World Journal of Cardiovascular Diseases, 2013, 3, 361-370 **WJCD**
http://dx.doi.org/10.4236/wjcd.2013.35056 Published Online August 2013 (http://www.scirp.org/journal/wjcd/)

Opiate exposure increases arterial stiffness, advances vascular age and is an independent cardiovascular risk factor in females: A cross-sectional clinical study*

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Received 11 May 2013; revised 11 June 2013; accepted 15 July 2013

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ABSTRACT

Background: Whilst several studies have demonstrated poor cardiovascular health in opiate dependence, its role as a cardiovascular risk factor has not been considered. Methods: Pulse wave analysis was undertaken by radial arterial tonometry (SphygmoCor) in female control and opiate-dependent patients and compared to lifetime opiate use. Results: 222 opiate dependent women were compared to 175 controls. Opiate dependent patients were receiving treatment with buprenorphine (83.3%), methadone (13.5%), or naltrexone (3.2%). Non log transformed chronologic age (CA) for the two groups was 33.58 ± 0.57 (opiate) vs. 32.62 ± 0.96 (controls) years (mean ± S.E.M.; P = 0.39). Vascular Reference Age (RA) 39.30 ± 1.28, vs. 35.03 ± 1.41 the RA-CA difference (5.73 ± 1.02 vs. 2.41 ± 0.91) and the RA/CA ratio (1.16 ± 0.03 vs. 1.07 ± 0.02; all P < 0.02), and all measurements of central arterial stiffness (P < 0.02) were significantly worse for opiates compared to controls. When adjusted for CA, RA and central augmentation pressure and index were all worse by themselves and in interaction with CA (all P < 0.005). At 60 years the modelled RA's were 83.79 and 67.52 years respectively. The opiate dose-duration interaction showed a dose-response effect with RA (P = 0.0033). After full adjustment for established cardiovascular risk factors, the dose-duration interaction remained significant ($P = 10^{-6}$), was included in 10 other terms, and dose or duration was included in 15 other interactions. Conclusion: These data show that lifetime opiate use is significantly associated with increased arterial stiffness and vascular age and suggest a dose-response relationship. This relationship is robust and persists after full multivariate adjustment. These findings carry far-reaching implications for opiate-induced generalized acceleration of organismal ageing.

Keywords: Arterial Stiffness; Heroin; Opiates; Vascular Ageing; Dependence; Human Ageing

1. INTRODUCTION

Opiate dependence which can arise from illegal drug use, inappropriate use of medically derived opiates, or iatrogenically as in the course of chronic pain management, is a major public health issue in modern western nations. Chronic pain is experienced by 90 million Americans over a lifetime, and is responsible for $100 billion in annual healthcare costs [1]. In 2004, it was estimated that 235 million scripts for opiates were prescribed. Indeed, it has been noted that in the USA in recent years overdoses with legal opiates outnumber those from heroin and cocaine combined [1]. Data from the Drug Abuse Warning Network suggested that from 1998-2009 non-heroin non-methadone opiate overdoses presenting to US Emergency Rooms rose from 2.2% of all ER presentations to 11.5% [2].

Whilst cardiovascular complications of amphetamine and cocaine addiction are well recognized, a small but increasing amount of empirical data exist linking long term opiate dependence with heart and vascular disease [3-5]. 17% of heroin dependent patients of 44 or more years of age in one study had coronary stenoses of more than 75% [6]. High rates of myocardial fibrosis, ventricular hypertrophy, interstitial fibrosis, perivascular fibrosis and severe coronary disease were identified in

*Conflict of Interest: Nil declared.
Sources of Funding: Nil.
Statement of Authorship: This work is the work of the authors in its entirety.
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another study; all worse in those patients treated with methadone [4]. In Australia, large state-wide autopsy population based study reviewing in excess of 20 years of opiate maintenance treatments from Sydney, showed a relative risk of cardiovascular disease of 2.2 (95% C.I. 1.8 - 2.7, Appendix 6) [7]. A careful angiographic study of 2405 patients showed that opium use was significantly related to coronary disease with odds ratio 1.8 (C.I. 1.8 - 4.7, P = 0.01), including a dose response effect with disease severity (P = 0.002) [8]. This relationship persisted when only non-smokers were considered (P < 0.001). These workers further showed that the coronary disease presents four years earlier in opium users [9]. Most of this literature however does not report on gender specific outcomes.

