Age-dependent electrocardiographic changes in Pgc-1β deficient murine hearts

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Summary
Increasing evidence implicates chronic energetic dysfunction in human cardiac arrhythmias. Mitochondrial impairment through Pgc-1β knockout is known to produce a murine arrhythmic phenotype. However, the cumulative effect of this with advancing age and its electrocardiographic basis have not been previously studied. Young (12-16 weeks) and aged (>52 weeks), wild type (WT) (n = 5 and 8) and Pgc-1β−/− (n = 9 and 6), mice were anaesthetised and used for electrocardiographic (ECG) recordings. Time intervals separating successive ECG deflections were analysed for differences between groups before and after β1-adrenergic (intraperitoneal dobutamine 3 mg/kg) challenge. Heart rates before dobutamine challenge were indistinguishable between groups. The Pgc-1β−/− genotype however displayed compromised nodal function in response to adrenergic challenge. This manifested as an impaired heart rate response suggesting a functional defect at the level of the sino-atrial node, and a negative dromotropic response suggesting an atrioventricular conduction defect. Incidences of the latter were most pronounced in the aged Pgc-1β−/− mice. Moreover, Pgc-1β−/− mice displayed electrocardiographic features consistent with the existence of a pro-arrhythmic substrate. Firstly, ventricular activation was prolonged in these mice consistent with slowed action potential conduction and is reported here for the first time. Additionally, Pgc-1β−/− mice had shorter repolarisation intervals. These were likely attributable to altered K+ conductance properties, ultimately resulting in a shortened QTc interval, which is also known to be associated with increased arrhythmic risk. ECG analysis thus yielded electrophysiological findings bearing on potential arrhythmogenicity in intact Pgc-1β−/− systems in widespread cardiac regions.

KEYWORDS
cardiac arrhythmias, cardiac conduction, electrocardiogram, ECG, peroxisome proliferator activated receptor-γ-coactivator-1 (PGC-1)

INTRODUCTION

Cardiac arrhythmias result from disruption of the normally coordinated sequence of atrial and ventricular excitation, often following altered ion channel function. The most common of these, atrial fibrillation (AF), affects in excess of 8 million individuals in Europe, and accounts for one in three arrhythmia-related hospital attendances. Similarly, ventricular arrhythmias are the commonest cause of sudden cardiac death (SCD), with a worldwide incidence of >300 000 deaths/year.1,2
Peroxisome proliferator activated receptor-γ coactivator-1 (PGC-1) transcriptional coactivators offer strategic targets for electrophysiological studies in such energetically deficient hearts. The PGC-1 family includes PGC-1α and PGC-1β; these are highly expressed in oxidative tissues such as the heart, brain and skeletal muscle. They are key regulators of mitochondrial mass, function, and cellular metabolism. They interact with cardiomyocyte nuclear receptor factor-1, estrogen related receptor-α and peroxisome proliferator-activated receptor-α in increasing mitochondrial biogenesis. They also upregulate expression of nuclear and mitochondrial-encoded mitochondrial proteins involved in fatty acid β-oxidation, the tricarboxylic acid cycle and electron transport. PGC-1 protein expression and the corresponding mitochondrial activity, is coordinated with several upstream stimuli reflecting the heart's energetic demand. Conversely, obesity, insulin resistance, type II diabetes mellitus, and ageing are associated with reduced PGC-1 protein expression and mitochondrial dysfunction.

