

# 1 **Carbonyl Reductase 1: a novel regulator of blood pressure in Down Syndrome**

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23 **Short Title:** Carbonyl reductase 1 and blood pressure

24 **Abstract**

25 Approximately one in every 800 children is born with the severe aneuploid condition of Down  
26 Syndrome, a trisomy of chromosome 21. Low blood pressure (hypotension) is a common condition  
27 associated with DS and can have a significant impact on exercise tolerance and quality of life. Little is  
28 known about the factors driving this hypotensive phenotype and therefore therapeutic interventions  
29 are limited. Carbonyl reductase 1 (CBR1) is an enzyme contributing to the metabolism of  
30 prostaglandins, glucocorticoids, reactive oxygen species and neurotransmitters, encoded by a gene  
31 (*CBR1*) positioned on chromosome 21 with the potential to impact blood pressure. Utilising telemetric  
32 blood pressure measurement of genetically modified mice, we tested the hypothesis that CBR1  
33 influences blood pressure and that its overexpression contributes to hypotension in Down Syndrome  
34 by evaluating possible contributing mechanisms *in vitro*. In a mouse model of Down Syndrome  
35 (Ts65Dn), which exhibits hypotension, CBR1 activity was increased and pharmacological inhibition  
36 of CBR1 increased blood pressure. Mice heterozygous null for *Cbr1* had reduced CBR1 enzyme  
37 activity and elevated blood pressure. Further experiments indicate that the underlying mechanisms  
38 include alterations in sympathetic tone and prostaglandin metabolism. We conclude that CBR1  
39 activity contributes to blood pressure homeostasis and inhibition of CBR1 may present a novel  
40 therapeutic opportunity to correct symptomatic hypotension in Down Syndrome.

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42 **Keywords:** Down Syndrome, blood pressure, carbonyl reductase 1, sympathetic drive

43 **Non-standard abbreviations and acronyms**

44 CBR1/Cbr1: Carbonyl reductase 1

45 DS: Down Syndrome

46 MESOR: Midline Estimating Statistic of Rhythm (Rhythm-adjusted mean)

47 TBARS: Thiobarbituric acid reactive substances

48 MDA: Malondialdehyde

49 MAP: Mean arterial pressure

50 ROS: Reactive oxygen species

51 8-OHdG: 8-hydroxy-2'-deoxyguanosine

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## 64 Introduction

65 Down Syndrome (DS) is the most common chromosomal disorder, affecting approximately 1 in every  
66 800 babies born<sup>1</sup>. 95 % of people with DS have a trisomy of chromosome 21 with resultant effects on  
67 development. Patients with DS are at risk of comorbidities including hypothyroidism, sleep apnoea,  
68 obesity, metabolic syndrome, psychiatric disorders and Alzheimer's disease<sup>2,3</sup>. Low blood pressure –  
69 hypotension - is common in both children and adults with DS<sup>4,6</sup>. This hypotension results in lower  
70 cardiorespiratory fitness and an inadequate blood pressure response to sub-maximal and maximal  
71 exercise<sup>7,8</sup>, limiting the ability to participate in many activities<sup>9</sup> which in turn impacts quality of life.  
72 DS patients also commonly have non-dipping nocturnal blood pressure and heart rate which may  
73 contribute to sleep disorders and an increased risk of cardiovascular events<sup>10-12</sup>. There is additionally a  
74 well-documented association between low blood pressure and the development of Alzheimer's  
75 disease which is particularly common in patients with DS<sup>5</sup>. This baseline hypotension makes the  
76 interpretation of blood pressure as a diagnostic tool for detecting other co-morbidities challenging<sup>4</sup>.  
77 Despite these impacts, the pathogenesis of hypotension in DS has not been elucidated; some have  
78 suggested that it is due to autonomic dysfunction since clinical studies report reduced sympathetic and  
79 increased parasympathetic tone in patients with DS<sup>13-17</sup>.

80 *CBR1*, the gene encoding the ubiquitously expressed enzyme carbonyl reductase 1<sup>18</sup>, is located in the  
81 'Down Syndrome critical region' of chromosome 21, the region that co-segregates with many of the  
82 developmental features of DS<sup>19,20</sup>. *CBR1* is a complex enzyme with a number of substrates and is  
83 most often studied for its role in metabolism of therapeutics such as doxorubicin<sup>21</sup>. *CBR1* is found in  
84 almost every cell including in the vasculature (endothelial and smooth muscle cells), the heart, liver,  
85 kidney and throughout the brain<sup>22, 23</sup> ([Tissue Cell Type - IGHG1 - The Human Protein Atlas](#)). *CBR1*  
86 plays a critical role in cellular homeostasis and blood flow regulation by preventing the accumulation  
87 of reactive oxygen species, vasoconstrictor prostaglandin E<sub>2</sub> and neuroactive metabolites such as  
88 monoamine oxidase inhibitor and endogenous indoles<sup>22-27</sup>. Recent data suggest that *CBR1* activity is  
89 important in regulating renal blood flow via prostaglandin metabolism<sup>28</sup>. Our work has also shown the  
90 role of *CBR1* in tissue metabolism of glucocorticoids<sup>29</sup> and its impact on glucose homeostasis in lean

91 mice<sup>30</sup>. In this study we used a transgenic murine model of *Cbr1* deletion, as well as pharmacological  
92 inhibition of CBR1 in a murine model of Down Syndrome, to address the hypothesis that CBR1/*Cbr1*  
93 plays a role in blood pressure regulation and that dysregulation of CBR1 contributes to hypotension in  
94 DS. We also explore the potential mechanisms by which this might occur.

## 95 **Materials and Methods**

### 96 **Animals**

97 All experiments were performed at the Queen's Medical Research Institute, University of Edinburgh  
98 in accordance with the UK's Animals (Scientific Procedures) Act under a UK Home Office Project  
99 Licence in accordance with EU Directive 2010/63/EU. Male B6EiC3Sn.BLiA-Ts(1716)65Dn/DnJ  
100 (Ts65Dn), a model of DS, with littermate controls were obtained from The Jackson laboratory (RRID:  
101 IMSR\_JAX:005252)<sup>31</sup>. This line contains a partial trisomy encompassing most of the human  
102 chromosome 21 orthologous region of mouse chromosome 16<sup>32</sup>, including *Cbr1*<sup>33</sup>. These animals  
103 have been well characterised with regards cerebellar volume which is reduced, as in DS<sup>31</sup>. They  
104 demonstrate increased locomotor activity and energy expenditure<sup>34</sup>, have reduced blood pressure<sup>35</sup>  
105 and impaired conscious respiration associated with a decreased neural drive<sup>36</sup>. Mice heterozygous for  
106 *Cbr1* (*Cbr1*<sup>+/-</sup>) were generated as previously described<sup>30</sup>, homozygosity of this gene deletion is foetal  
107 lethal<sup>37</sup>. Data from our group has previously shown that this model has an approximately 50 %  
108 reduction in CBR1 expression and activity<sup>30</sup>. Mice were maintained according to institutional  
109 guidelines, group housed at 21 ± 1 °C; humidity at 50 ± 10 % with a 12-hour light-dark cycle (light  
110 period 07:00-19:00) unless otherwise stated. Mice were randomly allocated to cage and all  
111 environmental factors were kept the same between cages to minimise bias. Unless otherwise  
112 specified, mice were killed by cervical dislocation. Mice were fed on a diet containing 0.3 % Na and  
113 0.7 % K by weight (RM1 diet, Special Diet Services, United Kingdom) throughout the experiment  
114 unless otherwise stated. None of the mice included at the start of the study were excluded from any  
115 analysis. Blood pressure was measured by telemetry in *Cbr1*<sup>+/-</sup> and *Cbr1*<sup>+/+</sup> littermate controls at  
116 baseline and during a high salt diet, and in Ts65Dn mice and their littermate controls at baseline and  
117 during treatment with hydroxy-PP-Me, an inhibitor of CBR1<sup>38,39</sup>. Hydroxy-PP-Me was synthesised

118 using modifications of methods previously described<sup>39</sup>. Renal function, vascular function, plasma  
119 renin, angiotensin and aldosterone were measured in mice heterozygous for *Cbr1* and their littermate  
120 controls.