The results of various genome wide association studies (GWAS) in coronary disease [10-13] have identified a "hotspot" on the senescence locus at chromosome 9p21.3, a site which is adjacent to genes CDKN2A and CDKN2B which code for P16INK4A, P15INK4B and P19ARF, and actually maps to the long non-protein coding senescence-associated RNA called ANRIL [14]. ANRIL has been shown to interact in cis with the promoter for CDKN2A (coding for P16), and to act via γ-interferon [15,16]. P16 activation is known to be associated with senescence induction in many tissues [17,18]. Such tissues secrete various factors including pro-inflammatory cytokines which further maintain and induce the senescent state [19]. It is of great interest therefore to note that opiates have long been known to interfere with tissue growth [20,21] by an effect which has been shown to be mediated by P16 and P21 [22,23]. Indeed, it has been shown that most tissues are under a normal tonic endorphin/enkephalin mediated negative growth suppression, which can be unmasked pharmacologically by opiate antagonists, or mechanistically with appropriate targeted siRNA's directed against the perinuclear receptor which mediates these activities called the opiate growth factor receptor [24].

Pulse Wave Analysis (PWA) by radial arterial tonometry is a technique which has been widely used in recent years to ascertain central arterial function, arterial stiffness and vascular compliance. It is able to differentiate the forward pressure wave originating from the heart from the backward projected pressure wave originating from peripheral resistance sites. The speed and amplitude of the reflected wave are a function of the stiffness of the arterial system, which in turn has been related to age in large population based studies. The SphygmoCor system automatically generates a vascular reference age (VA or RA) from the data output. The technique has several advantages in research into vascular health in opiate dependent persons, particularly, that it becomes sensitive to changes early in life in the third and fourth decade, prior to the time when many other vascular function tests become discriminative and is the age of most of our drug dependent cohort. PWA is also rapid, and can readily be repeated on subjects who re-present at a later time.

Importantly, cardiovascular ageing has been found to account for more than half the effect of aging in western populations [25]. For this reason, the importance of such studies extends well beyond its implications for cardiovascular medicine, and suggests that a demonstration of advanced vascular age is actually a surrogate marker for generalized organismal ageing.

As this clinic sees both general medical and opiate dependent patients and has experience with the PWA technique, we were ideally placed to formally test whether long term opiate dependence is associated with increased vascular stiffness and central arterial ageing. Data in males, in longitudinal studies and by pharmacological treatment types is presented in companion papers.

2. METHODS

Patient Selection and Treatment. Control females (N = 175) were recruited from patients having insurance or employment medical examinations, patients with minor physical health problems, such as ear blockages or minor psychological presentations (N = xx), university students (N = xx) and community volunteers (N = xx). 222 heroin dependent females maintained on methadone (13.5%), buprenorphine (83.3%), or implant naltrexone (3.2%) were recruited and sampled opportunistically at the time of their presentation to the clinic. Pharmacotherapy treatment was in accordance with established clinical practices either at this clinic or by their usual treating physicians. Naltrexone implants were not performed as part of this study, but patients who had them previously inserted for management of heroin dependence were studied on the occasion of their visit to the clinic. Naltrexone implants are not a registered therapeutic good in Australia, but are available to patients on a compassionate access scheme within the Special Access scheme of the Therapeutic Goods Administration. These techniques have been previously described [26]. Implants were obtained from Go Medical Industries in Perth, Western Australia, were of 1.7 g, and designed to last about five months.

PWA Measurements. Patients were not permitted to talk or sleep during the performance of PWA studies. Access to food, drink, and tobacco was not restricted. If patients identified use of alcohol prior to the testing, the studies from that day were discarded. PWA was performed with the Miller microtonometer, the SphygmoCor software and the AtCor preamplifier and hardware, obtained from AtCor in Sydney. Studies were performed over the right radial artery unless it was not available. The brachial blood pressure was taken from the contra-

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lateral arm to the study side using an Omron HEM 907 oscillometric device. Studies were done in quintuplicate wherever possible. A history of recent and lifetime drug use was taken from the patient at the time of study, and entered into the software's database. If patients' main opiate of abuse was not heroin, it was converted into morphine equivalents, and then into heroin equivalents, at a conversion rate of 500 mg of morphine per gram of street heroin (REF). The heroin dose was the dose usually taken at the time of study. Length of heroin use was the total period of opiate use from the time of first use to the present. Cardiovascular parameters were generated automatically by the software and outputted from it.