Overexpression of some PGC-1 family members results in increased mitochondrial density and oxidative capacity. Conversely, mice deficient in both Pgc-1α and Pgc-1β develop a perinatally lethal low cardiac output state and conduction disease. Cardiac phenotypes of mice lacking individual members of the PGC-1 family are less severe. Pgc-1α deficient murine hearts show normal baseline contractile function but develop cardiac failure following increased afterload. Studies on Pgc-1β deficient hearts are more limited, but nevertheless report normal baseline cardiac function despite their reduced mitochondrial content, but blunted heart rate responses following adrenergic stimulation. Furthermore, Langendorff-perfused Pgc-1β−/− hearts demonstrated increased arrhythmic propensity reflected in action potential (AP) duration alternans and increased frequencies of ventricular tachycardia (VT) following programmed electrical stimulation. Isolated Pgc-1β−/− cardiomyocytes also showed altered ion channel expression patterns, spontaneous diastolic Ca2+ transients, and pro-arrhythmic after-depolarisation events. The combination of apparently normal baseline contractile function but potentially pro-arrhythmic electrophysiological abnormalities make Pgc-1β−/− deficient hearts attractive models to investigate roles of mitochondrial impairment in arrhythmia in an absence of a confounding cardiac failure.
In contrast, genotype exerted independent effects, with the \( P_{gc-1} \beta^{-/-} \) mutation reducing heart rates observed following dobutamine challenge (\( P_{gc-1} \beta^{-/-} 8.30 \pm 0.28 \) Hz, \( n = 15 \); WT, 9.11 \( \pm 0.11 \) Hz, \( n = 13 \); \( P = .021 \); Figure 5). In contrast, there were no significant effects of either age or interactions between age and genotype. Similarly, ANOVA demonstrated independent significant effects of genotype (\( P = .011 \)) but not either age or interactive effects between genotype and age on maximum heart rates attained after dobutamine challenge. Thus, post hoc tests demonstrated lower peak heart rates in \( P_{gc-1} \beta^{-/-} \) than WT mice (mean peak heart rate 8.47 \( \pm 0.28 \) Hz vs 9.53 \( \pm 0.21 \) Hz, \( P = .0084 \), \( n = 13 \) vs 15 respectively).

Heart rate variabilities, reflecting autonomic influences on the heart, and known to be related to adverse mortality risk, expectedly altered within each experimental group following dobutamine challenge. However, the respective findings obtained before or following dobutamine did not vary between experimental groups. This was reflected in Poincare plots constructed for each mouse (Figure 6A, B), exemplified in pre- (A) and post-dobutamine (B), young (a,b) and aged (c,d), WT (a,c) and \( P_{gc-1} \beta^{-/-} \) mice (b,d). The dispersion of these points was quantified by the standard deviation of the \( \Delta RR \) interval before (Figure 6C) and following dobutamine challenge (Figure 6D). ANOVA demonstrated no significant differences in such dispersions before or after dobutamine addition between different experimental groups. These results attribute the present findings to an existence of sino-atrial node (SAN) as opposed to autonomic dysfunction in the \( P_{gc-1} \beta^{-/-} \) mice.

2.2 Age-related SA node disease in WT and \( P_{gc-1} \beta^{-/-} \) murine hearts

Although sinus rhythm was the prevailing rhythm in all groups, both WT and \( P_{gc-1} \beta^{-/-} \) mice demonstrated intermittent episodes of isorhythmic AV dissociation\(^b\) during the recording period. These episodes predominantly occurred in aged animals, affecting 3/6 aged WT mice and 4/8 aged \( P_{gc-1} \beta^{-/-} \) mice, but in only one young \( P_{gc-1} \beta^{-/-} \) and none of the young WT mice (Table 1). They most frequently occurred immediately following dobutamine challenge, whilst RR intervals were decreasing from their baseline, pre-treatment values. During these episodes, RR intervals were shorter than their corresponding PP intervals, but the ventricular complexes remained identical to those observed during sinus rhythm. The latter suggests a supraventricular (likely junctional) pacemaker focus driving such activity (Figure 2C).

2.3 \( P_{gc-1} \beta^{-/-} \) hearts display paradoxical atrioventricular node function

MANOVA analysis indicated that P-wave durations were not affected by either independent or interacting effects of age or genotype, whether before or following dobutamine challenge (Table 2B). In contrast, two alteration patterns of altered PR interval reflecting atrioventricular node (AVN) function were observed with dobutamine administration. These took the form of either positive or negative dromotropic effects of dobutamine appearing as increases...
or decreases in PR interval (Figure 7), taking place despite unchanged P-wave durations that were similar between experimental groups suggesting a continued normal atrial conduction (Table 2B).

MANOVA indicated that neither age nor genotype affected PR interval whether before or following dobutamine challenge (Table 2C). Nevertheless, the alteration in PR interval following dobutamine challenge demonstrated differing responses from WT and Pgc-1β−/− mice. All young (5 of 5) and aged (6 of 6) WT mice showed decreased PR intervals following dobutamine challenge. In contrast, the PR interval decreased in 5 of 9 and increased in the remaining four young Pgc-1β−/− mice. In aged Pgc-1β−/− mice the result was even more marked with only one mouse showing the expected positive dromotropic effect with dobutamine administration and 5 of 6 mice showing a paradoxical negative dromotropic effect in response to dobutamine. In addition to the SAN dysfunction seen with the Pgc-1β knockout there was compromised AVN conduction in a subset of mutant hearts, an effect exacerbated by increasing age.

