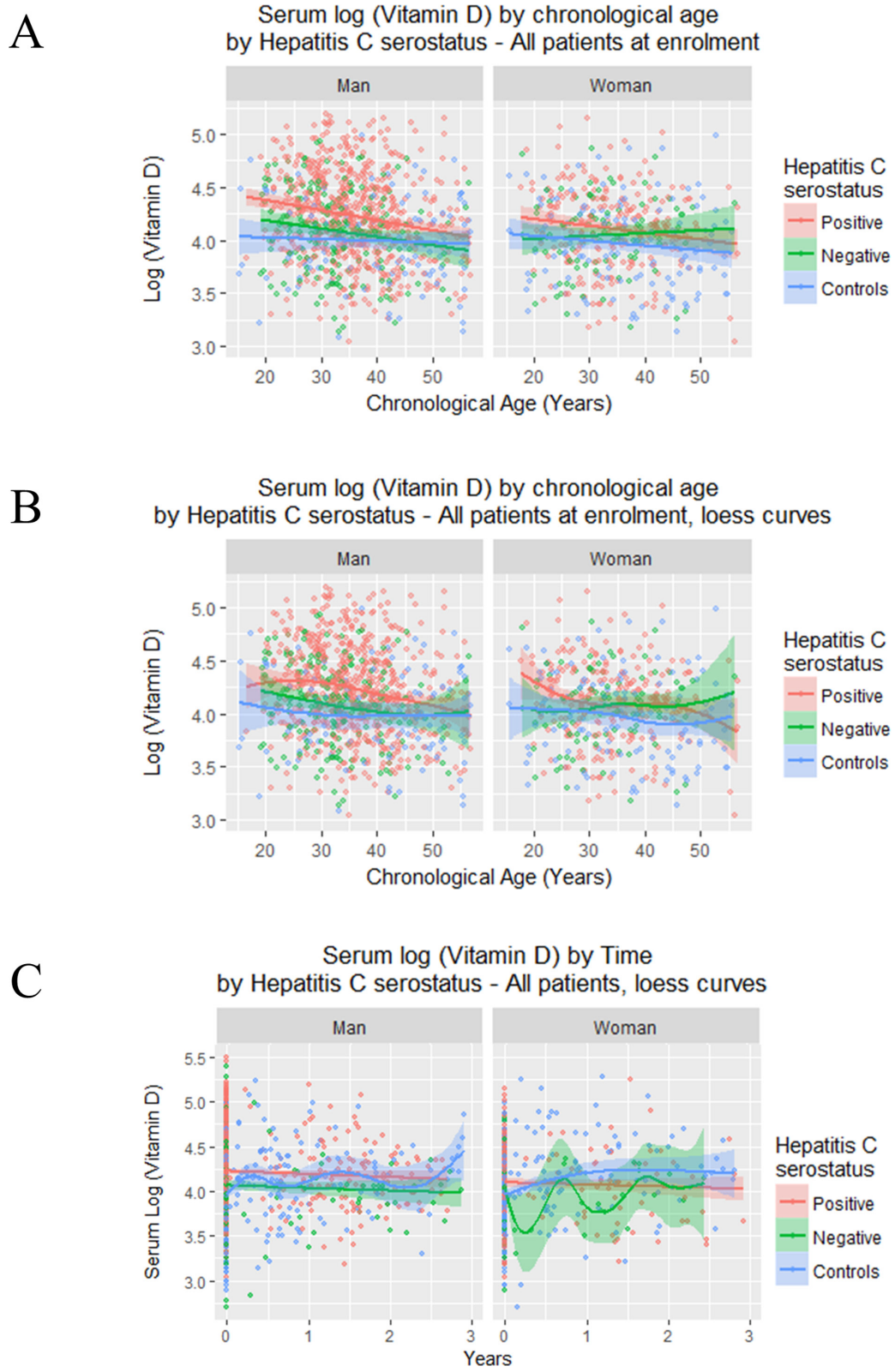

Figure 3 Logarithm vitamin D by hepatitis C serostatus by: (A) age and sex using linear lines of best fit; (B) age and sex using loess (localised polynomial) curves of best fit and (C) over the first 3 years by sex.

biomarkers.[37 38 49 51 63] In the ODPs described in the present study, vitamin D levels fell with age which would make vitamin D a negative biomarker of age in the drug-dependent cohort (tables 2–4, figures 1 and 3A,B and see the online supplementary figure 1). Moreover, vitamin D status was also shown to correlate with ALT in drug dependence which is a well-established biomarker of ageing.