Major indices calculated from this technique included the Vascular or Reference Age (VA, RA), Central Systolic Pressure (C_SP), Central Diastolic Pressure (C_DP), the Chronologic Age (CA), the Central Augmentation Pressure at Heart Rate 75 (C_AP_HR75), the Central Augmentation Pressure/Pulse Height Ratio at Heart Rate 75 (C_AGPH_HR75) also known as the Augmentation Index, Peripheral-Central Pulse Pressure Amplification Ratio (PPAmpRatio), Central Pulse Height (C_PH), Central Mean Pressure (C_MEANP), Central End Systolic Pressure (C_ESP), the Central Diastolic Time Index (C_DTI), the Central Tension Time index (C_TTI), the Central Diastolic Duration (C_DD), and an index of subendocardial perfusion known variously as the Central Stroke Volume Index (C_SVI), the Subendocardial Perfusion Ratio (SEVR) or the Buckberg ratio, which is defined as the C_TTI/C_DTI.

Statistics. Data are presented as mean ± S.E.M. EpiInfo 7.0.8.3 from CDC Atlanta, Georgia was used to perform Chi Squared tests to compare categorical variables. "Statistica" 7.1 from Statsoft, Oklahoma was used to compare continuous variables using student's t-tests. Separate variances were employed were Levene's test was significant. Data was log transformed as indicated by the Shapiro test in the interests of normality assumptions with the sole exception of CRP. CRP was transformed by the arcsinh transformation which is similar to log transformation, but it also accepts negative and zero arguments. Model appropriateness was determined by the outcome of Anova tests and Akiane Information Criteria (A.I.C.). Multiple-regression was performed in "R" 2.13.1 obtained from the Central "R" Archive Network mirror at the University of Melbourne. Graphs were drawn with the aid of Ggplot 2 software. $P < 0.05$ was considered significant.

Ethical Approval. Informed consent was obtained prior to the performance of the study in all patients. Patients undergoing naltrexone implants also gave informed written consent. This study was approved by the Human Research Ethics Committee (HREC) of Southcity Medical Centre, a recognized HREC by the National Health and Medical Research Council. All procedures complied with the Declaration of Helsinki. Relevant regulatory requirements were met throughout.

3. RESULTS

As shown in **Table 1** there were 175 control and 222 opiate dependent female patients. The chronological mean age (CA) of the two groups was 32.62 ± 0.96 and 33.58 ± 0.57 years (mean ± S.E.M.), respectively, was not statistically different ($t = 0.85$, $dF = 292.37$, $P = 0.39$). Significant differences between substance exposure and some laboratory values were also documented and have been previously reported [27]. 83.33% of the opiate dependent patients were treated with buprenorphine, 13.51% were treated with methadone, and 3.15% were treated with naltrexone. The mean dose of buprenorphine used was 6.83 ± 0.36 mg, and the mean dose of methadone used by these patients was 55.80 ± 6.04 mg.

Table 2 presents the results of the direct comparison of the two groups for central and peripheral cardiovascular parameters. The quality index (Operator Index) in the two groups was uniformly and similarly high. The vascular age, the difference between the vascular and chronologic ages, and the RA/CA ratio were all significantly elevated in the opiate dependent group. All five cited measures of arterial stiffness were elevated amongst addicted patients, except the pulse pressure amplification ratio, where depression is associated with age related stiffening.

Figures 1-4 present various plots of age, arterial stiffness, pressure and timing indices respectively against CA.

Judged by the AIC, the best way to model age related changes is the semi-log model. Using this technique, when patients achieve a CA of 60 years, the controls (intercept = 2.4868, slope = 0.0287) have a predicted modelled age of 67.52 years, and the opiate dependent patients (intercept = 2.4267, slope = 0.00333) of 83.79 years. This represents an elevation in the modelled age of 16.27 years or 24.10%.

When the (log) RA is regressed against the CA and addictive status, the addictive status is significantly predictive both as a factor in an additive model, and in interaction with CA. Details of these results and other results for a similar age dependent analysis of major central cardiovascular parameters are given in **Table 3**.