The presence of AVN dysfunction in mutant mice which also demonstrated impaired heart rate responses led us to examine whether paradoxical AV node dysfunction underlies or is associated with the blunted chronotropic responses. Such a comparison demonstrated that Pgc-1β−/− animals with a normal AVN response showed a mean heart rate of 9.10 ± 0.22 Hz (n = 6) following dobutamine challenge. In contrast, Pgc-1β−/− animals with a paradoxical AVN response to dobutamine showed a heart rate of 7.77 ± 0.34 Hz (n = 9). A two-tailed student t test confirmed that the difference was significant (P = .0061). Thus these findings suggest that the Pgc-1β−/− mutation is associated only with significantly altered AV nodal function in a subset of mutant mice, and that the presence of AV nodal dysfunction itself may be a marker for impaired heart rate responses.

2.4 Aged Pgc-1β−/− hearts display slowed ventricular activation

Ventricular activation is a synchronised, sequential process, whose onset is easily detected as the beginning of the Q wave deflection (Figure 1). Ventricular recovery is considered to begin at a time-point between the S wave trough, and the beginning of the R’ peak.

![FIGURE 2](image.png)
TABLE 1 Incidence of particular electrocardiographic features in the experimental groups

|                  | WT          | Pgc-1β<sup>−/−</sup> |
|------------------|-------------|----------------------|
|                  | Young       | Aged                 | Young       | Aged                 |
| (A) Ischaemic change |             |                      |             |                      |
| Ischaemic changes present | 0 | 2                    | 0 | 2                    |
| Ischaemic changes absent | 5 | 6                    | 9 | 4                    |
| (B) Arrhythmic ECG patterns |             |                      |             |                      |
| Sinus rhythm only | 5           | 4                    | 8           | 3                    |
| Isorhythmic AV dissociation | 0          | 4                    | 1           | 3                    |
| Ectopic beats   | 0           | 0                    | 0           | 1                    |

Electrocardiographic records obtained at baseline prior to pharmacological intervention in young WT (n = 5), aged WT (n = 8), young Pgc-1β<sup>−/−</sup> (n = 9) and aged Pgc-1β<sup>−/−</sup> (n = 6).

Examination of three independent ECG indicators, of QR, QS and QR' durations, of ventricular activation, suggested interacting effects of age and genotype upon its duration (Table 3). MANOVA demonstrated that genotype and age, whilst not exerting independent effects, showed interacting effects upon all the three parameters of

FIGURE 4 Correlations between heart rates observed pre- vs post-dobutamine challenge in Pgc-1β<sup>−/−</sup> and WT

FIGURE 3 Traces plotting heart rate response curves before and following dobutamine challenge in (A) young WT, (B) aged WT; (C) young Pgc-1β<sup>−/−</sup> and (D) aged Pgc-1β<sup>−/−</sup> mouse
QR \( (P = .032) \), QS \( (P = .040) \) and QR’ duration \( (P = .039) \). This was also the case with QR, QS, but not QR’ \( (QR: P = .029; \ QS: P = .029; QR’: P = .086) \) prior to and with all three parameters following \( QR: P = .016; QS: P = .022; QR’: P = .026 \) dobutamine challenge. Post hoc Tukey tests then demonstrated that prior to dobutamine administration, QT durations were longer in aged Pgc-1β−/− than aged WT \( (P = .030) \), with indications of such differences in aged compared to young Pgc-1β−/− mice \( (P = .059) \). QS intervals were longer in aged Pgc-1β−/− than either young Pgc-1β−/− \( (P = .040) \) or aged WT mice \( (P = .024) \). Following dobutamine administration, QT durations were longer in aged Pgc-1β−/− than either aged WT \( (P = .035) \) or young Pgc-1β−/− \( (P = .030) \). QS durations were longer in aged Pgc-1β−/− than either young Pgc-1β−/− \( (P = .035) \) or aged WT mice \( (P = .026) \). QR’ durations were longer in aged Pgc-1β−/− mice than either aged WT \( (P = .039) \) or young Pgc-1β−/− mice \( (P = .017) \).

