Reduction in Arterial Stiffness and Vascular Age by Naltrexone-Induced Interruption of Opiate Agonism, Particularly in Females

Running Head: "Vascular Age Reduction in Opiate Dependence"

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Relationship to Industry – Nil.

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Word Count: 4,498.

ABSTRACT

Objective: To prospectively study the effect of treatment type on opiate-related vasculopathy.

Background: Evidence consistently shows that opiate dependence is associated with increased rates of coronary and heart disease and other signs of accelerated ageing. The effect of treatment type is unknown.

Methods: 20 patients (16 males) were followed longitudinally over 6.5 years and studied by pulse wave analysis on opiate therapy, naltrexone, and opiate-free. Vascular Reference Age (RA) and measures of arterial stiffness were regressed using mixed effects models against time, Chronological Age (CA), biometric and biochemical parameters.

Results: The mean CA was 33.62$\pm$2.03 years. 74 studies were performed on opiates (16 cases of buprenorphine 4.11$\pm$1.17mg, two of methadone 57.5$\pm$12.5mg and two of heroin 0.75$\pm$0.25g), 38 on naltrexone, and 12 opiate-free. The opiate-free condition was associated with a lower apparent vascular age both in itself (males: P=0.0402, females: P=0.0360) and in interaction with time (males: P=0.0001; females: P=0.0004), and confirmed with other measures of arterial stiffness. The mean modelled RA was 35.57, 34.48 and 31.79 years in the opiate, naltrexone and opiate-free conditions respectively. The opiate-free condition was superior to opiate agonism after full multivariate adjustment (P=0.0131), with modelled RA/CA of 1.0173, 0.9563, and 0.8985. These changes were worse in females (all P<0.02).

Conclusions: These prospective data demonstrate that conversion from even mild opiate-agonism to opiate-free status improves vascular age and arterial stiffness, particularly in females. This subclinical endophenotype is consistent with other evidence showing signs of accelerated aging in these patients, and offers hope of novel therapeutic remediation.

INTRODUCTION

Opiate dependence is an increasingly common disorder with significant public health impacts for many reasons, including the increasing use of opiate analgesics for chronic pain in an aging population, an increasing rate of overdose deaths, and its association with blood borne virus pandemics.

A small but remarkably consistent literature has identified opiate dependence with increased rates of cardiovascular risk factors (hypertension, hypercholesterolaemia (1,2), tobacco consumption, diabetes (3,4), and raised hs-CRP (5)) and of clinically significant atherosclerotic disease. For example an Iranian group has found that opiate dependent males have an increased rate of coronary disease requiring surgical revascularization, and to need such revascularization an average of two years before non-dependent controls (6). Moreover in men, opiate dependence was the major risk factor for coronary disease over-riding conventional risk factors (7,8). A large 21 year review of an opiate substitution program in Sydney found that patients had a 2.2-fold risk of cardiovascular death (9). Similarly a large prospective population-wide screening program conducted by the NCI found a rate of coronary death to be elevated 1.90-fold, worse in females (H.R.=2.90, 95%C.I. 2.10-3.99; males HR=1.58, 95%C.I. 1.26-1.98) (10). A Sydney group found that 17% of patients over the age of 44 years dying from heroin overdose had a coronary stenosis of 75% or greater (11). These workers found that methadone treatment of heroin dependency appeared to exacerbate many forms of heart disease (12) consistent with a more complete pattern of opiate agonism by the longer acting agent. And finally a 33 year follow-up of Californian methadone registrants found a highly significant 3-6 fold elevation of years of life lost to fatal heart disease across all racial sub-groups (2). Opiates are known to impact stem cell activity (13) and be immunostimulatory (14), processes which are relevant to atherogenesis. Contrariwise, naltrexone, a broad spectrum opiate antagonist active at both classical (μ, δ, κ) and non-classical (perinuclear receptors and toll-like) receptors (TLR's), stimulates stem cell activity and suppresses immunostimulation (13,14).

Vascular ageing has been described as being one of the most important features of the ageing syndrome (15,16). For this reason cardiovascular age has been nominated as a surrogate for organismal age (15,16). The significance of the above findings therefore is amplified by the presence of elevated rates of various features of the ageing syndrome in opiate dependent patients such as hair graying (17), chronic periodontitis (18), osteopaenia (19), neuropsychiatric disorders (20), a senescence-mimetic polyclonal immunopathy (21,22), reduced circulating stem cell counts (23), and elevated rates of many malignancies (10,24).

Notwithstanding these tantalizing findings there is little cardiological research in opiate dependency. The availability of the rapid and portable SphygmoCor system from AtCor (Sydney) implies that measures of central arterial stiffness, and its correlate vascular age, are measurable in ambulatory patients as an important endophenotype of vascular ageing. The present study was prospectively designed to examine in a series of

patients followed longitudinally whether their treatment with opiates or opiate antagonists or perhaps the opiate-free lifestyle affected measures of their arterial stiffness and vascular age.