Interestingly very high levels of osteoporosis/osteopaenia have been noted in ODP groups by several

Table 4 Mixed effects final longitudinal models by hepatitis C serostatus

	Parameter					Model		
Variable	**Value**	**SE**	**df**	**t Value**	**P value**	**AIC**	**BIC**	**logLik**
Case-control series								
Linear model								
Status addicted	0.3814	0.1121	118	3.4025	0.0009	1046.232	1077.646	−516.1161
Age: status addicted	−0.0062	0.0026	118	−2.3991	0.0180			
Days	0.0001	0.0000	118	2.3023	0.0231			
Age: days	0.0000	0.0000	118	−2.2675	0.0252			
Quadratic model								
Status addicted	0.1798	0.0497	113	3.6176	0.0004	1077.582	1131.343	−526.7911
$(Age)^2$: days: status addicted	−0.0017	0.0005	113	−3.1549	0.0021			
Age: days: status addicted	0.0026	0.0009	113	2.8283	0.0055			
Days: status addicted	−0.0001	0.0000	113	−2.7344	0.0073			
Age: status addicted	−2.3528	0.8716	113	−2.6995	0.0080			
Age: days	−0.0009	0.0004	113	−2.5102	0.0135			
Days	0.0000	0.0000	113	2.1706	0.0321			
All patients								
Status addicted	0.4253	0.0588	514	7.2353	0.0000	2737.466	2776.991	−1361.733
Age: status addicted	−0.0072	0.0015	514	−4.8060	0.0000			
Days: status addicted	−0.0003	0.0001	514	−4.7578	0.0000			
Age: days	0.0000	0.0000	514	3.7269	0.0002			
All patients including sex								
Status addicted	0.3496	0.0637	512	5.4913	0.0000	2745.271	2796.080	−1363.636
Age: status addicted	−0.0072	0.0015	512	−4.7819	0.0000			
Age: days: status addicted	0.0000	0.0000	512	−4.4102	0.0000			
Sex male: status addicted	0.1127	0.0289	512	3.9009	0.0001			
Age: days	0.0000	0.0000	512	3.6454	0.0003			
Men								
Age: status addicted	−0.0084	0.0018	317	−4.6726	0.0000	1809.922	1851.731	−896.9608
Status addicted	0.4560	0.1124	317	4.0554	0.0001			
Days: status control	0.0002	0.0001	317	2.7606	0.0061			
Days: status addicted	−0.0001	0.0001	317	−2.7125	0.0070			
Women								
Age: days: status addicted	0.0000	0.0000	193	−3.3598	0.0009	984.101	1011.518	−486.0503
Status addicted	0.1027	0.0381	193	2.6984	0.0076			
Age: days	0.0000	0.0000	193	2.5055	0.0131			
All patients including biochemistry								
ALT: status addicted	0.1022	0.0191	442	5.3506	0.0000	2641.788	2703.304	−1309.894
Age: ALT: status addicted	−0.0018	0.0004	442	−4.4320	0.0000			
Age: days: ALT: globulin: status addicted	0.0000	0.0000	442	−3.3350	0.0009			
Age: days: ALT: globulin: status control	0.0000	0.0000	442	2.8871	0.0041			
ALT: globulin: status control	−0.0107	0.0050	442	−2.1345	0.0334			
Males with biochemistry								
ALT: status addicted	0.1022	0.0209	281	4.8779	0.0000	1862.039	1924.291	−919.0196
Age: ALT: status addicted	−0.0020	0.0005	281	−4.3398	0.0000			
Days: ALT: status addicted	−0.0001	0.0000	281	−3.9903	0.0001			
Days: ALT	0.0001	0.0000	281	2.8297	0.0050			

Continued

Table 4 Continued

	Parameter					Model		
Variable	Value	SE	df	t Value	P value	AIC	BIC	logLik
Age: days: ALT: globulin	0.0000	0.0000	281	−2.3787	0.0180			
Days: ALT: globulin	0.0003	0.0001	281	2.3190	0.0211			
Age: days: globulin	0.0000	0.0000	281	2.3006	0.0221			
Days: globulin	−0.0010	0.0005	281	−2.2099	0.0279			
Females with biochemistry								
Globulin: status addicted	0.2158	0.0669	150	3.2246	0.0015	979.508	1037.669	−476.754
Age: globulin: status addicted	−0.0076	0.0024	150	−3.1375	0.0021			
Age: dd: ALT: globulin: status addicted	0.0000	0.0000	150	−2.9772	0.0034			
ALT: globulin	−0.0394	0.0139	150	−2.8385	0.0052			
Age: ALT: globulin: status addicted	0.0016	0.0006	150	2.8339	0.0052			
Age: days: ALT	0.0000	0.0000	150	2.5096	0.0132			
Age: days	0.0000	0.0000	150	−2.3426	0.0205			