### 2.5 | Pgc-1β−/− hearts show shortened ventricular recovery times after adrenergic challenge

Age and genotype exerted contrasting effects on ventricular recovery times. Genotype affected all three measures of such recovery (RTc, RT and STc durations; \( P = .0098, P = .0014 \) and \( P = .0029 \), respectively) (Table 4). In contrast, age did not significantly affect any of these recovery parameters, nor were there any interactive effects of age and genotype. Post hoc testing showed that (for all parameters) the difference lay in findings obtained post-dobutamine challenge; there were no differences due to age, genotype or their interaction prior to dobutamine administration. Following dobutamine administration all three parameters showed a marked effect of genotype \( (P = .015, P = .021 \) and \( P = .0067 \) respectively) but no other effects. Post hoc Tukey tests showed that Pgc-1β−/− showed significantly shorter RTc, RT and STc intervals than WT mice \( (P = .0053, P = .018 \) and \( P = .0080 \) respectively). Thus, all three recovery parameters were highly concordant confirming that the Pgc-1β ablation significantly shortened ventricular recovery parameters (Table 5).

#### 2.6 | Emergence of a short-QT phenotype in Pgc-1β−/− animals

The QTc interval has traditionally been used as a marker for repolarization abnormalities in that the electrocardiographic phenotype is usually caused by a defect in ventricular recovery. However, it is more accurate to describe the QTc interval as a parameter that describes the combined durations of both activation and recovery i.e. the duration of ventricular excitation. The onset of ventricular activation is represented by the Q wave deflection; the C wave trough was taken to represent the end of ventricular recovery and hence used for calculation of the QT interval in the present study (Figure 1). Genotype, but neither age

### TABLE 2 Electrocardiographic features related to sino-atrial, atrio-ventricular and atrial conduction

| Feature | WT Young | Aged | Pgc-1β−/− Young | Aged |
|---------|----------|------|-----------------|------|
| (A) Heart rate response | | | | |
| Mean heart rate prior to dobutamine challenge (Hz) | 6.29 ± 0.15 | 7.08 ± 0.16 | 6.32 ± 0.38 | 6.81 ± 0.59 |
| Mean heart rate following dobutamine challenge (Hz) | 9.10 ± 0.19 | 9.12 ± 0.15 | 8.33 ± 0.40 | 8.25 ± 0.39 |
| Peak heart rate following dobutamine challenge (Hz) | 9.32 ± 0.21 | 9.66 ± 0.32 | 8.51 ± 0.40 | 8.41 ± 0.39 |
| (B) Atrial conduction | | | | |
| P-wave duration prior to dobutamine challenge (ms) | 26.08 ± 0.50 | 25.57 ± 1.06 | 26.06 ± 0.47 | 27.64 ± 0.67 |
| P-wave duration following dobutamine challenge (ms) | 25.43 ± 0.58 | 26.08 ± 0.79 | 26.21 ± 0.48 | 26.90 ± 0.86 |
| (C) AV conduction | | | | |
| Mean PR interval prior to dobutamine challenge (ms) | 54.20 ± 2.57 | 63.26 ± 4.89 | 56.35 ± 5.56 | 66.62 ± 4.25 |
| Mean PR interval following dobutamine challenge (ms) | 52.53 ± 2.22 | 53.61 ± 2.76 | 58.38 ± 5.41 | 76.95 ± 9.54 |
| Hearts showing decreased PR interval following dobutamine challenge | 5 of 5 | 6 of 6 | 5 of 9 | 1 of 6 |
| Hearts showing increased PR interval following dobutamine challenge | 0 of 5 | 0 of 6 | 4 of 9 | 5 of 6 |

Electrocardiographic features gave (A) heart rates responses in studies of young WT \( (n = 5) \), aged WT \( (n = 8) \), young Pgc-1β−/− \( (n = 9) \) and aged Pgc-1β−/− mice \( (n = 6) \), in which two of the aged WT showed AV dissociation within the ECG analysis window. Studies of atrial (B) and AV (C) conduction were therefore based on young WT \( (n = 5) \), aged WT \( (n = 6) \), young Pgc-1β−/− \( (n = 9) \) and aged Pgc-1β−/− mice \( (n = 6) \) respectively.
specific ECG waveform components more closely than did previous
mediated extracardiac changes which in the clinical setting are also
Ca$^{2+}$ conduction shortening of the QT intervals than their WT counterparts with most of the effect arising from (brane excitability and intracellular Ca$^{2+}$ryanodine receptor function alterations in which affect surface mem
increases sarcolemmal ATP-sensitive K$^{+}$ channel (sarcKATP) open prob
in turn affecting maximum Na$^{+}$ current and L-type Ca$^{2+}$ channel inactivation kinetics and late Na$^{+}$ current. They also affect
ryanodine receptor function alterations in which affect surface mem
brane excitability and intracellular Ca$^{2+}$ homeostasis. Mitochondria
are also the main cardiomyocyte ATP source and ATP/ADP depletion increases sarcemmal ATP-sensitive K$^{+}$ channel (sarcK$^{2+}$) open prob
abilities affecting action potential duration (APD), effective refractory period (ERP) and heterogenous current sinks potentially causing current-load mismatch. These cellular mechanisms could in turn potentially give rise to potentially pro-arrhythmic effects on cell-cell coupling, AP conduction and AP repolarisation. They may also be an appearance of alternans and Ca$^{2+}$ mediated triggering phenomena. The Pgc-1β genetic modification has thus been associated with altered ion channel function and ventricular arrhythmias in Langendorff-perfused heart preparations.