METHODS

Patient recruitment and Drug Usage. Substance dependent patients were recruited opportunistically during the time of their clinic visits before and after naltrexone implant insertion. Data relating to tobacco, alcohol and drug use drug use histories was also documented. The current form of opiate administration (heroin. methadone, morphine or buprenorphine) was recorded. Time was measured from the time of the first naltrexone implant insertion. The study was performed May 2006 – December 2012.

Arterial Tonometry. Patients were lain supine for five minutes to rest. No restrictions on smoking or food or beverage intake were imposed prior to the study. Patients were not allowed to talk or fall asleep during the study. The brachial blood pressure was taken over the left upper arm using an oscillatory device Omron HEM 907 Intellisense professional blood pressure monitor. Radial arterial tonometry was performed using the SphygmoCor 8.0 hardware (Atcor, Sydney). The right radial artery was used unless it was unavailable or unsuitable. Studied were conducted in quintuplicate. Acceptable studies had an Operator Index greater than 70 and were not inconclusive. Each day's readings were averaged. The main parameters of interest were the chronological age (CA), the Vascular Reference Age (RA), the difference between the two (RA-CA), the ratio of the RA and CA (RA/CA), the central aortic augmentation pressure (C_AP) corrected to a heart rate of 75 bpm (C_AP_HR75), the central aortic compliance defined as the central augmented pulse pressure divided by the central pulse height (C_AGPH) corrected to a pulse rate of 75bpm (C_AGPH_HR75, also referred to as the "Augmentation Index"). Other indices of interest were the central pulse height (C_PH), the central augmentation load (C_AL), the peripheral-central pulse pressure amplification ratio (PPAmpRatio), the maximum peripheral dP/dT (P_MAX_DPDT), the peripheral systolic and diastolic pressures (SP, DP), the central systolic and diastolic pressures (C_SP, C_DP), the central mean pressure (C_MEANP) and central end systolic pressure (C_ESP), the heart or pulse rate (HR), the ejection duration in milliseconds, (ED), the central stroke volume index (C_SVI) or Buckberg index, the central tension-time index (C_TTI) and the central diastolic-time index (C_DTI).

Naltrexone implants. Naltrexone implants used in this clinic were obtained from "Go Medical" Industries in Subiaco, Perth, Western Australia. Patients had a single naltrexone implant inserted under local anaesthetic most often beneath the skin of the left iliac fossa, by standard surgical techniques which have been previously described (25). The legal framework within which this work was undertaken was the Special Access Scheme which allows Australian patients with serious illnesses access to new and investigational drugs, pursuant to formal notification of the Federal Health Department Therapeutic Goods Administration (TGA). Patients were detoxified prior to implant insertion.

Statistics. Data is listed as mean $\pm$ S.E.M.. Continuous data was transformed as appropriate informed by the Shapiro-Wilks test and boxcox analysis in "R" (2.15.2, Central "R" Archive Network at University of

Melbourne). For RA and CA, RA/CA, weight and SP this was the reciprocal transformation. Repeated measures multiple regression was performed using the non-linear mixed effects nlme package. The comparator condition was the opiate treated condition. In each case the random effects were unity and the case number. The "log-likelihood value" is abbreviated to "LogLik". Model estimates for the various treatment conditions were calculated by inserting the appropriate mean values into the final models using untransformed parameters. Graphs were drawn in "R" with ggplot2. $P < 0.05$ was considered significant.

Ethics. After appropriate consultation and advice patients gave informed consent to the study Pulse Wave Analysis (PWA) procedures. Patient confidentiality was respected and maintained throughout. All patients were carefully and fully advised of the risks and benefits of the naltrexone implant insertion and gave formal written consent prior to this procedure. The study was approved by the Human Research Ethics Committee of the Southcity Medical Centre, and conformed with both the Declaration of Helsinki and the code of ethical practice of the National Health and Medical Research Council of Australia.

RESULTS

20 patients (16 males) were studied on 124 occasions. 721 studies were performed (5.81±0.12, mean ± S.E.M.). The mean age at enrollment was 33.62±2.03 years. The mean study duration per patient was 460±82.22 days (range 11-2,414 days). 14 patients were followed for 360 days or more. The mean age on the study was 34.88±1.96 years. Baseline patient data are given in Table 1. The time base from the first naltrexone implant varied from -915 to 2393 days (-2.50 to 6.55 years). There were 74 PWA studies done during opiate treatment, 38 on naltrexone, and 12 whilst opiate-free. In 16 cases the opiate used was buprenorphine (4.11±1.17mg), in 2 cases it was methadone (57.5±12.5mg), and in two cases heroin (0.75±0.25g).

Figure 1 shows the various measures of arterial stiffness over time by treatment type. As the PPAmpRatio normally declines with age its projection has been inverted. At repeated measures regression, opiate-free status was a significant determinant of RA both as a factor (est. = 0.0122, dF=99, P=0.0008) and in interaction with time (est.=-0.0000156, dF=99, P=0.0043, model AIC=-714.79, LogLik=356.39). The mean modelled RA during each treatment was 35.57, 34.48 and 31.79 years under the opiate, naltrexone and opiate-free conditions respectively.