Possible dose-response relationship with lifetime opiate exposure in opiate exposed individuals was examined in an interactive model of the log of the RA/CA ratio against both heroin dose and duration. In the final model (Adj. $R^2 = 0.193$, $F = 8.73$, $dF = 1391$, $P = 0.0033$) the only remaining significant variable was the dose-duration interaction (est. = 0.0057 ± 0.0019, $t = 2.954$, $P = 0.0033$).

Persistence of a dose-response effect after adjustment

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Table 1. Socio-demographic parameters.

Parameter	Controls	Addiction	P Value
No.	175	222	
Biometrics			
Chronologic_Age*	32.62 (0.96)	33.58 (0.57)	0.0439
Height (m)	165.74 (0.51)	165.52 (0.44)	0.7429
Weight (kg)	66.33 (0.93)	63.99 (0.82)	0.0583
BMI (kg/m^2)	24.17 (0.34)	23.35 (0.28)	0.0635
Substance Abuse			
Smokers, No. (%)	29 (16.57%)	200 (90.09%)	0.0000
Cigarettes/d	2.18 (0.45)	14.66 (0.62)	0.0000
Minutes Post-Cigarette	150.51 (27.76)	106.41 (20.26)	0.1976
Heroin Dose (g)	0 (0)	0.57 (0.06)	0.0000
Heroin Duration (Years)	0.11 (0.11)	12.05 (0.6)	0.0000
Heroin Dose-Duration (g-Years)	0.01 (0.01)	8.78 (1.79)	0.0000
Laboratory Values			
Cholesterol (mmol/l)	4.72 (0.1)	4.55 (0.07)	0.1956
Triglyceride (mmol/l)	1.17 (0.08)	1.27 (0.05)	0.3057
HDL (mmol/l)	1.45 (0.06)	1.38 (0.04)	0.3830
LDL (mmol/l)	2.72 (0.14)	2.55 (0.07)	0.2360
ALT (IU/l)	25.96 (1.57)	54.58 (7.55)	0.0003
AST (IU/l)	25.01 (1.12)	46.44 (5.52)	0.0002
Glucose (mmol/l)	4.73 (0.11)	5.27 (0.21)	0.0915
Creatinine (mcmol/l)	71.33 (1.59)	70.33 (0.95)	0.5756
Urea (mmol/l)	4.9 (0.12)	4.35 (0.11)	0.0030
Albumin (g/l)	44.28 (0.32)	43.49 (0.26)	0.0798
Globulin (g/l)	29.83 (0.41)	31.57 (0.35)	0.0033
CRP (mg/l)	2.73 (0.6)	5.75 (0.74)	0.0017
ESR (mm/hr)	9.03 (0.83)	15.47 (1.01)	0.0000
Lymphocytes ($\times 10^{-9}$/l)	2.37 (0.08)	2.47 (0.06)	0.3538
Monocytes ($\times 10^{-9}$/l)	0.51 (0.03)	0.54 (0.02)	0.3117

*—Statistics for log transformed data presented; data presented as mean (S.E.M.).

for established cardiovascular risk factors was also investigated. **Table 4** shows the result of a multiple linear regression of (log) RA against interactive terms in CA, heroin dose and duration, cigarette consumption, HDL, and CRP and additive terms in BMI, brachial systolic pressure, cholesterol and height. A factor related to time since cigarette consumption was found not to be signficant in exploratory modelling and so was omitted. The parameters for this model were Adj. $R^2 = 0.5053$, $F = 4.652$, $dF = 33.85$, $P = 5.97 \times 10^{-9}$.

The table arranges the results in ascending order of P values. Amongst the final 33 terms remaining in the model, the heroin dose: duration interaction is noted to occur in 11 terms, and the heroin dose and duration separately in a further 15 terms. Indeed, the first such heroin dose: duration interaction is that with CA, which has an est. = 0.1196 ± 0.0228, t = −5.300, and $P = 9.00 \times 10^{-7}$. Interactions involving CA, tobacco consumption, CRP and HDL are prominent. CA, CRP, tobacco and brachial systolic pressure are independently predictive.

4. DISCUSSION

Study data indicated dramatic differences in central arterial function, stiffness and vascular age in opiate exposed compared to non-exposed women, on direct bivariate comparison, and when corrected for chronologic age (CA). In a fully adjusted multivariate model including all major cardiovascular risk factors the dose: duration interaction is independently significant and it is also interactively significant with other major risk factors such as age, tobacco consumption and HDL, thereby demon-

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Table 2. Selected cardiovascular parameters.