AIC, Akaike Information Criterion; ALT, alanine aminotransferase; BIC, Bayesian Information Crieterion; log Lik, Log Likelihood ratio.

authors.[5 64–66] Clearly the higher levels of vitamin D seen in this study are not in accordance with such a finding. However, an inverse effect may be mediated by either a higher level of VDBG or a block to the metabolism of vitamin D to the active form 1,25-dihydroxycholecalciferol. Such a finding must await further studies. Moreover, the generally immunologically activated milieu of opiate dependence is now increasingly well characterised.[35 37 39 49 63] It may be that the immune active environment, with higher levels of interleukin-1, interleukin-6, tumor necrosis factor-alpha and Monocyte Chemotactic Protein 1/Chemokine (C-C motif) Ligand 2 (MCP-1/CCL2) among other key cytokines,[67] is the dominant force acting on bone mineralisation and overwhelms any relatively minor effect related to vitamin D metabolism. Interestingly melanocortin receptor-1 has been identified in the skin.[68] P53-induced photoactivation of pro-opiomelanocortin synthesis has been shown to be linked with cutaneous β-endorphin release, elevated pain thresholds and naloxone-inducible withdrawal after sun exposure. It is therefore potentially possible that ODPs may be self-medicating the possibility of withdrawal by elevating their rate of sun exposure.[69] Further intriguing conceptual possibilities emerge. Opioid dependence is characterised by a subtle disruption of normal metabolism[39 70] to the extent where patients have been compared with prediabetics.[50 56 71 72] Moreover, and in common with many addictions, opioid dependence is characterised by a marked immune stimulation[36 37 39 58 73] and simulation or at least phenocopying of the ageing process.[6 7 9 39 45 74] It turns out that nuclear hormone receptors (NHRs) of various classes facilely integrate such immunometabolic signalling.[75–80] NHRs of the oestrogen, androgen, pregnane, peroxisome proliferator activator receptor, liver X receptor and rexinoids are involved along with the vitamin D receptor.[75 78 79] Importantly, there is significant heterodimerisation and apparent promiscuity with many of these receptors[81–83] including the vitamin D receptor[84–90] and involvement of these pathways in diverse cell processes including stem cell regeneration,[91–96] atherogenesis[78 97–101] and cancer.[83 102–106] As altered metabolism, immunosenescence, cancerogenesis and stem cell failure are all well described in the ageing literature,[28 29 107–109] it would appear that pathophysiologically, phenotypically and clinically important processes may be impacted by the changes reported in the present paper.

This study had various strengths and limitations. The large sample size, prospective design, longitudinal nature and real-world sampling for the groups were major strengths. As the study was taken from 'real-world participants' and as the opioid use has previously been shown to be typical of that reported in many other clinical series, we feel that these results may be generalisable to opioid-dependent populations elsewhere. As the drug use data were not available in these patients, it was not possible to compare drug use levels with vitamin D status or calculate dose–response relationships. Similarly, anthropometric including body mass index data and sun exposure information is not available. The extent of vitamin D supplementation used by patients is also unknown, but it is believed that its use would be more widespread among controls than in ODP, thereby acting in the reverse direction. Socioeconomic and occupational data were also not available to the present study. While every attempt has been made to adjust the findings for measured confounding variables, the involvement of unmeasured confounding in the present results is not known. Nevertheless, the robustness of the present findings to various data manipulations including longitudinal and multivariate adjustment suggests that the finding is genuine. These findings could be supported by future mechanistic and interventional studies. The unavailability of VDBG assay

or studies of vitamin D nuclear receptor to the present work were limitations.

In summary, the present work quantitated vitamin D status for the first time in a patient cohort dependent on illicit opioids and demonstrated higher vitamin D levels in the opioid-dependent group both as a group mean, in case–control and after adjustment for age, sex and selected laboratory markers in various linear regression models. The modelled level was 8.47% higher in the OPD than in controls. The effect was not simply attributable to hepatitis C infection. The cause of this elevation was not clear from the present report. Vitamin D levels fell with age in ODPs, making it a negative biomarker of ageing in this cohort. This finding is of interest due to the described involvement of NHRs (including vitamin D receptor) with inflammatory and metabolic pathways which may be of clinical significance and may relate to the well-described ageing phenotype observed in opioid-dependent populations. Future studies should consider including detailed parametric drug use histories, occupational exposure to sunlight and measurement of VDBG or vitamin D receptor activity and active vitamin D metabolites in further exploring this issue.