Of the stiffness measures, significant differences were also seen for the Augmentation Index with the opiate-free status being significant both as a factor (est. = -7.4071, dF=99, P=0.0050) and in interaction with time (est.=-0.0127, dF=99, P=0.0016, model AIC=857.93, LogLik=-420.96). When the C_AP_HR75 was considered, the time: opiate-free interaction was superior to opiate agonism (est.=0.0036, dF=99, P=0.0283), and there was a trend for opiate-free status as a factor (est.=-1.9642, dF=99, P=0.0687; model AIC=649.49, LogLik=-316.74). When the C_PH was considered, the time: naltrexone interaction was better than the opiate treatment (est.=-0.0032, dF=101, P=0.0068, model AIC=797.06, LogLik=-392.53).

Figure 2 shows the RA/CA ratio, and its adjustment for various other parameters. The CA, height and SP were shown in preliminary analyses to be the major significant determinants of RA. Biochemical parameters were not significant in any analyses. When the RA/CA ratio was regressed against interactions between time, height, SP, and treatment type with an additive term for weight in a mixed effects model, the opiate-free status was again found to be significant both as a factor alone, and in interaction with time and with time and SP as shown in Table 2. These results model a mean RA/CA ratio of 1.0173, 0.9563, and 0.8985 under opiate, naltrexone and opiate-free conditions.

When RA/CA was regressed interactively against cumulative time in treatment on opiates, naltrexone and opiate-free, the interaction between cumulative opiate and opiate-free time trended towards significance (est.=-0.0000003, P=0.0548, model AIC=56.47, LogLik=-24.23).

Figure 3 shows the various measures of arterial stiffness by sex. In each case the effect is much worse in females. These effects are quantitated using mixed effects models in Table 3 where the various parameters of arterial stiffness are regressed against Sex. Note that the sign of the estimates for PPAmpRatio and P_Max_DPDT is reversed as these parameters decrease with age. These major measures are shown separately for each sex by treatment condition in Supplementary Figures 1 and 2.

In respect to RA/CA the opiate-free condition was superior in both males and females both as a factor (males: est.=0.3186, P=0.0402; females: est.=0.3285, P=0.0360) and in interaction with time (males: est.=-0.0001, P=0.0320, model AIC=90.72, LogLik=-37.36; females: est.=-0.00044, P=0.0460, model AIC=48.35, LogLik=-16.18).

No patient suffered a significant major adverse effect as a result of the treatment or their PWA studies during the observation period on the study.

DISCUSSION

The main findings of this study were that the opiate-free state was associated with a reduction of arterial stiffness indices and vascular age, and by extension, in apparent biological age. That this effect was highly statistically significant in this numerically small study suggests that it is a real effect. The effect was consistent across several indices of arterial stiffness, remained after full statistical adjustment for other confounding factors, and was seen in each sex separately. Interestingly the deleterious cardiovascular effect of opiates was seen most dramatically in females.

The importance of arterial stiffness as a cardiovascular risk factor has been noted by a large body of literature. It was particularly encouraging therefore that the adverse changes described in the present work were amenable to amelioration by conversion of the patients' treatment to antagonist based treatment and then the opiate-free state, as opposed to the more commonly recommended protocols featuring indefinite opiate agonism (26).

The magnitude of the effect observed was noted to be relatively small. Compared to a mean age on the study of 34.88 years, the modelled RA's were 35.57, 34.48 and 31.79 in the opiate, naltrexone and opiate-free conditions respectively, representing 102.98%, 98.85% and 91.14%. These are equivalent to differential effects on vascular age of 3.13% (opiate – naltrexone) and 10.84% (opiate – opiate-free). These data are in good agreement with the modelled RA/CA ratios after full multivariate mixed effects adjustment which found 1.0173, 0.9563 and 0.8985 in the three conditions, suggesting differentials of 6.10% and 11.88% respectively.

These findings need to be interpreted in the light of at least two major considerations. Firstly opiate agonism frequently occurs, and is often recommended, for protracted periods spanning many decades. Hence a 6.1% effect as found here continued over 30 years is equivalent to a 590.82% effect in amplification, and an 84.86% in diminution if continued at a similar rate for the whole period because of the compounding interest effect over protracted periods of time. For an 11.88% effect the similar 30 year results are 2847.21% in amplification and 97.75% in reduction. Hence whilst the quanta observed here are not large, over great amounts of time they can become very considerable. This begins to address the uniformity of the deleterious nature of the pathologies identified by various authors in the field (9-12,27,28).

Secondly the level of opiate exposure to which these patients were submitted was relatively low level. Most studies were performed on patients on low dose buprenorphine. As a partial opiate agonist, buprenorphine is likely to have more moderate opiate agonist effects than full agonists such as physeptone. Moreover the mean dose employed in these patients is much lower than that commonly reported (29). For this reason it may be that the findings reported here represent a lower bound on the magnitude of the observed effects.