### 121 **Blood pressure measurement**

122 Ten-week-old male mice (*Cbr1*<sup>+/-</sup>, *Cbr1*<sup>+/+</sup>, Ts65Dn mice and wild-type littermates (n=8/group)) had  
123 PA-C10 radio-telemetry devices (Data Science International, USA) implanted into the carotid artery  
124 under isoflurane anaesthetic (4 % induction, 2-3 % maintenance). Buprenorphine (0.1 mg/kg  
125 Vetergesic; Ceva Animal Health Ltd, Libourne, France) was administered subcutaneously prior to  
126 recovery and per os (Vetergesic jelly) for the first four days. Mice were randomly assigned to the  
127 order of surgery. Mice underwent a one-week post-surgical recovery period as basal diurnal  
128 rhythmicity of the measures was re-established. Data were obtained for the following 7 days. For the  
129 duration of the experiment, five consecutive one minute blood pressure and heart rate readings were  
130 taken every 30 min at an acquisition rate of 1kHz.

131 Ts65Dn mice and their wild-type controls then received hydroxy-PP-Me for 1 week during which  
132 data were collected. Hydroxy-PP-Me was administered intraperitoneally at a dose of 30mg/kg based  
133 on previously published data<sup>39</sup>. Previous work from our group showed there was no effect of  
134 intraperitoneal injection alone on blood pressure<sup>40</sup>. *Cbr1*<sup>+/-</sup> mice and *Cbr1*<sup>+/+</sup> littermates did not  
135 receive the CBR1 inhibitor but did receive a high-salt diet (3 % Na) for 7 days (see supplementary  
136 data). Raw data are available on request from the authors.

### 137 **CBR1 activity**

138 CBR1 activity, as measured by reduction of the substrate doxorubicin, was quantified in hepatic, brain  
139 or cardiac cytosol from Ts65DN animals and their littermate controls with or without administration  
140 of hydroxy-PP-Me (n=6/group), as previously described<sup>41-43</sup>. Briefly cytosol from homogenised tissue  
141 was extracted by ultracentrifugation, the protein quantified by Bradford protein assay. Cytosol was  
142 incubated with 50 µM doxorubicin, the reaction was started by addition of co-factor NADPH whose

143 oxidation was measured at 340 nm at 37 °C over 3 minutes. Enzymatic velocities were calculated by  
144 linear regression of the change in absorbance over time.

### 145 **Urine collection and analysis**

146 For collection of urine, mice were housed in metabolic cages for 48 hours (n=8-10/group). Urinary  
147 catecholamines adrenaline and noradrenaline were measured by enzyme linked immunoassay  
148 (ELISA) (CatCombi ELISA Kit, Creative Diagnostics, DEIA1663). Prostaglandin E<sub>2</sub> metabolite was  
149 measured by ELISA (Cayman Chemical, 514531) according to the manufacturer's protocol. Urinary  
150 8-hydroxy-2'-deoxyguanosine (8-OHdG) was measured by ELISA (Abcam, ab201734) according to  
151 manufacturer's instructions.

### 152 **Quantitative qPCR**

153 Liver, heart and kidneys from mice were harvested and snap frozen in liquid nitrogen at post-mortem.  
154 One kidney from each animal was separated into cortex and medulla prior to freezing. RNA was  
155 isolated using RNeasy kits (Qiagen, US), and quantified by spectrophotometry (NanoDrop-1000,  
156 Thermo Fisher Scientific, UK) and 500ng cDNA synthesised using high-capacity RNA-to-cDNA kit  
157 (Thermo Fisher Scientific, UK). mRNA abundance of relevant transcripts was measured by  
158 quantitative RT-PCR using the Universal Probe Library (Roche, UK). Triplicates of each sample and  
159 standard curve were run on the LightCycler 480 (Roche, UK). Expression was normalized to the mean  
160 concentration of housekeeping genes.

161 **Table 1 Details of Primers used in qPCR**

Gene Symbol, full name	Accession Number	Forward Primer (3' → 5')	Reverse Primer (5' → 3')	T <sub>m</sub>	Product Length
<i>18S ribosomal RNA (Rn18s)</i>	NR_003278.3	GTAACCCGTTGAACCCC ATT	CCATCCAATCGGTAGTA GCG	58.09	151
		Reverse Primer (5' → 3')	CCATCCAATCGGTAGTA GCG	57.93	
<i>Cbr1, Carbonyl Reductase 1</i>	NM_007620.3	Forward Primer (3' → 5')	CCCAGATGTCTGCAAGGA G	60.18	142
		Reverse Primer (5' → 3')	TCTGTGATGGTCTCGCTTCG	59.83	

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## 163 **Renal function and salt handling**

164 *Cbr1*<sup>+/-</sup> and wild-type littermates (n=6/group) were anaesthetized (thiobutabarbital; Inactin; Sigma-  
165 Aldrich, Darmstadt, Germany; 120 mg/kg intraperitoneally) and the jugular vein cannulated and  
166 isotonic saline containing 0.25 % fluorescein isothiocyanate-inulin (FITC-inulin) infused. The carotid  
167 artery was cannulated for blood sampling and measurement of BP (Powerlab, AD Instruments, UK).  
168 Following baseline measurements hydrochlorothiazide was injected intravenously (2 mg/kg  
169 hydrochlorothiazide in 0.9 % NaCl and 1 % DMSO)<sup>44</sup>. Arterial blood was sampled every 40 minutes  
170 on three occasions, separated using Haematospin 1400 (Hawksley, UK) and haematocrit read using  
171 Microhaematocrit Reader (Hawksley, UK). FITC-Inulin was measured by fluorescence (Tecan  
172 Sunrise, Tecan Lifesciences, Switzerland) in urine and arterial samples for calculation of glomerular  
173 filtration rate.

## 174 **Histological examination**

175 Following perfusion fixation, kidneys were collected from 8-week-old male *Cbr1*<sup>+/-</sup> mice and *Cbr1*<sup>+/+</sup>  
176 littermates (n=4/group). These were longitudinally sectioned and routinely processed through graded  
177 alcohol into paraffin prior to sectioning at 2 µm and staining with haematoxylin and eosin (H&E).  
178 The sections were examined by a board-certified veterinary pathologist.