Contributors ASR: designed the study, performed the analysis, prepared the figures and wrote the first draft of the paper. GKH: wrote and the final draft and assisted with the statistical analysis.

Funding This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent Obtained.

Ethics approval The study was given ethical approval by the Human Research Ethics Committee of the Southcity Medical Centre which has been accredited by the National Health and Medical Research Centre.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement The data for this paper may be obtained from the authors upon specific request.

REFERENCES

1. Abelha-Aleixo J, Fonseca R, Bernardo A, *et al*. Vitamin D - immunomodulatory actions and new potentialities. *Acta Reumatol Port* 2014;39:355-6.
2. Bidulescu A, Morris AA, Stoyanova N, *et al*. Association between Vitamin D and Adiponectin and Its Relationship with Body Mass Index: The META-Health Study. *Front Public Health* 2014;2:193.
3. Stefanska B, Salamé P, Bednarek A, *et al*. Comparative effects of retinoic acid, vitamin D and resveratrol alone and in combination with adenosine analogues on methylation and expression of phosphatase and tensin homologue tumour suppressor gene in breast cancer cells. *Br J Nutr* 2012;107:781–90.
4. Stöcklin E, Eggersdorfer M. Vitamin D, an essential nutrient with versatile functions in nearly all organs. *Int J Vitam Nutr Res* 2013;83:92–100.
5. Kim TW, Alford DP, Malabanan A, *et al*. Low bone density in patients receiving methadone maintenance treatment. *Drug Alcohol Depend* 2006;85:258–62.
6. Cheng GL, Zeng H, Leung MK, *et al*. Heroin abuse accelerates biological aging: a novel insight from telomerase and brain imaging interaction. *Transl Psychiatry* 2013;3:e260.
7. Reece AS, Hulse GK. Impact of lifetime opioid exposure on arterial stiffness and vascular age: cross-sectional and longitudinal studies in men and women. *BMJ Open* 2014;4:e004521.
8. Reece AS. Differing age related trajectories of dysfunction in several organ systems in opiate dependence. *Aging Clin Exp Res* 2012;24:85–96.
9. Reece AS, Davidson P. Deficit of circulating stem–progenitor cells in opiate addiction: a pilot study. *Subst Abuse Treat Prev Policy* 2007;2:19–28.
10. Turner MK, Hooten WM, Schmidt JE, *et al*. Prevalence and clinical correlates of vitamin D inadequacy among patients with chronic pain. *Pain Med* 2008;9:979–84.
11. Wawrzyniak S, Mikołajewska E, Kuczko-Piekarska E, *et al*. Association of vitamin D status and clinical and radiological outcomes in a treated MS population in Poland. *Brain Behav* 2017;7:e00609.
12. *Vitamin D for the Treatment or Prevention of Multiple Sclerosis: a review of the clinical effectiveness*. Ottawa (ON: Health Canda, 2016. https://www.cadth.ca/vitamin-d-treatment-or-prevention-multiple-sclerosis-review- clinical-effectiveness.
13. Burton JM, Costello FE. Vitamin D in multiple sclerosis and central nervous system demyelinating disease–a review. *J Neuroophthalmol* 2015;35:194–200.