The clear sex differential in opiate-related cardiovascular pathology has only been reported in a single previous study to our knowledge (10). The detailed findings of the Iranian group were mentioned in the Introduction. Curiously 17/26 pathologies reported in their Table 3 were worse in females (Wilcoxson matched pairs test $Z=2.28$, $P=0.022$). This result is clearly consistent with the effect observed here. It is also consistent with a larger cross-sectional study of which the present result forms a part (manuscript in preparation). It suggests that the cardiovascular effects described herein may be part of a larger framework in which the toxicopathological effects of long term opiate agonism are more pronounced on females across the whole organism.

The structural implications of the present findings are unknown. Like other welldocumented cohorts our patients have on occasion suffered from devastating major cardiovascular end organ complications usually at very young ages. There is therefore good evidence of fixed severe atherosclerotic disease in this cohort. The demonstration of a reduced vascular age and lowered arterial stiffness in this condition is conceptualized as a reduction primarily in arterial tone. Its exact interpretation however must await further structural studies conducted longitudinally in this group.

Some of the most fascinating questions to emerge from this study relate to the possible mechanisms which are likely to account for these changes apparently over a relatively short period. Whilst this is not a mechanistically focussed study some concise remarks may be of interest. As stated above, opiates are known to have effects both on stem cells (13) and on immune modulation (14). It seems likely that the immunostimulatory effects also induce well described immunosuppressive effects (30). Opiate ligation of TLR4 is particularly potent and is coupled downstream to numerous immune-effector pathways including IL-1, IL-6, TNFα, MCP-1/CCL2, TGFβ, MAP kinase, several interferon response factors, NF-κB and sphingosine-1-phosphate signalling (14). Importantly it now appears that there are many biological interactions between stem cell behaviour and immune response. Cytokines are known to inhibit most stem cells (31). Two key stem cell master genes, Oct4 and Nanog, have cytokine response sequences in their promoters particularly for the gp130 cytokine series and STAT (Signal Transducer and Activator of Transcription) -1 and -3 (32,33). Indeed it was recently shown that generalized DNA unwrapping occurs from nucleosomes loosening the epigenetic control of the histones, and facilitating widespread gene transcription as in nuclear re-programming in induced pluripotent stem cells, under the control of TLR3 (34). At least two key cytokines, high molecular group box 1 (35) and VEGF (36) undergo regulated trafficking across the nuclear pore so as to control widespread gene transcription cassettes. Furthermore there are three-way interactions between stem cells, immunity and metabolic regulation, further confusing the roles of cardiovascular disease mechanism induction and risk factor potentiation alluded to above (36,37).

Naltrexone (38) and the opiate-free state may also benefit patients by reducing their use of stimulants including tobacco.

The present work has various strengths and limitations. Major design strengths include the application of modern cardiovascular investigative technologies to the problem of clinical opiate dependence which in itself is distinctly novel; the long period of follow-up achieved for many patients; the ability to follow-up patients with these clinical characteristics, who are notoriously transient and itinerant; and the serial "N of one" design where each patient served as their own control. Limitations of the study included its small size, the absence of mechanistic studies, and the lack of a formal instrument to quantitate lifetime drug and treatment exposure. When the study is replicated it is recommended that spot testing for drugs and alcohol be undertaken at every encounter, particularly since these importantly confound the interpretation of arterial stiffness testing. It is also hoped that other tests of cardiovascular health will be applied to this problem and the monitoring of its various treatments.

In conclusion we have shown that the elevated vascular age and arterial stiffness associated with opiate dependence can be reduced by the opiate-free condition. The effect is estimated at being 6-11%, is robust to multivariate adjustment, is seen in both sexes but worse in females, and is likely to become signficant over several decades. It is believed to underlie the pattern of accelerated ageing occurring in opiate dependent patients in many tissue beds. Further research is indicated.

Acknowledgement.

The statistical assistance of Dr. Mervyn Thomas of Emphron Informatics is gratefully acknowledged.

Conflict of Interest.

The authors have no conflict of interest to declare.

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Figure Captions

Figure 1.: Arterial Stiffness Indices by Time Since First Naltrexone Implant.
See Methods section for abbreviations.

Figure 2.: Adjusted Ageing Indices by Time Since First Naltrexone Implant
See Methods section for abbreviations. Ht – Height, Wt – Weight.

Figure 3.: Arterial Stiffness Indices by Time Since First Naltrexone Implant by Sex
See Methods section for abbreviations.