## 179 **Vascular Function**

180 Eight-week-old male *Cbr1*<sup>+/-</sup> mice and wild-type littermates (n=6/group) fed a control diet (0.3% Na)  
181 were subject to cervical dislocation after which second order mesenteric arteries were immediately  
182 harvested, submerged in physiological salt solution (PSS; mM: 119.0 NaCl, 4.7 KCl, 2.5 CaCl<sub>2</sub>, 1.17  
183 MgSO<sub>4</sub>, 25.0 NaHCO<sub>3</sub>, 1.81 EDTA, 5.5 D-glucose) and cleaned of adherent perivascular adipose  
184 tissue. Wire myography (DMT, Denmark) was used to evaluate the reactivity of the vessels. Vessels  
185 were equilibrated under passive tension. Vessel viability was assessed using consecutive stimulations  
186 with high potassium physiological saline solution (KPSS, 125 mM) followed by a washout period.  
187 Cumulative concentration-response curves were obtained for vasoconstrictors, phenylephrine (1x10<sup>-9</sup>  
188 – 1x10<sup>-4</sup> M), noradrenaline (1x10<sup>-9</sup> – 1x10<sup>-4</sup> M), 5-hydroxytryptamine (5HT) (1x10<sup>-9</sup> – 1x10<sup>-4</sup> M) and

189 endothelin 1 ( $1 \times 10^{-12}$  –  $1 \times 10^{-6}$  M). Following contraction with phenylephrine to produce 80 % of the  
190 KPSS response, a cumulative concentration-response curve was obtained for acetylcholine ( $1 \times 10^{-9}$  –  
191  $1 \times 10^{-4}$  M) and sodium nitroprusside ( $1 \times 10^{-9}$  –  $1 \times 10^{-4}$  M).

## 192 **Markers of oxidative stress**

193 Plasma was collected from animals at cull. Brains were harvested at post-mortem, snap frozen in  
194 liquid nitrogen and stored at  $-80$  °C. Total anti-oxidant capacity was measured in plasma using a  
195 colorimetric assay based on reduction of ferric ions ( $\text{Fe}^{3+}$ ) to ferrous ions ( $\text{Fe}^{2+}$ ) using a  
196 phenanthroline substance according to manufacturer's instructions (ThermoFisher EEA022).  
197 Malondialdehyde (MDA) was measured in plasma and brain homogenate by quantifying the adduct  
198 generated when MDA in the sample reacts with thiobarbituric acid (TBA) (Abcam, ab118970, Lipid-  
199 Peroxidation Kit).

## 200 **Plasma analysis**

201 Plasma aldosterone, corticosterone and 11-dehydrocorticosterone were measured by liquid  
202 chromatography tandem mass spectrometry as previously described<sup>30</sup>. Plasma renin was measured by  
203 ELISA (Abcam, ab193728).

## 204 **Statistical Analysis**

205 Power calculations were used to determine sample size (G\*Power<sup>45</sup> RRID:SCR\_013726) for reliable  
206 detection of differences in blood pressure as measured by telemetry. They were based on previously  
207 published differences in blood pressure between Ts65Dn mice and their wild-type littermates<sup>35</sup>. A  
208 sample size of 7/group was determined to be sufficient to give 80 % power to detect a difference with  
209 a significance of  $P < 0.05$  using Cohen's d effect size; we used 8 animals/group to allow for any  
210 complications of telemetry but we did not have to exclude any animals from analysis. For the renal  
211 function and tissue analysis we used 6-9 animals/group.

212 All data were tested for normality using the Kolmogorov-Smirnoff normality test, and the appropriate  
213 parametric or nonparametric statistical tests were used accordingly. All statistical tests used were two-

214 tailed. Statistical comparisons were made using a Student's t-test or Mann-Whitney U test or two-way  
215 ANOVA tests with appropriate post hoc tests (Tukey's) for multiple groups. The asterisks in the  
216 figures indicate statistical significance: \* $P < 0.05$ , \*\* $P < 0.01$ , and \*\*\* $P < 0.001$ . All graphs were  
217 plotted with GraphPad Prism software (RRID:SCR\_002798) or R ggplot (RRID:SCR\_014601).  
218 Blood pressure data were analysed in two ways: first by comparison of the medians of blood pressure  
219 and heart rate during the inactive and active periods; and second by cosinor analysis which takes into  
220 account the circadian rhythm of these measures. This included calculation of the amplitude and  
221 MESOR. Amplitude is a measure of the magnitude of fluctuation in blood pressure and heart rate over  
222 the course of 24 hours. The amplitude allows us to determine the extent of drop or dipping in blood  
223 pressure which should occur during the inactive period. MESOR refers to the midline estimating  
224 statistic of rhythm, it is the baseline or average value around which a circadian rhythm fluctuates,  
225 unlike a mean blood pressure alone MESOR reflects the centre point of the biological rhythm.  
226 Cosinor analysis was conducted and visualised using the R packages Circacompare and  
227 Limorhyde<sup>46,47</sup>.

## 228 **Results**

### 229 **Blood pressure in *Cbr1*<sup>+/-</sup> mice**

230 Mice heterozygous for *Cbr1* had increased median systolic pressure during both the active and  
231 inactive periods and increased diastolic and mean arterial pressure during the inactive phase compared  
232 to *Cbr1*<sup>+/+</sup> littermate controls (Table 1). There was no difference in median heart rate between *Cbr1*<sup>+/-</sup>  
233 and *Cbr1*<sup>+/+</sup> littermate controls.

234 The blood pressure and heart rate of both *Cbr1*<sup>+/-</sup> and *Cbr1*<sup>+/+</sup> littermate controls could be modelled  
235 with a cosine curve indicating a circadian rhythm, as expected. The rhythm-adjusted mean (MESOR,  
236 midline estimating statistic of rhythm) of systolic, diastolic and mean arterial pressure was increased  
237 in *Cbr1*<sup>+/-</sup> mice compared with *Cbr1*<sup>+/+</sup> controls (Fig 1, Table 1). There was no difference in the  
238 amplitude between the groups for any blood pressure parameter measured. This indicates that blood  
239 pressure was increased in *Cbr1*<sup>+/-</sup> mice during both the active and inactive period and that the

240 magnitude of the inactive dipping was not affected by genotype (Fig 1, Table 1). The MESOR of  
241 heart rate was significantly higher in *Cbr1*<sup>+/-</sup> mice compared with *Cbr1*<sup>+/+</sup> controls (Fig 1, Table 1).  
242 The amplitude did not differ between the groups for heart rate indicating *Cbr1*<sup>+/-</sup> mice retained a  
243 dipping of blood pressure in the inactive phase (Fig 1, Table 1).

#### 244 **Inhibition of CBR1 in a mouse of model of DS**

245 We hypothesised that a mouse model of DS, Ts65Dn, would have relative hypotension and that  
246 pharmacological inhibition of CBR1 would increase blood pressure.

247 We first confirmed that Ts65Dn mice had higher hepatic and cardiac mRNA levels and CBR1 activity  
248 (Fig. S1) compared with littermate controls. We then determined the extent of inhibition of CBR1  
249 activity by the drug. Administration of the selective CBR1 inhibitor, hydroxy-PP-Me, reduced hepatic  
250 and brain CBR1 activity in Ts65Dn mice to equivalent to the wild type mice but did not reduce  
251 cardiac CBR1 activity (Fig S1).

252 Blood pressure was measured at baseline and during treatment with hydroxy-PP-Me. Median systolic,  
253 diastolic and mean arterial pressure during both the inactive period and active period were  
254 significantly lower in Ts65Dn mice compared with wild-type littermates (Table 2). Heart rate was  
255 significantly higher in the Ts65Dn mice compared with littermate controls (Table 2).

256 Cosinor analysis also showed that the MESOR (the rhythm-adjusted means) of the systolic, diastolic  
257 and mean arterial pressures were significantly lower in Ts65Dn mice compared with wild-type  
258 littermates (Fig. 2, Table 3). MESOR of heart rate was significantly higher in the Ts65Dn mice  
259 compared with littermate controls (Fig. 2, Table 3). The amplitude of the circadian rhythm was not  
260 different between the groups for systolic pressure or heart rate. The amplitude of diastolic pressure  
261 and mean arterial pressure (MAP) was larger in the Ts65Dn mice compared with wild-type controls,  
262 corresponding to an increase in both active period blood pressure peak and inactive period blood  
263 pressure dip (Fig. 2, Table 3).