Parameter	Controls	Addiction	P Value
Operator Index	86.23 (0.53)	87.1 (0.45)	0.2138
Ages			
Vascular_Age*	35.03 (1.41)	39.30 (1.28)	0.0083
Difference	2.41 (0.91)	5.73 (1.02)	0.0156
RA/CA	1.07 (0.02)	1.16 (0.03)	0.0197
Log (RA/CA)	0.02 (0.02)	0.08 (0.02)	0.0732
Arterial Stiffness			
C_AP_HR75	4.29 (0.4)	5.96 (0.34)	0.0016
C_AGPH_HR75	11.66 (1.07)	15.81 (0.83)	0.0023
C_PH (mmHg)	33.49 (0.59)	35.8 (0.5)	0.0029
PPAmpRatio	149.62 (1.59)	144.32 (1.38)	0.0120
P_AI	64.35 (1.48)	69.7 (1.29)	0.0066
Timing			
HR (bpm)	69.42 (0.84)	70.63 (0.71)	0.2681
Ejection_Duration (msec)	331.06 (1.51)	326.27 (1.34)	0.0179
C_SVI	141.15 (2.21)	138.86 (1.71)	0.4066
C_DTI	2976.29 (29.29)	2971.69 (27.11)	0.9089
C_TTI	2175.61 (30.12)	2191.48 (25.16)	0.6841
C_Diastolic Duration (msec)	558.05 (10.1)	544.46 (8.02)	0.2866
Pressures			
SP	118.11 (0.9)	118.68 (0.77)	0.6320
DP	69.03 (0.7)	68 (0.69)	0.3029
Central_SP	103.82 (0.93)	105.21 (0.81)	0.2575
Central_DP	70.35 (0.72)	69.5 (0.69)	0.3967
Central_ESP	92.86 (0.89)	94.12 (0.8)	0.2929
Central_MEANP	85.93 (0.76)	86.17 (0.7)	0.8161

*—Statistics for log transformed data presented; abbreviations as in methods; data presented as mean (S.E.M.).

Table 3. Age dependent multiple regression of central CVS parameters.

Parameter	Variable	Estimate (Std. Error)	t Value	Pr (> |t|)	Adjusted R^2	F	DF1, DF2	Model P
RA	Opiate. Status	0.095 (0.034)	2.8200	0.0050	0.4912	192.10	2394	<2.0E−16
RA	CA: Opiate. Status	0.003 (0.001)	3.2140	0.0014	0.4942	194.40	2394	<2.0E−16
C_AP_HR75	Opiate. Status	1.3344 (0.3697)	3.610	0.0003	0.5158	211.90	2394	<2.0E−16
C_AP_HR75	CA: Opiate. Status	0.041 (0.0106)	3.869	0.0001	0.5181	213.80	2394	<2.0E−16
C_AGPH_HR75	Opiate. Status	3.3319 (0.9692)	3.438	0.0006	0.4820	185.30	2394	<2.0E−16
C_AGPH_HR75	CA: Opiate. Status	0.0999 (0.0278)	3.588	0.0004	0.4834	186.30	2394	<2.0E−16
C_SVI	Opiate. Status	−28.371 (9.274)	−3.059	0.0024	0.0159	3.13	3393	0.0255
C_SVI	CA: Opiate. Status	0.4766 (0.2121)	2.247	0.0252	0.0159	3.13	3393	0.0255
C_SP/CA	Opiate. Status	−0.0513 (0.027)	−1.900	0.0582	0.0065	3.61	1395	0.0582

Abbreviations as in Methods.

strating a positive dose-response. Opiate dose or duration exposure featured in 26 of the terms in the final regression model, which accounted for 50.5% of the variance in (log) RA. The most powerful interaction we demonstrated was that between the dose-duration interaction and age, which had a P value < 10^{-6} (**Table 4**). Based on the regression models established, at a CA of 60 years, controls would have a mean vascular age of 67.52 years

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Table 4. Final model of CVS risk factors.