TABLES

Table 1.: Baseline Patient Characteristics

	Mean (+S.E.)	Range
Biometric		
Chronologic Age (CA)	33.62_(2.03)	24.4-54.1
Height (cm)	176.00_(1.62)	160-190
Weight (kg)	84.10_(4.03)	63-122
BMI (kg/m^2)	27.26_(1.35)	18.84-40.3
Substance Use		
Heroin Dose (g)	0.37_(0.05)	0.1-1
Heroin Duration (Yr)	11.375_(1.59)	3-31
Heroin Dose-Duration (g-yr)	4.85_(1.47)	0.75-31
Cigarettes / Day	10.85_(2.07)	0-25
Selected Laboratory Values		
ALT (IU/l)	67.21_(20.27)	14-360
Glucose(mmol/l)	5.62_(0.28)	4-8.6
Platelets ($x10^9$/ml)	258.05_(14.22)	168-415
ESR (mm/hr)	11.22_(1.96)	1-34
hs-CRP (g/l)	4.18_(0.97)	0.1-13.9
Cholesterol (mmol/l)	4.84_(0.32)	2.3-7.3
Triglycerides (mmol/l)	1.25_(0.16)	0.3-2.8
LDL (mmol/l)	2.99_(0.27)	1.13-4.33
HDL (mmol/l)	1.36_(0.11)	0.64-2.25
Cardiovascular Parameters		
CVS Ageing		
Operator Quality Index	88.80_(1.21)	79-99
Vascular Age (RA)	35.00_(4.68)	20-86
RA/CA	1.00_(0.07)	0.65-1.58
RA-CA	2.01_(2.77)	-10.83-31.58
Augmentation		
C_AP_HR75	2.60_(1.46)	-7-16
C_AGPH_HR75	6.25_(3.55)	-19-40
C_PH	36.45_(1.92)	21-62
C_T1R	1480_(2.29)	133-180
C_AL	6.93_(1.24)	1-5
PPAmpRatio	158.80_(3.69)	119-187
P_MAX_DPDT	921.90_(46.05)	382-1182
Pressures		
SP	128.60_(2.9)	103-161
DP	71.00_(1.99)	58-89
C_SP	108.80_(2.72)	87-136
C_DP	72.45_(2.06)	59-91
C_MEANP	89.05_(2.22)	73-105
C_ESP	95.80_(2.42)	78-114
Timing		
HR	70.90_(3.25)	52-95
ED	316.45_(4.85)	274-352
C_SVI	147.20_(8.5)	86-216
C_TTI	2232.05_(118.45)	1456-3231
C_DTI	3106.60_(74.96)	2717-3871

Abbreviations as per Methods Section

Table 2.: Final Mixed Effects Multiple Regression

	Value	Std.Error	DF	t-value	p-value
Time:Clean.Status	-0.0048	0.0018	93	-2.7675	0.0068
Clean.Status	2.9680	1.1733	93	2.5296	0.0131
Time:Clean.Status:SP	0.5064	0.2029	93	2.4956	0.0143
SP:Naltrexone.Status	120.6398	55.6741	93	2.1669	0.0328

Model AIC=56.19, BIC=94.25, LogLik=-14.09.

Table 3.: Effect of Sex on Key CVS Parameters

	Value	Std.Error	DF	t-value	Model AIC	Model LogLik	p-value
RA/(CA*Ht*Wt*SP)	-0.2687	0.0969	18	-2.7720	-124.24	66.12	0.0126
C_AP_HR75	-8.8623	2.7689	18	-3.2007	615.81	-303.91	0.0050
C_AGPH_HR75	-24.7016	6.2097	18	-3.9779	831.06	-411.53	0.0009
PPAmpRatio	26.0517	6.8353	18	3.8114	944.53	-468.26	0.0013
P_MAX_DPDT	252.0485	93.3518	18	2.7000	1552.70	-772.34	0.0147
C_AL	-6.5555	1.4843	16	-4.4167	489.49	-240.74	0.0004

For abbreviations, see Methods Section.