264 Treatment with hydroxy-PP-Me significantly increased the MESOR of systolic, diastolic and mean  
265 arterial pressure of Ts65Dn mice from baseline but decreased the MESOR in the wild type mice (Fig.

266 2, Table 3). There was a decrease in the amplitude of the rhythm in both wild-type and Ts65Dn mice  
267 corresponding to a reduction in the inactive phase dip in blood pressure i.e. inhibition of CBR1  
268 blunted the fall in blood pressure (Table 3). Amplitude and MESOR of heart rate were significantly  
269 reduced by treatment in both groups of mice (Table 3).

## 270 **Mechanisms altering blood pressure**

271 To determine if the blood pressure phenotype observed in *Cbr1*<sup>+/-</sup> mice was salt-sensitive, the animals  
272 were given a high-salt diet (3 % sodium) and blood pressure was measured by telemetry for 7 days.  
273 During high salt feeding the mean systolic, diastolic and mean arterial pressure (MAP) increased in  
274 both groups but the difference between the groups remained constant (Table S1) demonstrating that  
275 salt sensitivity was similar between the groups. We confirmed that there were no differences in renal  
276 function as measured by glomerular filtration rate between *Cbr1*<sup>+/-</sup> mice and *Cbr1*<sup>+/+</sup> littermate  
277 controls (Fig. S2). Renal histology determined by light microscopy of haematoxylin and eosin-stained  
278 sections was normal in both genotypes (Fig. S2). The components of the renin-angiotensin-  
279 aldosterone system were not different between the groups (Fig. S3).

280 We then examined vascular function in *Cbr1*<sup>+/-</sup> animals and found no differences in the response of  
281 mesenteric vessels to vasoconstrictors or vasodilators to those of *Cbr1*<sup>+/+</sup> littermate controls (Fig. S4).

282 Plasma glucocorticoids (corticosterone and its inactive form 11-dehydrocorticosterone) measured by  
283 liquid chromatography tandem mass spectrometry were not different between the groups (Fig. S5).

284 Next, we examined known functions of CBR1 which may influence blood pressure by changing the  
285 vascular microenvironment. We explored the potential for CBR1 to impact oxidative stress,  
286 sympathetic tone and prostaglandin metabolism.

## 287 **Oxidative stress**

288 CBR1 mediates detoxification of ROS making this a potential mechanism by which it influences  
289 blood pressure. We therefore looked at measures of whole-body oxidative stress (total antioxidant  
290 capacity), lipid peroxidation (TBARS assay) and urinary 8-hydroxy-2'-deoxyguanosine (8-oxo-dG) as

291 well as brain specific malondialdehyde (MDA). There were no differences in plasma or urinary  
292 measures of oxidative stress but brain MDA concentrations were increased in *Cbr1*<sup>+/-</sup> animals  
293 compared with *Cbr1*<sup>+/+</sup> littermate controls (Fig. S6).

### 294 **Sympathetic Activity**

295 We determined if urinary excretion of catecholamines noradrenaline and adrenaline, as a proxy for  
296 sympathetic drive, were altered in *Cbr1*<sup>+/-</sup> compared with *Cbr1*<sup>+/+</sup> littermate controls. Twenty-four-  
297 hour urinary excretion of noradrenaline and adrenaline was measured in mice housed in metabolic  
298 cages. Urinary excretion of noradrenaline but not adrenaline was increased in *Cbr1*<sup>+/-</sup> animals  
299 compared with their littermate controls (Fig. 3 A and C). We also showed that the mouse model of DS  
300 demonstrated decreased urinary excretion of noradrenaline but not adrenaline (Fig. 3 B and D).  
301 Administration of hydroxy-PP-Me normalised noradrenaline excretion in Ts65Dn animals (Fig. 3).

### 302 **Prostaglandin excretion**

303 CBR1 inactivates prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and converts it to prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>), a mediator of  
304 blood pressure. As such we measured excretion of the metabolites of substrate PGE<sub>2</sub> in urine of mice  
305 heterozygous for *Cbr1* (*Cbr1*<sup>+/-</sup>) compared with their littermate controls (*Cbr1*<sup>+/+</sup>) and found that  
306 *Cbr1*<sup>+/-</sup> mice had increased excretion indicating reduced systemic metabolism (Fig. 3). The opposite  
307 was true of Ts65Dn animals compared with littermate controls, but this was normalised by  
308 administration of hydroxy-PP-Me (Fig. 3).

309

## 310 Discussion

311 In this study we present the first demonstration of carbonyl reductase 1 (CBR1) as a novel regulator of  
312 blood pressure. Our data indicate that increased CBR1 contributes to hypotension observed in a  
313 mouse model of Down Syndrome. Additionally, mice heterozygous for *Cbr1*, with a 50 % reduction  
314 in enzyme activity in all tissues<sup>30</sup>, had increased systolic, diastolic and mean arterial pressure. In the  
315 absence of changes in renal function, salt sensitivity or vascular reactivity, the most plausible drivers  
316 of altered blood pressure are the observed alterations in sympathetic tone and prostanoid metabolism,  
317 inferred from urinary catecholamine and prostaglandin excretion.

318 It is suggested that, in DS, blunted sympathetic control is associated with exercise intolerance and low  
319  $VO_2$  max<sup>17,48</sup> and is also implicated in sleep apnoea in these patients<sup>11</sup>. Others have shown a reduced  
320 catecholamine response to exercise in adults with DS<sup>49</sup> and a lack of vasoconstriction in response to  
321 sympathoexcitation<sup>50</sup>. Hypotension and exercise intolerance can have a significant impact on quality  
322 of life for people with DS; limiting exercise, contributing to sleep disturbances and potentially  
323 accelerating the onset and progression of Alzheimer's disease<sup>3,5</sup>. There are currently no specific  
324 treatments available for hypotension in DS as the pathophysiology is unknown. Our study suggests  
325 that decreasing CBR1 activity either genetically or pharmacologically can increase sympathetic tone,  
326 particularly noradrenaline release, which can impact blood pressure control.

327 We cannot be sure of the mechanism by which CBR1 influences sympathetic tone; the protein is  
328 expressed throughout the brain and adrenal medulla and its effects could be direct or indirect. We  
329 found changes in both systemic prostaglandin metabolism and brain oxidative stress relative to CBR1  
330 activity, both of which could indirectly affect sympathetic output.  $PGE_2$ , a substrate of CBR1<sup>51</sup>, is  
331 known to induce hypertension and catecholamine release when administered intracerebroventricularly  
332 to rats<sup>52,53</sup> and yet have the opposite effect when given systemically<sup>54</sup>. Reduced levels of  $PGE_2$  in the  
333 brain are found in the Ts1Cje rodent model of Down Syndrome and this is reversed when the copy  
334 number of the *Cbr1* gene is restored<sup>55</sup>. Our results are consistent with this, demonstrating that mice  
335 with reduced CBR1 activity had reduced metabolism (and hence increased excretion) of  $PGE_2$   
336 metabolites. We did not identify the source of this increased  $PGE_2$  but given we did not see

337 differences in plasma renin, and systemic vascular function was unaffected, we might hypothesise that  
338 the increases were localised in the brain and thereby influencing sympathetic activity or alternatively  
339 acting directly on the cerebral vasculature.