14. Tizaoui K, Kaabachi W, Hamzaoui A, *et al*. Association between vitamin D receptor polymorphisms and multiple sclerosis: systematic review and meta-analysis of case-control studies. *Cell Mol Immunol* 2015;12:243–52.
15. Bagur MJ, Murcia MA, Jiménez-Monreal AM, *et al*. Influence of Diet in Multiple Sclerosis: A Systematic Review. *Adv Nutr* 2017;8:463–72.
16. Xie Z, Chen J, Zheng C, *et al*. 1,25-dihydroxyvitamin D3 -induced dendritic cells suppress experimental autoimmune encephalomyelitis by increasing proportions of the regulatory lymphocytes and reducing T helper type 1 and type 17 cells. *Immunology* 2017;152:414–24.
17. Parnell GP, Booth DR. The Multiple Sclerosis (MS) Genetic Risk Factors Indicate both Acquired and Innate Immune Cell Subsets Contribute to MS Pathogenesis and Identify Novel Therapeutic Opportunities. *Front Immunol* 2017;8:425.
18. Bettencourt A, Boleixa D, Guimarães AL, *et al*. The vitamin D receptor gene FokI polymorphism and Multiple Sclerosis in a Northern Portuguese population. *J Neuroimmunol* 2017;309:34–7.
19. Jelinek GA. Determining Causation from Observational Studies: A Challenge for Modern Neuroepidemiology. *Front Neurol* 2017;8:265.
20. Jorde R. RCTS are the only appropriate way to demonstrate the role of vitamin D in health. *J Steroid Biochem Mol Biol* 2017: doi: 10.1016/j.jsbmb.2017.05.004. [Epub ahead of print 5 May 2017].
21. Grey A, Rix-Trott K, Horne A, *et al*. Decreased bone density in men on methadone maintenance therapy. *Addiction* 2011;106:349–54.
22. Reece AS. Absolute and age-dependent elevations of serum calcium and phosphate and their products in clinical opiate dependence. *J Subst Use* 2014;19:125–33.
23. Reece AS, Hulse GK. Impact of opioid pharmacotherapy on arterial stiffness and vascular ageing: cross-sectional and longitudinal studies. *Cardiovasc Toxicol* 2013;13:254–66.
24. Behmard S, Sadeghi A, Mohareri MR, *et al*. Positive association of opium addiction and cancer of the bladder. Results of urine cytology in 3,500 opium addicts. *Acta Cytol* 1981;25:142–6.
25. Mousavi MR, Damghani MA, Haghdoust AA, *et al*. Opium and risk of laryngeal cancer. *Laryngoscope* 2003;113:1939–43.
26. Masjedi MR, Naghan PA, Taslimi S, *et al*. Opium could be considered an independent risk factor for lung cancer: a case-control study. *Respiration* 2013;85:112–8.
27. Nellen JF, Smulders YM, Jos Frissen PH, *et al*. Hypovitaminosis D in immigrant women: slow to be diagnosed. *BMJ* 1996;312:570–2.
28. Hadley EC, Lakatta EG, Morrison-Bogorad M, *et al*. The future of aging therapies. *Cell* 2005;120:557–67.
29. Kirkwood TB. Understanding the odd science of aging. *Cell* 2005;120:437–47.
30. Adamczak DM, Nowak JK, Frydrychowicz M, *et al*. The role of Toll-like receptors and vitamin D in diabetes mellitus type 1–a review. *Scand J Immunol* 2014;80:75–84.
31. Aguilar-Jiménez W, Feria MG, Arcia ED, *et al*. Molecules Involved in the Vitamin-D Pathway Correlate with Higher mRNA Expression of