Fig. 1 – Stiffness Measures

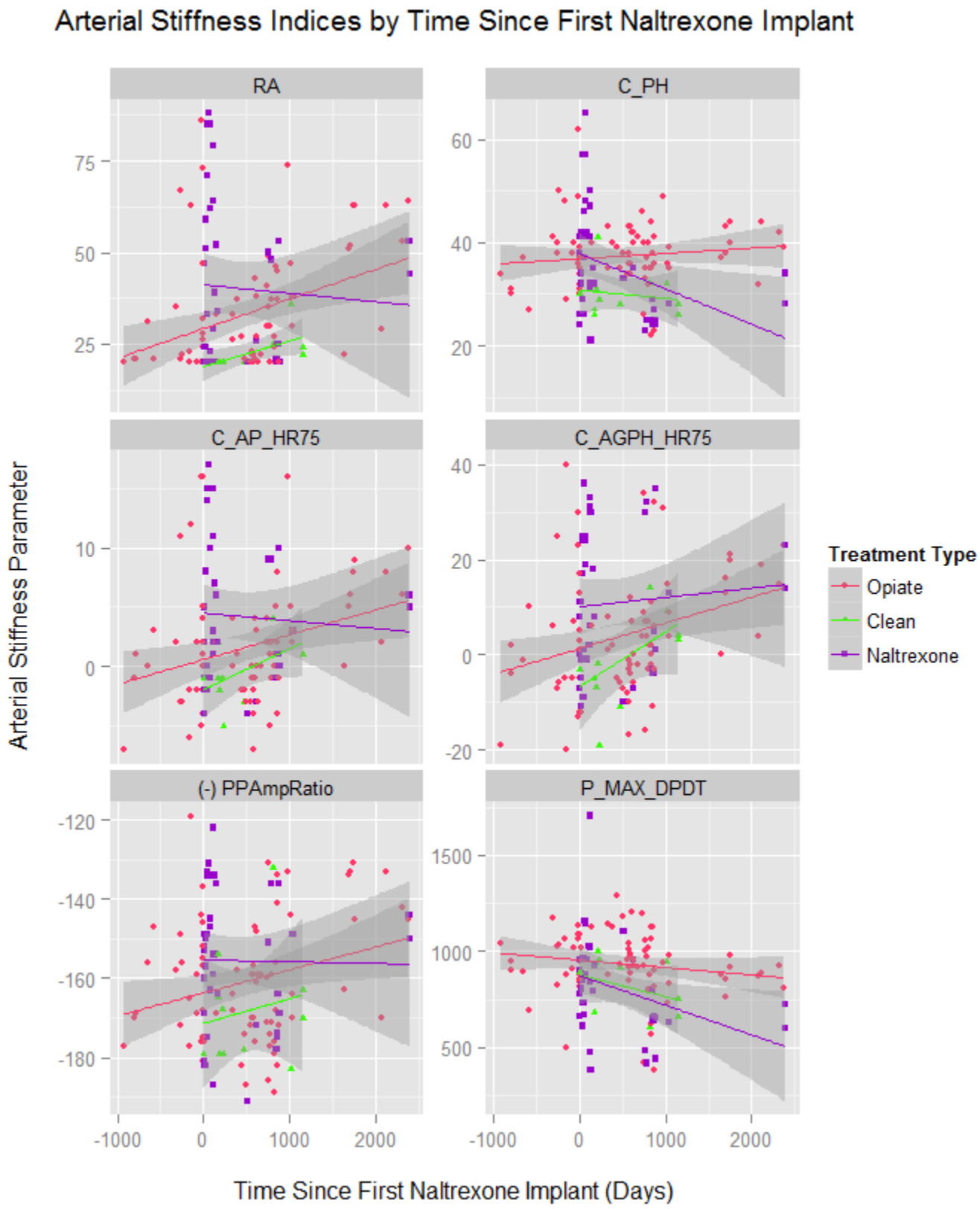


Fig 2 – Adjusted Age

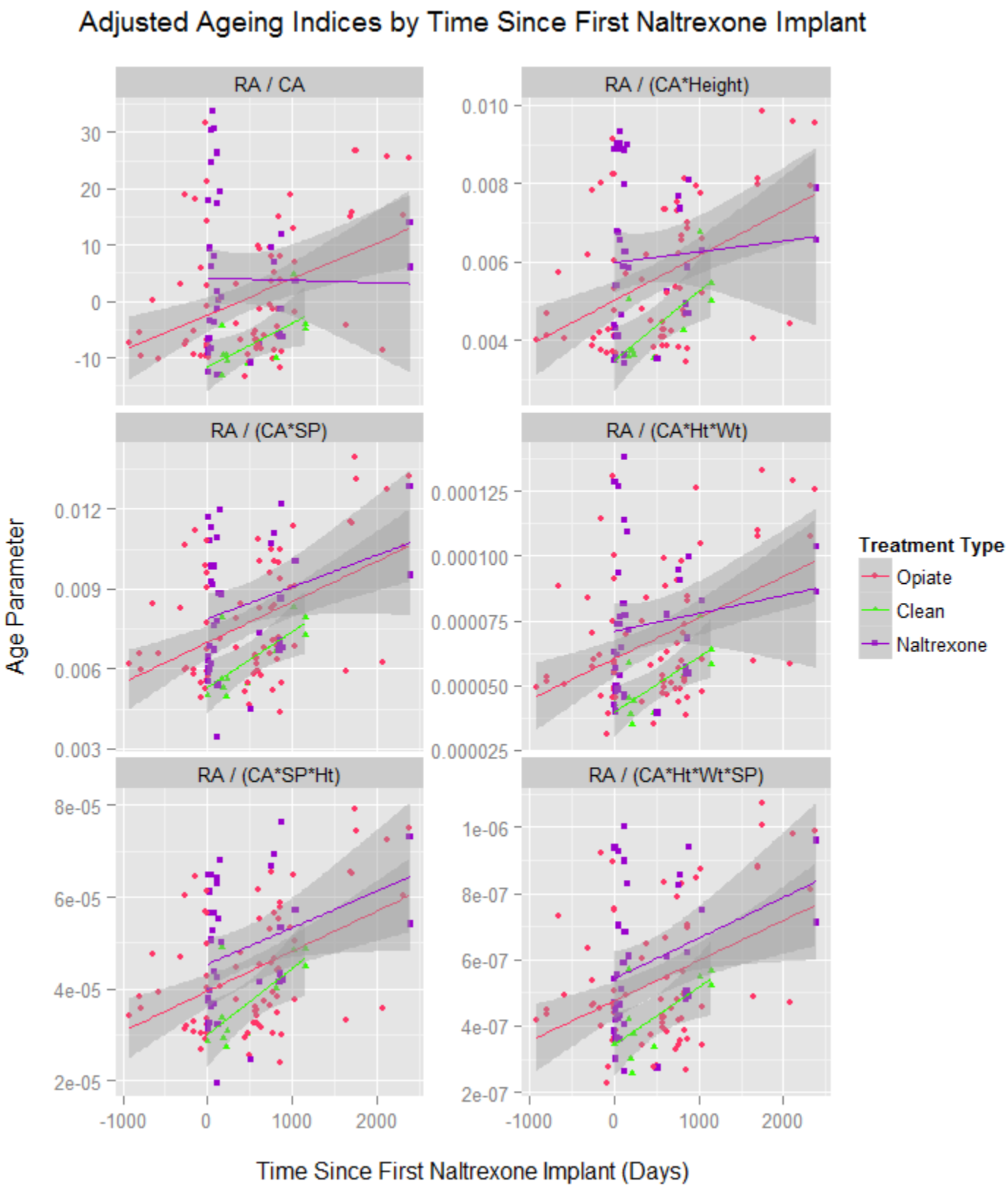
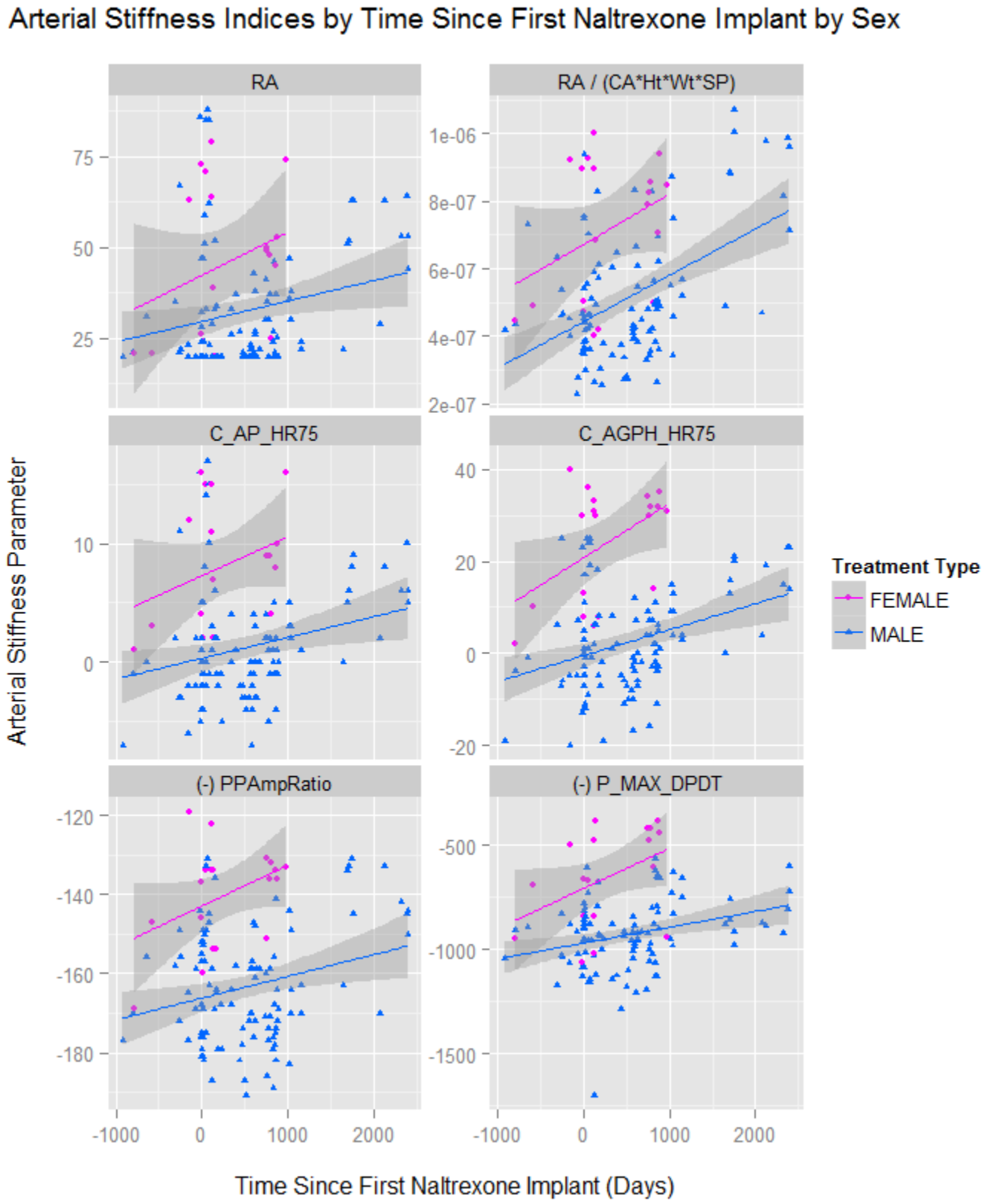