340 CBR1 may also impact sympathetic tone or blood pressure by alterations in oxidative stress.  
341 Oxidative stress appears to stimulate central sympathetic outflow in various models of hypertension<sup>56</sup>  
342 but little is reported in relation to hypotension. CBR1 is known to reduce oxidative stress centrally  
343 where it inactivates highly reactive lipids<sup>57</sup> and this was apparent in our work which showed increased  
344 levels of MDA in the brains of mice deficient in *Cbr1*. Serum MDA levels have consistently been  
345 found to be elevated in patients with hypertension<sup>58</sup> and are thought to be a marker of increased  
346 systemic oxidative stress. However, the casual direction in hypertension is unclear<sup>56</sup>. Interestingly, our  
347 findings were confined to the brain and we found no evidence of a systemic increase in markers of  
348 oxidative stress in *Cbr1*<sup>+/-</sup> mice. This is consistent with the normal vascular and renal function we saw  
349 in these animals and it is also likely that compensatory mechanisms come into play when *Cbr1* is  
350 lacking or that 50 % of normal levels are sufficient to protect cells elsewhere. To our knowledge our  
351 work is the first to demonstrate that a reduction or imbalance in oxidative stress may contribute to  
352 hypotension and we proffer that a perfect balance is required throughout to maintain optimal blood  
353 pressure.

354 CBR1 could also affect the sympathetic nervous system more directly; for example, it was recently  
355 described as the predominant pathway by which the endogenous monoamine oxidase inhibitor, isatin,  
356 is inactivated<sup>26,59</sup>. Increases in isatin have been associated with hypertension<sup>60</sup>. It is most likely that a  
357 combination of all these proposed mechanisms play a part in the phenotype and our data suggest that  
358 there is a critical and optimal level of CBR1 activity which maintains homeostasis in the  
359 microvascular environment. Indeed inhibition of CBR1 in wild type animals increased the MESOR of  
360 blood pressure whilst still decreasing the amplitude of each blood pressure parameter and heart rate  
361 suggesting that compensation is possible when CBR1 is not elevated.

362 Despite inhibition of CBR1 with hydroxy-PP-Me resulting in tissue-specific rather than systemic  
363 enzyme inhibition there was still a blunting of the normal inactive phase dip in blood pressure and an



364 increase in noradrenaline excretion in this mouse model of DS. This suggests there is merit in  
365 pursuing CBR1 inhibition by this or other compounds<sup>61,62</sup> as a therapeutic intervention in patients for  
366 whom hypotension impacts quality of life. It is interesting to note that inhibition of CBR1 reduced  
367 blood pressure in the wild type mice in whom CBR1 levels were “normal” so it seems likely that a  
368 critical balance of CBR1 activity is required to maintain a normal vascular microenvironment and  
369 blood pressure; as such, partial inhibition may be an attractive therapeutic option.

370 Whilst we have focused on the role of *Cbr1* in DS, our work has wider implications. In the general  
371 population there is wide variation in CBR1 expression and activity levels between the sexes and  
372 between ethnic groups<sup>63</sup> and our data suggest that *Cbr1* may be a novel gene influencing blood  
373 pressure. Inhibitors of CBR1, particularly flavonoids, exist in many foodstuffs and food supplements<sup>64</sup>  
374 and are often advocated as supplements for people with metabolic disease. Pharmacological inhibitors  
375 of CBR1 are being explored for use as adjunctive therapy in chemotherapeutic regimes which include  
376 doxorubicin because CBR1 metabolises doxorubicin to cardiotoxic daunorubicin which limits its use,  
377 particularly in DS patients<sup>21,37,65</sup>. Our data suggest that inhibition of *Cbr1* should be used with caution  
378 in those with or susceptible to hypertension.

379 It is important to acknowledge the limitations of these studies. We used mice which were  
380 heterozygous for *Cbr1* in every tissue, we therefore cannot ascertain which tissue or cell type is most  
381 important in the hypotensive phenotype. We acknowledge the limitations of inferences made in mice  
382 in such a complex human syndrome as DS, the role or importance of *Cbr1* in human blood pressure  
383 control may differ from that in mice. Our power calculations demonstrated we were sufficiently  
384 powered to determine a difference in blood pressure between genotypes and with the inhibitor and  
385 blood pressure was measured in the same animals with and without inhibitor which is a major strength  
386 of the study. However, the study may have been underpowered to detect more subtle differences in  
387 physiological changes which speak to the underlying mechanisms.

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393 **Clinical Perspectives**

394 • Down Syndrome (DS) is the most common chromosomal disorder, affecting approximately 1  
395 in every 800 babies born. Hypotension is common amongst children and adults with DS and  
396 often impacts quality of life. The pathophysiology of DS-associated hypotension is poorly  
397 understood.

398 • In this study we identified Carbonyl Reductase 1 (*CBRI/Cbr1*) as a driver of the hypotensive  
399 phenotype in DS. Inhibition of *CBRI* in a hypotensive rodent model of DS resulted in an  
400 increased blood pressure. Mice heterozygous for *Cbr1* have increased blood pressure.  
401 Mechanistic studies show that changes in sympathetic drive, oxidative stress and prostanoid  
402 metabolism underpin the effects of *CBRI* on blood pressure.

403 • Our data suggest that *CBRI* may be a potential therapeutic target in those DS patients for  
404 whom low blood pressure impacts their quality of life.

405

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413 **Data Availability:**

414 The data included in this study are available from the corresponding authors upon reasonable request.

415 **Disclosures/Conflicts:** None

416

## 417 **List of Supplementary Materials**

418 • Table S1

419 • Fig. S1 to S6

420

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633

634

## 635 **Figure Legends**

636 ***Fig. 1: Cbr1 deletion results in elevated blood pressure regardless of cardiac or circadian phase.***

637 *The left hand column [A, B, and C] show the five-hour rolling averages and minimum and maximum*  
638 *systolic, diastolic and mean arterial blood pressure of wild type mice (blue) and mice heterozygous*  
639 *for Cbr1 (red) (n=8/group). [D, E and F] show the cosinor analysis, curves fitted and spread of data*  
640 *points for the 7-day measurement period for systolic, diastolic and mean arterial pressure.*

641 ***Fig. 2: Ts65Dn mice have lower blood pressure and higher heart rate compared with Wt***

642 ***mice.*** *[A, B, and C] show five-hour rolling averages and minimum and maximum systolic,*  
643 *diastolic and mean arterial pressures measured by telemetry in Ts65Dn mice (pink) and their wild-*  
644 *type (Wt) littermate controls (purple) (n=8/group) over the course of 7 days of baseline measurements*  
645 *and then during daily treatment with CBR1 inhibitor hydroxy-PP-Me (+inhibitor) for 7 days. The*  
646 *dotted line denotes the start of inhibitor treatment. [D, E and F] show the cosinor analysis and curves*  
647 *fitted for the baseline and +inhibitor periods in Ts65Dn and Wt mice.*

648 ***Fig. 3: Cbr1 deletion and inhibition results in increased sympathetic drive and prostaglandin***

649 ***metabolism .*** *Urinary noradrenaline excretion in a 24-hour period was increased in mice*  
650 *heterozygous for Cbr1 (Cbr1<sup>+/-</sup>) compared with their littermate controls (Cbr1<sup>+/+</sup>) [A] (n=6/group)*  
651 *and the opposite was true of Ts65Dn mice who had reduced noradrenaline excretion [B]*  
652 *(n=8/group). Urinary adrenaline excretion was not significantly different in Cbr1<sup>+/-</sup> or Ts65Dn*  
653 *animals compared with wild-type controls [C, D]. [E] Urinary prostaglandin E<sub>2</sub> excretion was*  
654 *increased in mice heterozygous for Cbr1 (Cbr1<sup>+/-</sup>) compared with littermate controls (Cbr1<sup>+/+</sup>)*  
655 *(n=8/group). PGE<sub>2</sub> excretion was decreased in Ts65Dn animals compared to controls and this was*  
656 *corrected by administration of the inhibitor[F] (n=8-11/group). Data were analysed by t-test or by*  
657 *ANOVA with post-hoc Tukey and are presented as group mean ± standard deviation (\*P < 0.05,*  
658 *\*\*P < 0.01, \*\*\*P < 0.001, and \*\*\*\*P < 0.0001).*