Anti-HIV Molecules in HIV Exposed Seronegative Individuals. *AIDS Res Hum Retroviruses* 2014;30(Suppl 1):A100.
32. Aguilar-Jiménez W, Zapata W, Caruz A, *et al*. Variants in Vitamin D Pathway and Antiviral Response Genes Interact to Modulate the Natural Resistance to HIV-1 Infection. *AIDS Res Hum Retroviruses* 2014;30(Suppl 1):A217–18.
33. Giangreco AA, Dambal S, Wagner D, *et al*. Differential expression and regulation of vitamin D hydroxylases and inflammatory genes in prostate stroma and epithelium by 1,25-dihydroxyvitamin D in men with prostate cancer and an in vitro model. *J Steroid Biochem Mol Biol* 2015;148:156–65.
34. Kongsbak M, von Essen MR, Levring TB, *et al*. Vitamin D-binding protein controls T cell responses to vitamin D. *BMC Immunol* 2014;15:35.
35. Brunton LL, Lazo JS, Parker KL. eds. *Goodman and Gilman's the Pharmacologic Basis of Therapeutics. Eleventh Edition ed*. New York: McGraw Hill, 2006.
36. Reece AS. Chronic immune stimulation as a contributing cause of chronic disease in opiate addiction including multi-system ageing. *Med Hypotheses* 2010;75:613–9.
37. Reece AS. High-sensitivity CRP in opiate addiction: relative and age-dependent elevations. *Cardiovasc Toxicol* 2012;12:149–57.
38. Reece AS. Epidemiologic and molecular pathophysiology of chronic opioid dependence and the place of naltrexone extended-release formulations in its clinical management. *Subst Abuse* 2012;6:SART.S9031.
39. Reece AS. Evidence of accelerated ageing in clinical drug addiction from immune, hepatic and metabolic biomarkers. *Immun Ageing* 2007;4:6–15.
40. Claesson MJ, Jeffery IB, Conde S, *et al*. Gut microbiota composition correlates with diet and health in the elderly. *Nature* 2012;488:178–84.
41. Takahashi K, Sugi Y, Nakano K, *et al*. Epigenetic control of the host gene by commensal bacteria in large intestinal epithelial cells. *J Biol Chem* 2011;286:35755–62.
42. Vijay-Kumar M, Aitken JD, Carvalho FA, *et al*. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. *Science* 2010;328:228–31.
43. Foster JA, McVey Neufeld KA. Gut-brain axis: how the microbiome influences anxiety and depression. *Trends Neurosci* 2013;36:305–12.
44. Reece S. Dental health in addiction. *Aust Dent J* 2009;54:185–6.
45. Reece AS. Dentition of addiction in Queensland: poor dental status and major contributing drugs. *Aust Dent J* 2007;52:144–9.
46. Reece AS. Hair graying in substance addiction. *Arch Dermatol* 2007;143:115–8.
47. López-Otín C, Blasco MA, Partridge L, *et al*. The hallmarks of aging. *Cell* 2013;153:1194–217.
48. Reece AS. Chronic hepatitis as an important contributor to the immunosenescence of parenteral drug addiction. *Addiction Biology* 2008;14:214–26.
49. Reece AS. Relative and age-dependent stimulation of soluble and cellular immunity in opiate dependence. *J Addict Med* 2012;6:10–17.
50. Reece AS. Manifold implications of forgotten hyperglycemia in clinical opiate dependence. *Drug Chem Toxicol* 2013;36:55–66.
51. Reece AS. Chronic viral hepatitis is a significant contributor to the immunosenescent phenotype of parenteral drug addiction. *Addict Biol*2009;14:214–26.
52. Reece AS. Clinical implications of addiction related immunosuppression. *J Infect* 2008;56:437–45.
53. Reece AS, Thomas MR, Norman A, *et al*. Dramatic acceleration of reproductive aging, contraction of biochemical fecundity and healthspan-lifespan implications of opioid-induced endocrinopathy-FSH/LH ratio and other interrelationships. *Reprod Toxicol* 2016;66:20–30.
54. University of Sydney. *Help for melanoma patients*. Sydney: University of Sydney, 2013, http://sydney.edu.au/news/84.html?newsstoryid=11465 (accessed 8 Nov 2014).
55. Reece AS, Hulse GK. Hypothalamic Pathophysiology in the Neuroimmune, Dysmetabolic and Longevity Complications of Chronic Opiate Dependency. *J Forensic Toxicology and Pharmacology* 2014;3:3–46.
56. Ceriello A, Quatraro A, Giugliano D. Opiate addict as diabetic patient? *Diabetes Care* 1988;11:443.
57. Reece AS, Hulse GK. Elevation of the ACTH/cortisol ratio in female opioid dependent patients: A biomarker of aging and correlate of metabolic and immune activation. *Neuro Endocrinol Lett* 2016;37:325–36.
58. Hutchinson MR, Shavit Y, Grace PM, *et al*. Exploring the neuroimmunopharmacology of opioids: an integrative review of mechanisms of central immune signaling and their implications for opioid analgesia. *Pharmacol Rev* 2011;63:772–810.