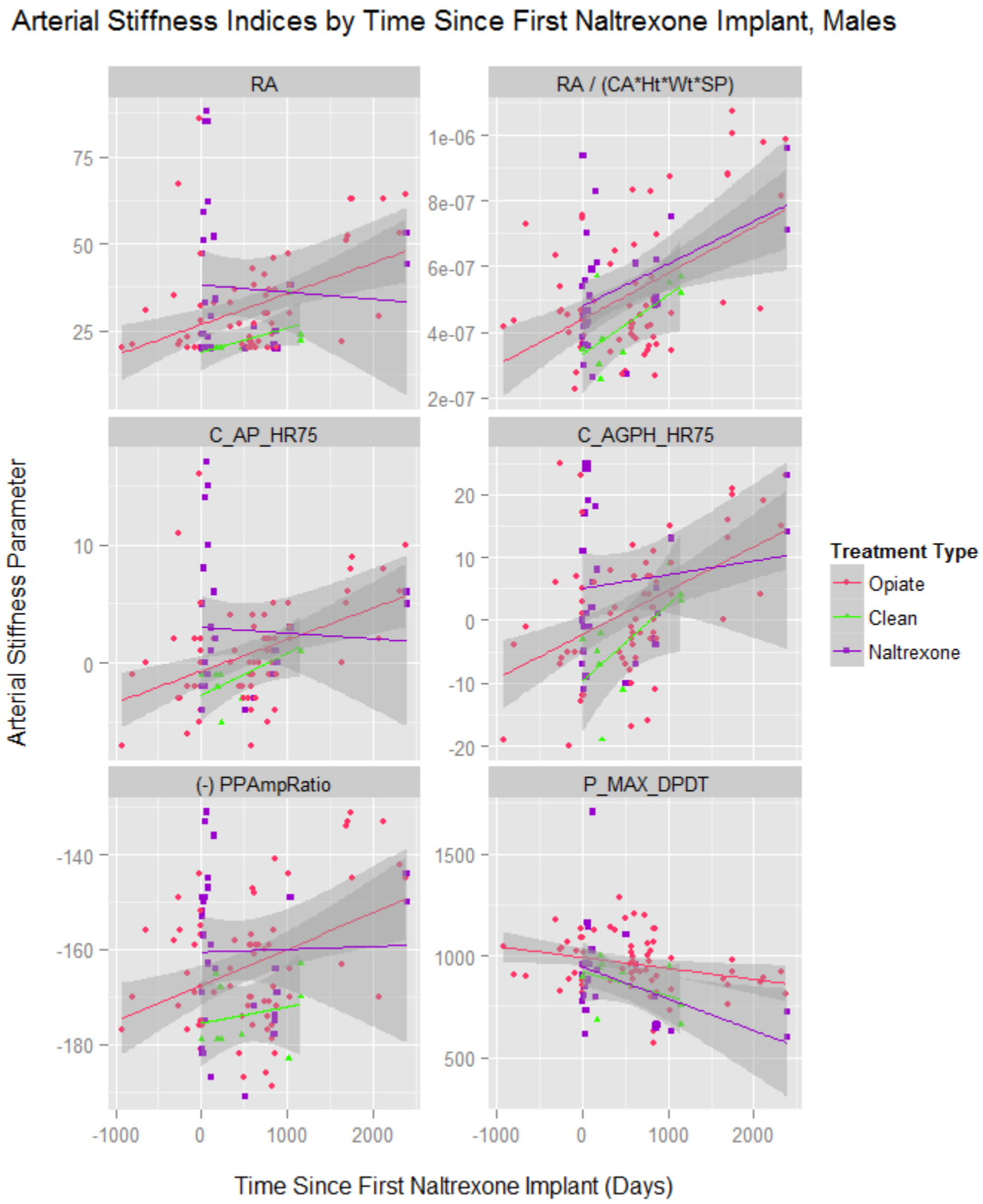


Fig. 3, Sex


Arterial Stiffness Indices by Time Since First Naltrexone Implant by Sex
RA
RA / (CA*Ht*Wt*SP)
C_AP_HR75
C_AGPH_HR75
(-) PPAmpRatio
(-) P_MAX_DPDT
Arterial Stiffness Parameter
Time Since First Naltrexone Implant (Days)
Treatment Type
FEMALE
MALE

Suppl. Fig. 1, Males



Suppl. Fig. 2, Females