**Table 1: Median (+IQR) blood pressure and heart rate and cosinor analysis of these parameters in mice heterozygous for *Cbr1* (*Cbr1*<sup>+/-</sup>) and their littermate controls (*Cbr1*<sup>+/+</sup>) during the inactive and active period (n=8/group). The rhythm-adjusted mean (MESOR), amplitude of each parameter and the outcome of statistical comparison of genotypes by Mann-Whitney U test are shown. \*<0.05, \*\*<0.001, \*\*\*<0.0001**

	Inactive Period		Active Period		MESOR			Amplitude	
	<i>Cbr1</i> <sup>+/+</sup>	<i>Cbr1</i> <sup>+/-</sup>	<i>Cbr1</i> <sup>+/+</sup>	<i>Cbr1</i> <sup>+/-</sup>	<i>Cbr1</i> <sup>+/+</sup>	<i>Cbr1</i> <sup>+/-</sup>	<i>Cbr1</i> <sup>+/+</sup>	<i>Cbr1</i> <sup>+/-</sup>	
<b>Systolic (mmHg)</b>	110.2 (105.8, 113.5)	115.0 (111.9, 116.6)***	125.1 (122.5, 129.2)	127.8 (124.3, 131.1)*	116.9 (116.7, 117.1)	121.2 (121.0, 121.4)***	10.0 (9.7, 10.4)	10 (9.6, 10.3)	
<b>Diastolic (mmHg)</b>	80.31 (76.47, 84.92)	83.26 (81.42, 85.19)*	94.53 (89.77, 98.67)	95.97 (94.36, 98.85)	87.6 (87.4, 87.8)	89.4 (89.2, 89.6)***	9.3 (9.2, 9.5)	9.4 (9.2, 9.6)	
<b>MAP (mmHg)</b>	91.17 (86.45, 94.39)	93.96 (91.96, 95.73)**	105.9 (100.9, 108.4)	106.6 (104.7, 110.1)	96.4 (96.1, 96.6)	99.8 (99.6, 100.1)***	9.3 (8.9, 9.6)	9.5 (8.9, 9.7)	
<b>Heart Rate (bpm)</b>	455.9 (434.0, 479.5)	473.5 (442.3, 496.4)	521.2 (501.8, 553.9)	530.1 (512.8, 561)	492.7 (490.6, 494.8)	500.6 (498.5, 502.8)***	56.4 (53.4, 59.4)	58.0 (55.0, 61.1)	

mmHg – millimetres of mercury; MAP - mean arterial pressure; bpm – beats per minute

**Table 2: Blood pressure and heart rate of Ts65Dn mice and their wild-type littermate controls (Wt) during the inactive and active period (n=8/group). Data are median and interquartile range. Genotypes were compared using a Mann-Whitney U test.**

	Inactive Period			Active Period		
	Wt	Ts65Dn	P-Value	Wt	Ts65Dn	P-Value
<b>Systolic (mmHg)</b>	114.2 (111.3, 122.1)	106.6 (101.9, 109.9)	<0.0001	124.8 (122.3, 133.3)	116.4 (110.8, 119.8)	<0.0001
<b>Diastolic (mmHg)</b>	89.95 (85.76, 100.4)	82.79 (78.95, 85.86)	<0.0001	100.6 (96.16, 109.1)	92.21 (88.94, 94.16)	<0.0001
<b>MAP (mmHg)</b>	98.31 (94.11, 107.5)	90.43 (86.55, 93.51)	<0.0001	108.5 (105.1, 117.2)	100.2 (97.13, 101.5)	<0.0001
<b>Heart Rate (bpm)</b>	517.4 (496.8, 550.2)	570.7 (550.9, 600.9)	<0.001	591.7 (579, 608.9)	630.7 (618.1, 665.4)	<0.0001