Variable	Parameter Estimates		
	Estimate_(S. E.)	t Value	Pr (> |t|)
CA	2.244 (0.3621)	6.196	0.000000
Cigarettes: CRP	0.0548 (0.0102)	5.359	0.000001
CA: H. Dose: H. Dura. n	−0.1196 (0.0226)	−5.3	0.000001
H. Dura. n: Cigarettes: CRP	−0.0046 (0.0009)	−5.091	0.000002
CA: H. Dose: Cigarettes: HDL: CRP	−0.1412 (0.0278)	−5.076	0.000002
CA: H. Dose: HDL: CRP	3.056 (0.6055)	5.047	0.000003
CA: H. Dose: Cigarettes: HDL	1.685 (0.3351)	5.028	0.000003
CRP	−1.111 (0.2237)	−4.965	0.000004
CA: H. Dose: H. Dura. n: HDL: CRP	−0.2134 (0.0449)	−4.758	0.000008
CA: H. Dose: H. Dura. n: Cigarettes	0.0057 (0.0012)	4.755	0.000008
H. Dose: Cigarettes: HDL	−5 (1.055)	−4.74	0.000009
CA: H. Dose: HDL	−29.4 (6.708)	−4.383	0.000033
CA: H. Dose: H. Dura. n: HDL	2.533 (0.5862)	4.321	0.000042
H. Dose: HDL	84.36 (20.76)	4.063	0.000107
CA: H. Dose: H. Dura. n: Cigarettes: HDL: CRP	0.0086 (0.0022)	3.989	0.000140
H. Dose: H. Dura. n: HDL	−7.555 (1.969)	−3.837	0.000239
CA: H. Dose: H. Dura. n: Cigarettes: HDL	−0.1146 (0.0304)	−3.772	0.000298
CA: H. Dose: CRP	−1.309 (0.3512)	−3.727	0.000349
H. Dose	2.164 (0.5981)	3.617	0.000504
H. Dose: CRP	4.644 (1.297)	3.58	0.000572
CA: H. Dose: H. Dura. n: CRP	0.1494 (0.0419)	3.571	0.000589
CA: Cigarettes: HDL	−0.2205 (0.0638)	−3.454	0.000864
H. Dura. n: Cigarettes: HDL: CRP	0.0061 (0.0018)	3.395	0.001046
Cigarettes: HDL	0.7377 (0.2177)	3.388	0.001069
H. Dose: H. Dura. n: Cigarettes: HDL	0.341 (0.1024)	3.332	0.001279
Cigarettes	−0.0447 (0.0134)	−3.328	0.001296
CA: H. Dose: Cigarettes	−0.0297 (0.0091)	−3.277	0.001520
H. Dose: H. Dura. n: CRP	−0.4326 (0.146)	−2.962	0.003964
H. Dura. n: CRP	0.1944 (0.0669)	2.905	0.004681
CA: H. Dose: H. Dura. n: Cigarettes: CRP	−0.0009 (0.0003)	−2.673	0.009015
SP	0.9822 (0.3713)	2.645	0.009717
CA: H. Dura. n: HDL: CRP	−0.014 (0.0054)	−2.595	0.011135
CA: H. Dura. n: CRP	−0.0361 (0.0171)	−2.115	0.037373

CA—Chronologic Age; H. Dose—Usual Heroin Dose; H. Dura. n—Lifetime Duration of Heroin Use.

and opiate dependent patients a mean vascular age of 83.79 years, an elevation of 16.27 years or 24.1%. These results demonstrate that long term opiate dependence is an independent and interactive cardiovascular risk factor in females.

Figures 1, **2**, and **4** show a very clear separation between the age, arterial stiffness and timing data for the opiate exposed and control groups. Even when the mean CA of the two groups was assessed as significantly different using log-transformed data, the magnitude of this is less than one year, which is much less than the demonstrated differences in the various measures of vascular age which was 4.2 years. The changes in vascular age were paralleled by significant changes in measures of central arterial stiffness, subendocardial perfusion and systolic pressure (**Table 3**).