59. Reece AS, Norman A, Hulse GK. Acceleration of cardiovascular-biological age by amphetamine exposure is a power function of chronological age. *Heart Asia* 2017;9:30–8.
60. Reece AS, Norman A, Hulse GK. Cannabis exposure as an interactive cardiovascular risk factor and accelerant of organismal ageing: a longitudinal study. *BMJ Open*. 2016;6:e011891.
61. Cooke NE, Haddad JG. Vitamin D binding protein (Gc-globulin). *Endocr Rev* 1989;10:294–307.
62. Haddad JG, Walgate J. 25-Hydroxyvitamin D transport in human plasma. Isolation and partial characterization of calcifidiol-binding protein. *J Biol Chem* 1976;251:4803–9.
63. Reece AS. Epidemiologic and molecular pathophysiology of chronic opioid dependence and the place of naltrexone extended-release formulations in its clinical management. *Subst Abuse* 2012;6:115–33.
64. Arnsten JH, Freeman R, Howard AA, *et al*. Decreased bone mineral density and increased fracture risk in aging men with or at risk for HIV infection. *AIDS* 2007;21:617–23.
65. Pedrazzoni M, Vescovi PP, Maninetti L, *et al*. Effects of chronic heroin abuse on bone and mineral metabolism. *Acta Endocrinol* 1993;129:42–5.
66. Shorr RI, Griffin MR, Daugherty JR, *et al*. Opioid analgesics and the risk of hip fracture in the elderly: codeine and propoxyphene. *J Gerontol* 1992;47:M111–15.
67. Neri S, Bruno CM, Pulvirenti D, *et al*. Randomized clinical trial to compare the effects of methadone and buprenorphine on the immune system in drug abusers. *Psychopharmacology* 2005;179:700–4.
68. Swope VB, Jameson JA, McFarland KL, *et al*. Defining MC1R regulation in human melanocytes by its agonist α-melanocortin and antagonists agouti signaling protein and β-defensin 3. *J Invest Dermatol* 2012;132:2255–62.
69. Fell GL, Robinson KC, Mao J, *et al*. Skin β-endorphin mediates addiction to UV light. *Cell* 2014;157:1527–34.
70. Cooper OB, Brown TT, Dobs AS. Opiate drug use: a potential contributor to the endocrine and metabolic complications in human immunodeficiency virus disease. *Clin Infect Dis* 2003;37(Suppl 2):S132–6.
71. Passariello N, Giugliano D, Ceriello A, *et al*. Impaired insulin response to glucose but not to arginine in heroin addicts. *J Endocrinol Invest* 1986;9:353–7.
72. Passariello N, Giugliano D, Quatraro A, *et al*. Glucose tolerance and hormonal responses in heroin addicts. A possible role for endogenous opiates in the pathogenesis of non-insulin-dependent diabetes. *Metabolism* 1983;32:1163–5.
73. Cabral GA. abuse D immune modulation, and AIDS. *J Neuroimmune Pharmacol* 2006;1:280–95.
74. Bachi K, Sierra S, Volkow ND, *et al*. Is biological aging accelerated in drug addiction? *Curr Opin Behav Sci* 2017;13:34–9.
75. Chinetti G, Fruchart JC, Staels B. Transcriptional regulation of macrophage cholesterol trafficking by PPARalpha and LXR. *Biochem Soc Trans* 2006;34:1128–31.
76. Guillemot-Legris O, Mutemberezi V, Muccioli GG. Oxysterols in Metabolic Syndrome: From Bystander Molecules to Bioactive Lipids. *Trends Mol Med* 2016;22:594–614.
77. Kaul D. Molecular link between cholesterol, cytokines and atherosclerosis. *Mol Cell Biochem* 2001;219:65–71.
78. Manna PR, Sennoune SR, Martinez-Zaguilan R, *et al*. Regulation of retinoid mediated cholesterol efflux involves liver X receptor activation in mouse macrophages. *Biochem Biophys Res Commun* 2015;464:312–7.
79. Rigamonti E, Chinetti-Gbaguidi G, Staels B. Regulation of macrophage functions by PPAR-alpha, PPAR-gamma, and LXRs in mice and men. *Arterioscler Thromb Vasc Biol* 2008;28:1050–9.
80. Sallam T, Jones MC, Gilliland T, *et al*. Feedback modulation of cholesterol metabolism by the lipid-responsive non-coding RNA LeXis. *Nature* 2016;534:124–8.
81. Cave MC, Clair HB, Hardesty JE, *et al*. Nuclear receptors and nonalcoholic fatty liver disease. *Biochim Biophys Acta* 2016;1859:1083–99.
82. Baker AH, Watt J, Huang CK, *et al*. Tributyltin engages multiple nuclear receptor pathways and suppresses osteogenesis in bone marrow multipotent stromal cells. *Chem Res Toxicol* 2015;28:1156–66.
83. Wu Y, Yu DD, Yan DL, *et al*. Liver X receptor as a drug target for the treatment of breast cancer. *Anticancer Drugs* 2016;27:373–82.
84. Jusakul A, Khuntikeo N, Haigh WG, *et al*. Identification of biliary bile acids in patients with benign biliary diseases, hepatocellular carcinoma and cholangiocarcinoma. *Asian Pac J Cancer Prev* 2012;13 Suppl:–77–82.