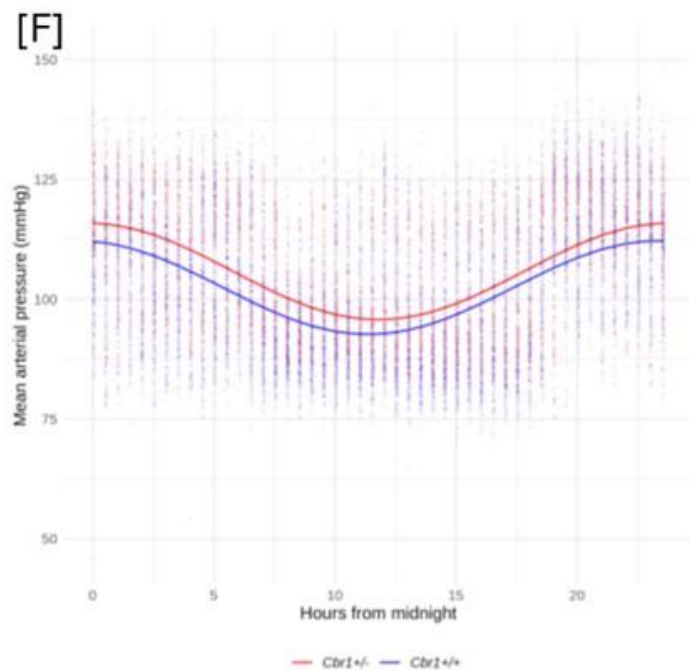
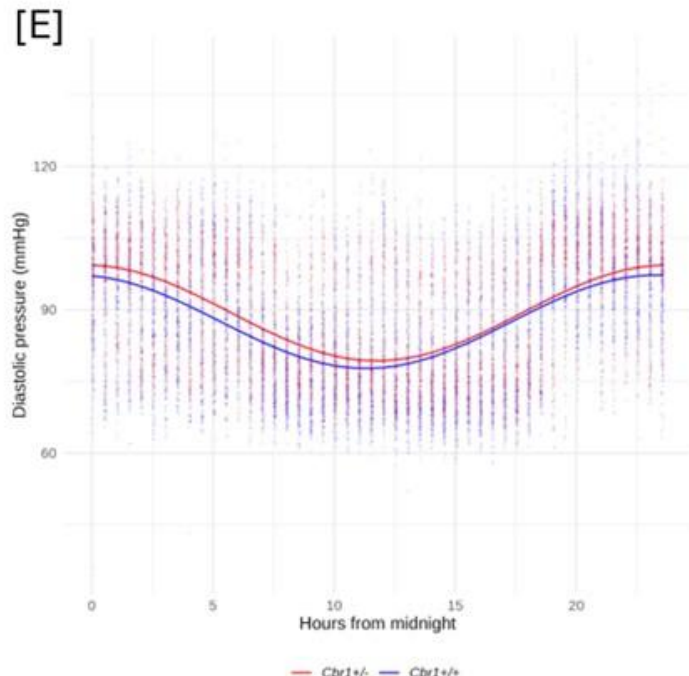
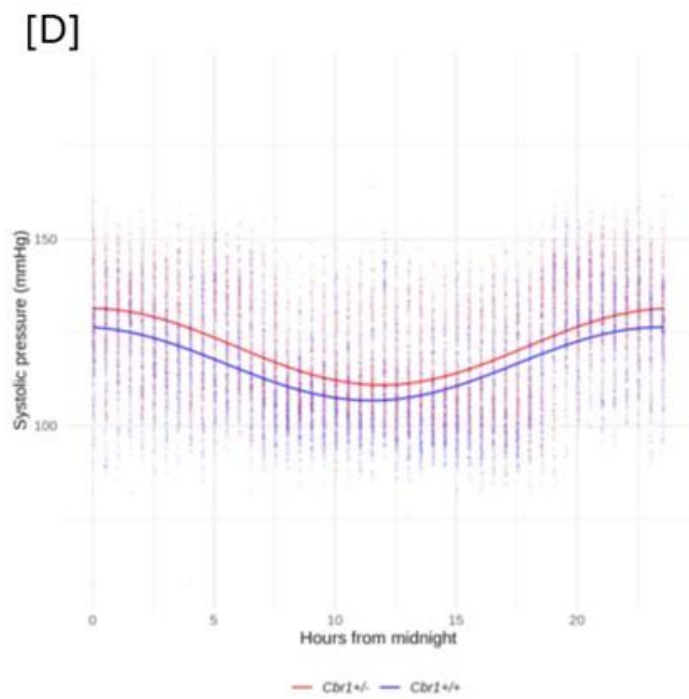
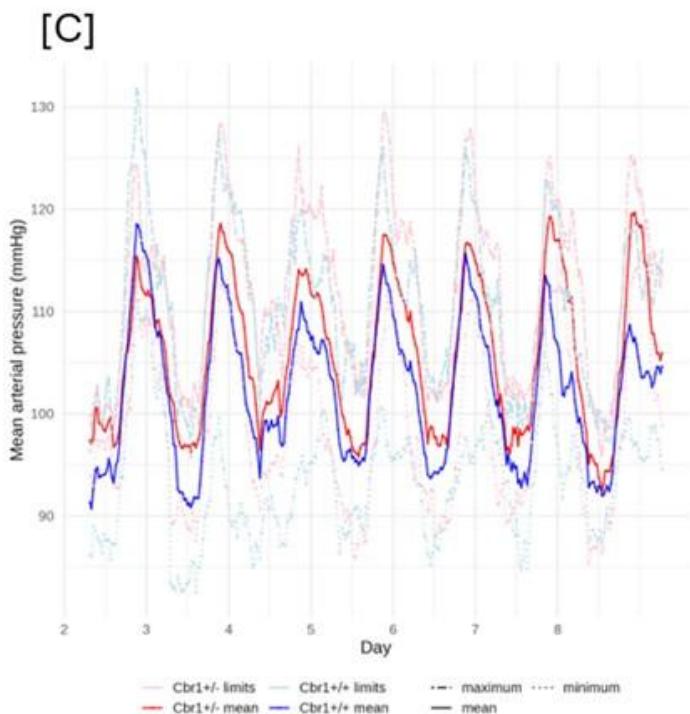
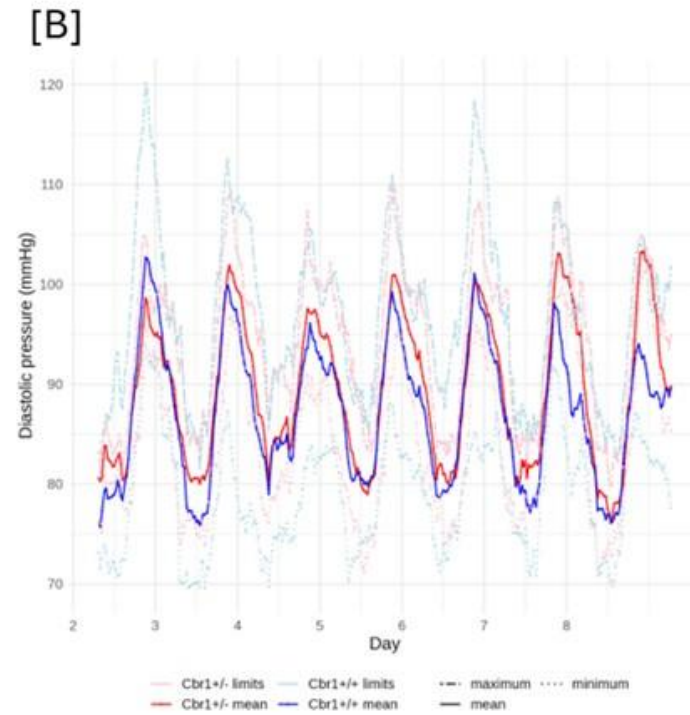
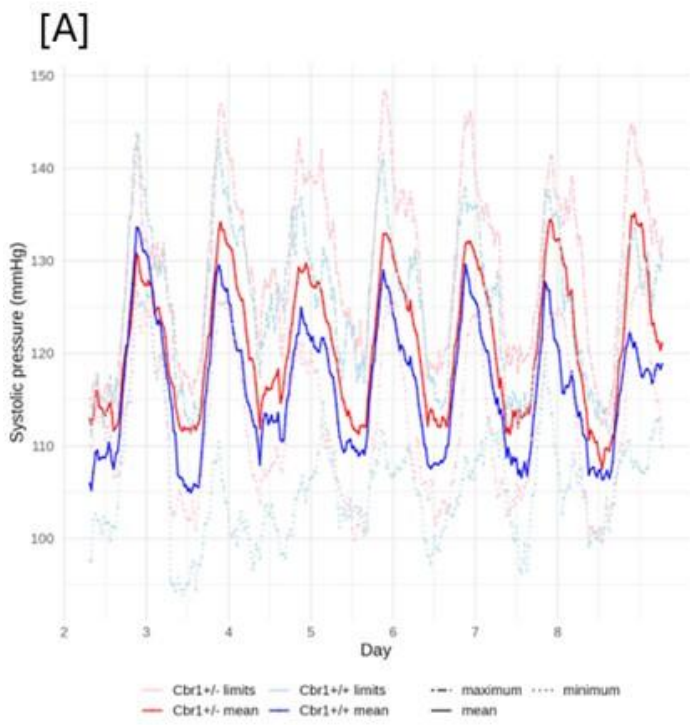
mmHg – millimetres of mercury; MAP - mean arterial pressure; bpm – beats per minute

**Table 3: Cosinor analysis of blood pressure measured by telemetry in Ts65Dn mice and their wild-type littermate controls during the baseline period and during treatment with CBR1 inhibitor, hydroxy-PP-Me (n=8/group). The rhythm-adjusted mean (MESOR), amplitude of each parameter for each genotype during baseline and treatment and the outcome of statistical comparison by 2-way ANOVA and Tukey post-hoc test.**