It should also be noted that although this study has considered all the opiate dependent patients together, in fact this group is heterogeneous when judged by treatment type (methadone (13.5%), buprenorphine (83.3%),

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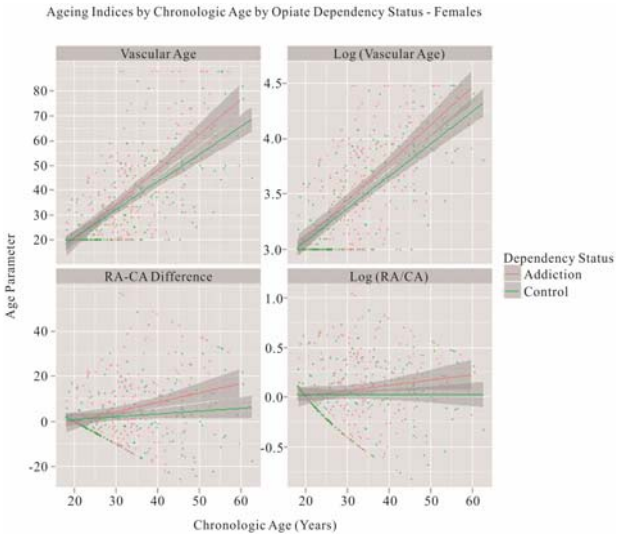

Figure 1. Ageing indices by chronologic age by opiate dependency status.

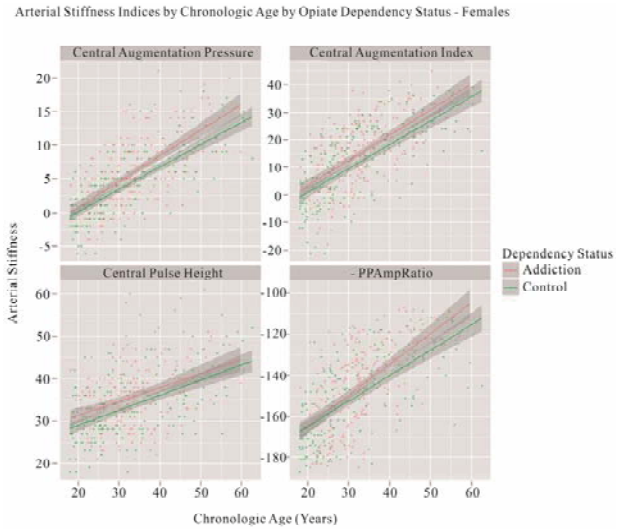

Figure 2. Arterial stiffness by chronologic age by opiate dependency status.

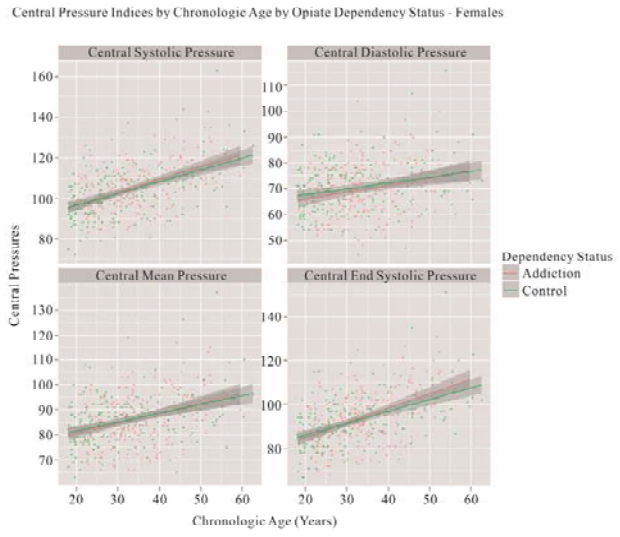

Figure 3. Central pressures by chronologic age by opiate dependency status.

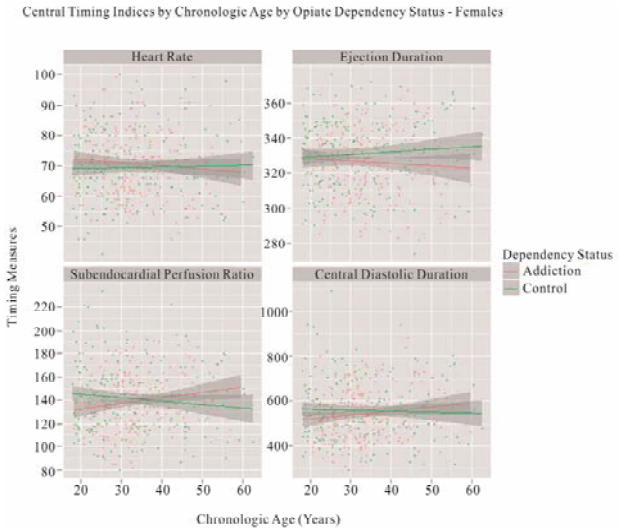

Figure 4. Central timing indices by chronologic age by opiate dependency status.