85. Kiss M, Czimmerer Z, Nagy L. The role of lipid-activated nuclear receptors in shaping macrophage and dendritic cell function: From physiology to pathology. *J Allergy Clin Immunol* 2013;132:264–86.
86. Krasowski MD, Ni A, Hagey LR, *et al*. Evolution of promiscuous nuclear hormone receptors: LXR, FXR, VDR, PXR, and CAR. *Mol Cell Endocrinol* 2011;334:39–48.
87. Kuver R. Mechanisms of oxysterol-induced disease: insights from the biliary system. *Clin Lipidol* 2012;7:537–48.
88. Széles L, Póliska S, Nagy G, *et al*. Research resource: transcriptome profiling of genes regulated by RXR and its permissive and nonpermissive partners in differentiating monocyte-derived dendritic cells. *Mol Endocrinol* 2010;24:2218–31.
89. vinh quốc Luong K, Nguyễn LT. The beneficial role of vitamin D in systemic lupus erythematosus (SLE). *Clin Rheumatol* 2012;31:1423–35.
90. Zhou H, Hylemon PB. Bile acids are nutrient signaling hormones. *Steroids* 2014;86:62–8.
91. Bijsmans IT, Milona A, Ijssennagger N, *et al*. Characterization of stem cell-derived liver and intestinal organoids as a model system to study nuclear receptor biology. *Biochim Biophys Acta* 2017;1863:687–700.
92. Kruse MS, Suarez LG, Coirini H. Regulation of the expression of LXR in rat hypothalamic and hippocampal explants. *Neurosci Lett* 2017;639:53–8.
93. Wang JZ, Fang Y, Ji WD, *et al*. LXR agonists promote the proliferation of neural progenitor cells through MEK-ERK pathway. *Biochem Biophys Res Commun* 2017;483:216–22.
94. Cimadamore F, Amador-Arjona A, Chen C, *et al*. SOX2-LIN28/let-7 pathway regulates proliferation and neurogenesis in neural precursors. *Proc Natl Acad Sci U S A* 2013;110:E3017–26.
95. Pinto CL, Kalasekar SM, McCollum CW, *et al*. Lxr regulates lipid metabolic and visual perception pathways during zebrafish development. *Mol Cell Endocrinol* 2016;419:29–43.
96. Theofilopoulos S, Arenas E. Liver X receptors and cholesterol metabolism: role in ventral midbrain development and neurodegeneration. *F1000Prime Rep* 2015;7:37.
97. Huwait EA, Singh NN, Michael DR, *et al*. Protein Kinase C Is Involved in the Induction of ATP-Binding Cassette Transporter A1 Expression by Liver X Receptor/Retinoid X Receptor Agonist in Human Macrophages. *J Cell Physiol* 2015;116:2032–8.
98. Jiang M, Li X. Activation of PPARγ does not contribute to macrophage ABCA1 expression and ABCA1-mediated cholesterol efflux to apoAI. *Biochem Biophys Res Commun* 2017;482:849–56.
99. Manna PR. Retinoid regulated macrophage cholesterol efflux involves the steroidogenic acute regulatory protein. *Data Brief* 2016;7:940–5.
100. Pourcet B, Gage MC, León TE, *et al*. The nuclear receptor LXR modulates interleukin-18 levels in macrophages through multiple mechanisms. *Sci Rep* 2016;6:25481.
101. Tran M, Wang L. Preserving LXR by inhibiting T39: A step closer to treating atherosclerosis and steatohepatitis? *Hepatology* 2017;65:741–4.
102. Courtaut F, Derangère V, Chevriaux A, *et al*. Liver X receptor ligand cytotoxicity in colon cancer cells and not in normal colon epithelial cells depends on LXRβ subcellular localization. *Oncotarget* 2015;6:26651–62.
103. Derangère V, Chevriaux A, Courtaut F, *et al*. Liver X receptor β activation induces pyroptosis of human and murine colon cancer cells. *Cell Death Differ* 2014;21:1914–24.
104. Lin CY, Vedin LL, Steffensen KR. The emerging roles of liver X receptors and their ligands in cancer. *Expert Opin Ther Targets* 2016;20:61–71.
105. Tsui KH, Chung LC, Feng TH, *et al*. Divergent effect of liver X receptor agonists on prostate-specific antigen expression is dependent on androgen receptor in prostate carcinoma cells. *Prostate* 2015;75:603–15.
106. Wu Y, Yu DD, Hu Y, *et al*. LXR ligands sensitize EGFR-TKI-resistant human lung cancer cells in vitro by inhibiting Akt activation. *Biochem Biophys Res Commun* 2015;467:900–5.
107. Campisi J. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. *Cell* 2005;120:513–22.
108. Chien KR, Karsenty G. Longevity and lineages: toward the integrative biology of degenerative diseases in heart, muscle, and bone. *Cell* 2005;120:533–44.
109. Lombard DB, Chua KF, Mostoslavsky R, *et al*. DNA repair, genome stability, and aging. *Cell* 2005;120:497–512.