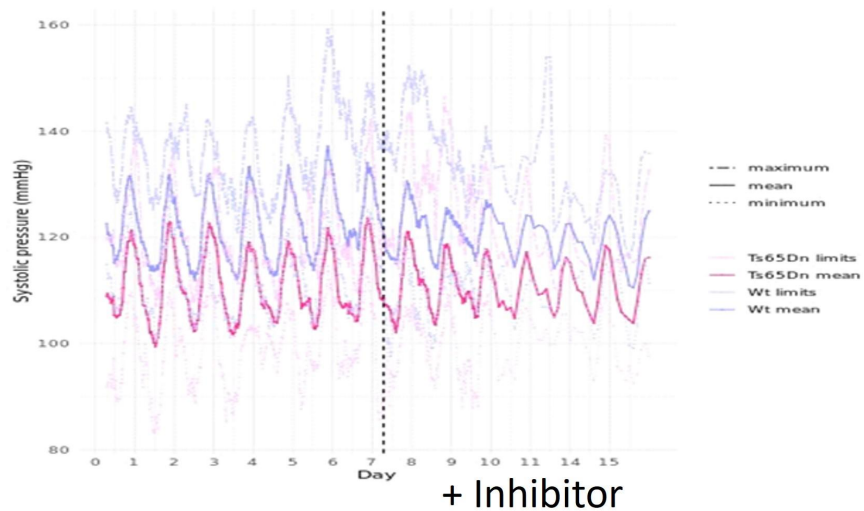
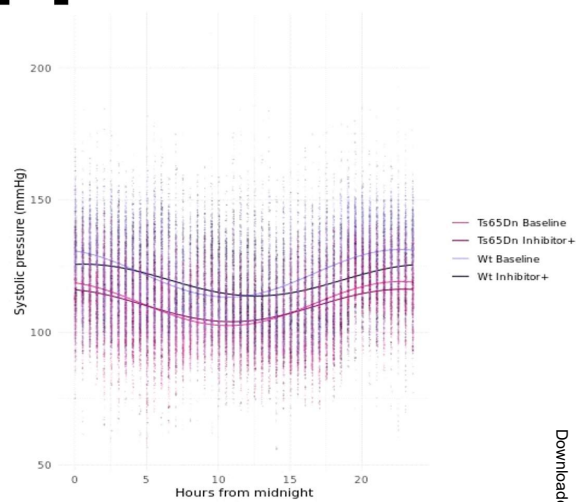
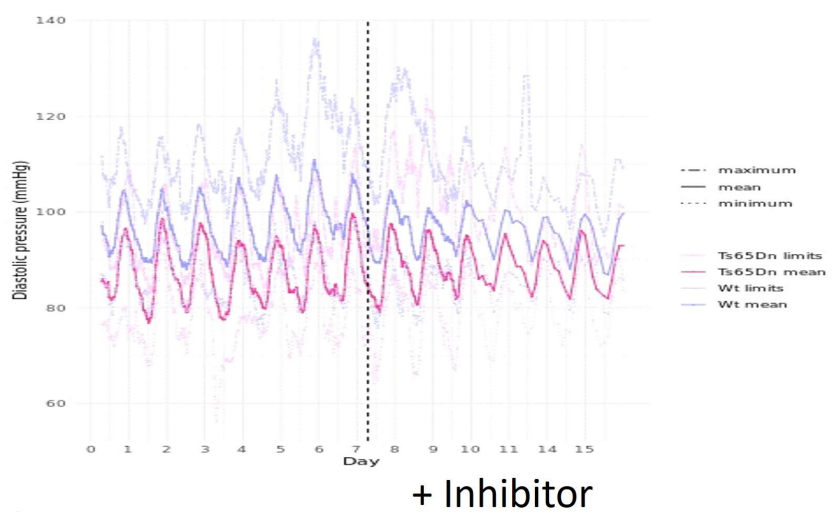
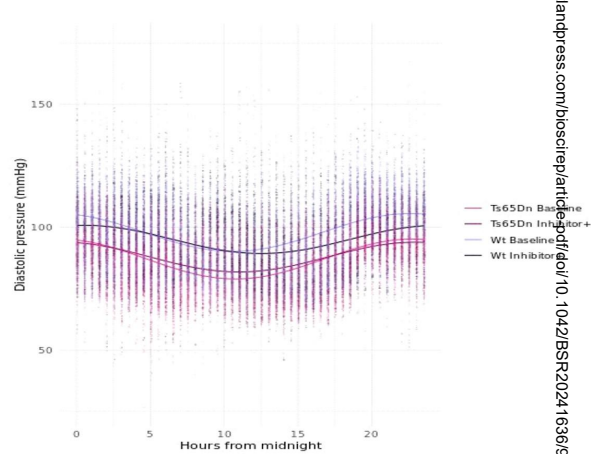
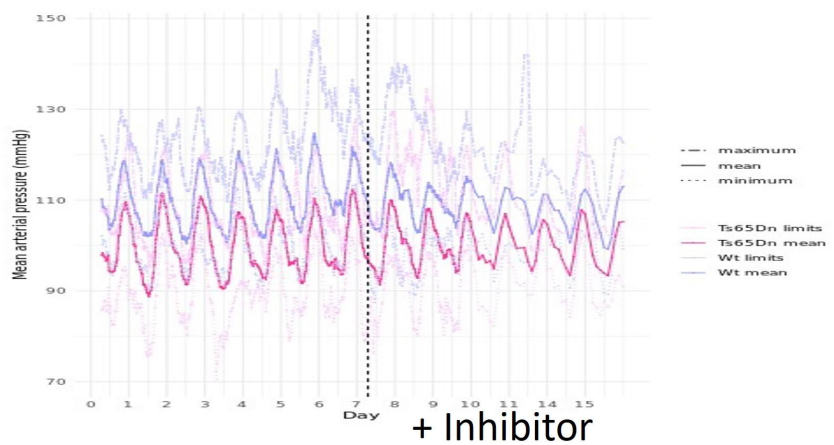
	Baseline			Treatment			Change with Treatment (P-Value)		
	Wt	Ts65Dn	Wt vs Ts65Dn (P-Value)	Wt	Ts65Dn	Wt vs Ts65Dn (P-Value)	Wt	Ts65Dn	Wt vs Ts65Dn (P-Value)
<b>SYSTOLIC</b>									
MESOR (mmHg)	122.09 (121.8, 122.3)	110.9 (110.6, 111.1)	<0.001	120.0 (119.8, 120.2)	111.4 (111.3, 111.6)	<0.001	<0.001	<0.01	<0.01
Amplitude (mmHg)	8.9 (8.6, 9.3)	8.8 (8.5, 9.2)	0.13	5.9 (5.6, 6.2)	6.2 (5.9, 6.5)	0.19	<0.001	<0.001	<0.001
<b>DIASTOLIC</b>									
MESOR (mmHg)	97.5 (97.2, 97.7)	87.1 (86.8, 87.3)	<0.001	95.3 (95.1, 95.4)	88.0 (87.8, 88.2)	<0.001	<0.001	<0.001	<0.001
Amplitude (mmHg)	7.7 (7.4, 7.9)	8.5 (8.2, 8.8)	<0.001	5.6 (5.3, 5.9)	6.1 (5.9, 6.3)	<0.01	<0.001	<0.001	<0.001
<b>MAP</b>									
MESOR (mmHg)	105.7 (105.4, 105.9)	95.0 (94.8, 95.2)	<0.001	103.5 (103.3, 103.7)	95.5 (95.3, 95.6)	<0.001	<0.001	<0.01	<0.01
Amplitude (mmHg)	8.1 (7.8, 8.4)	8.6 (8.3, 9.0)	<0.01	5.7 (5.5, 6.0)	6.2 (5.9, 6.4)	<0.05	<0.001	<0.001	<0.001
<b>HEART RATE</b>									
MESOR (bpm)	557.5 (556.0, 558.8)	602.5 (601.1, 603.9)	<0.001	539.8 (538.5, 541.1)	564.2 (562.9, 565.5)	<0.001	<0.001	<0.001	<0.001
Amplitude (bpm)	70.4 (68.4, 72.4)	69.1 (67.1, 71.1)	0.39	37.4 (35.5, 39.3)	58.1 (56.3, 59.9)	<0.001	<0.001	<0.001	<0.001

mmHg – millimetres of mercury; MAP – mean arterial pressure; bpm – beats per minutes







**[A]****[D]****[B]****[E]****[C]****[F]**