or implant naltrexone (3.2%)). Unpublished data from this project (manuscript submitted) shows that the type of pharmacotherapy used to treat opiate dependence has a very material impact on the central cardiovascular outcomes. Other studies in the literature are consistent with this view [4,8]. 83.3% of our patients were treated with buprenorphine with a mean dose of 6.83 ± 0.36 mg which is an unusually low dose judged by literature standards. As a partial μ-agonist buprenorphine is a relatively mild intoxicant compared to other treatments. For this reason we consider that the results reported herein represent a best case scenario for opiate dependence and likely a lower bound of their cardiovascular toxicological effect.

The strength of association and the uniformity of the results presented raise the question as to possible mechanisms of action by which opiates might be impacting the cardiovasculature. At the outset it is important to note that opiate use has been associated with exacerbation of most of the major cardiovascular risk factors including smoking [8], cholesterol [28], poor dietary habits, weight gain [29], hypertension [30,31], and hyperglycaemia and diabetes [32]. Higher levels of immune activity have also been shown [27]. Indeed the present study also demonstrated elevated levels of ESR, CRP and globulins in our patients (**Table 1**).

The pro-senescence activities of opiates have been noted in the Introduction. This effect is likely compounded by the affects of opiates to stimulate or prime apoptosis [33] and to induce immune responses both

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through toll-like receptor 4 [34] and chemokines [35]. Senescent tissues have also been shown to secrete various substances including interleukins -6 and -8 [19] which are highly toxic to most stem cells niches [36] and perpetuate the senescent phenotype. From a mechanistic point it is possible that in opiate dependent patients, there is a pro-senescence stimulus mediated via ANRIL and P16, compounded by apoptotic activities, further impacted by heightened immune activity, which is further exacerbated by the interactive effect of immune stimulation on stem cell vulnerability to produce the observed phenotype of accelerated ageing in all organ beds examined. The relative hypercalcaemia likely exacerbates these changes and contributes to the increased vascular stiffness.

The importance of cardiovascular age as a surrogate marker for generalized organismal ageing was also noted in the introduction [25]. The present findings are therefore consistent with other data which shows acceleration of age dependent changes in bone, hair, teeth and stem cells [37-39], and with the hypothesis that opiate dependent patients are ageing in a more accelerated manner across all tissue beds [4,6,30] (see also Appendix 6 [7]).

This study had a number of limitations, primarily related to its observational design. Design of a randomised study however has ethical concerns with the randomisation of opiate or non-opiate dependent persons to treatment or non treatment by an opiate pharmacotherapy. Additionally when log as opposed to non-log transformed data was used there was a significant difference between the control and drug dependent group. Further, a systematic drug history was not collected in a format which facilitated easy statistical analysis: a key feature that would be required in future prospective work. Future iterations of this study would also need to give careful consideration to the treatment make-up of the opiate dependent condition. Ideally the numbers in the treatment groups would be broadly comparable, and treatment assignment might be randomized between the various conditions. Further studies might also consider investigating the mechanism of these changes with prospectively collected immune, stem cell and senescence related parameters of circulating cells able to be quantified and studied.

In summary, this study provides evidence suggesting an increased vascular age including central arterial stiffness in opiate dependent patients, and demonstrated a dose-response relationship between these features and lifetime opiate exposure. This relationship is robust and persists after adjustment for other cardiovascular risk factors. At age 60, this is equivalent to a 24.1% advancement in cardiovascular age above controls. These findings are consistent with the observations of other workers, and have the advantage that by studying a subclinical endophenotype, they are performed on living patients. These results are also consistent with an emerging body of evidence showing accelerated ageing in all body systems in opiate dependent patients. Whilst the present study has not presented evidence for likely mechanisms of these changes, they are consistent with the known pro-senescence, pro-apoptotic, immunostimulatory activities of opiates, and the interactions of these effects. Opiates are also known to exacerbate classical cardiovascular risk factors. Various suggestions for further research are made.

5. ACKNOWLEDGEMENTS

The authors would like to thank Dr Mervyn Thomas of Emphron for assistance with the statistics and graphical design.

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