ECG alterations accompanying the associated mitochondrial dys
function were therefore investigated in intact anaesthetised Pgc-1β$^{-/-}$ mice lacking the transcriptional coactivator Pgc-1β. The present study therefore yielded electrophysiological features associated with Pgc-1β ablation in the in vivo system with intact autonomic innervation and normal cardiac mechanical function, building upon earlier reports from the cellular studies and ex vivo hearts. The pharmacological manoeuvres involving dobutamine challenge in the latter studies would largely involve β1-adrenergic receptor activity, whereas the present in vivo studies could potentially further involve β2-adrenergic receptor mediated extracardiac changes which in the clinical setting are also known to influence arrhythmic risk.

The present experiments characterised the intervals separating specific ECG waveform components more closely than did previous

3 | DISCUSSION

The ECG yields much prescient and strategic clinical electrophysiologi
cal information as a primary investigational tool. Its recent experimental application had demonstrated valuable insights into electrophysiological abnormalities in murine hearts modelling clinical arrhythmic conditions. Cellular energetic dysfunction following metabolic disturbances is increasingly recognised as important factor in the aetiology of such atrial and ventricular arrhythmias. Destabilisation of inner mitochondrial membrane potentials results in approximately 10-fold increases in re
active oxygen species production, which in turn affecting maximum Na$^{+}$ current and K$^{+}$ current, sarcolemmal K$^{+}$ATP channel function, Na$^{+}$ and L-type Ca$^{2+}$ channel inactivation kinetics and late Na$^{+}$ current. They also affect ryanodine receptor function alterations in which affect surface membrane excitability and intracellular Ca$^{2+}$ homeostasis. Mitochondria are also the main cardiomyocyte ATP source and ATP/ADP depletion increases sarcemmal ATP-sensitive K$^{+}$ channel (sarcK$^{2+}$) open probabilities affecting action potential duration (APD), effective refractory period (ERP) and heterogenous current sinks potentially causing current-load mismatch. These cellular mechanisms could in turn potentially give rise to potentially pro-arrhythmic effects on cell-cell coupling, AP conduction and AP repolarisation. They may also be an appearance of alternans and Ca$^{2+}$ mediated triggering phenomena. The Pgc-1β genetic modification has thus been associated with altered ion channel function and ventricular arrhythmias in Langendorff-perfused heart preparations.

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Quantitative statistical analysis of these steady state param
eters then employed two way factorial MANOVA testing for interacting and non-interacting effects of age and genotype before and after dobutamine challenge. The presence of significant differences then prompted further, two way factorial ANOVA to ascertain whether the difference occurred before or following dobutamine application. Finally, appropriate Tukey HSD tests assessed for particular differences between individual parameters. Peak heart rates following dobutamine challenge were analysed by themselves by a two way fac
torial ANOVA followed by post hoc Tukey tests.

The ECG analysis demonstrated a range of age-dependent ab
normalities associated with the Pgc-1β$^{-/-}$ genotype. The predomi
nant ECG pattern in both young and aged, WT and Pgc-1β$^{-/-}$ mice was one of sinus rhythm. Any ischaemic ECG changes observed were associated with age but were not specific to Pgc-1β$^{-/-}$ or WT gen
otypes, suggesting that there was no underlying difference in vasc
ular as opposed to primary cardiomyocyte function between the two groups. However, we demonstrated for the first time blunted chronotropic responses to dobutamine challenge in an intact Pgc-
1β$^{-/-}$ mammalian system despite heart rate variabilities suggesting unchanged autonomic backgrounds. Previous reports had demon
strated compromised heart rate responses in ex vivo Langendorff-perfused Pgc-1β$^{-/-}$ hearts following dobutamine challenge. These results together suggest that the impaired heart rate response of Pgc-1β$^{-/-}$ hearts does not reflect generalised autonomic dysfunction but rather alterations in the intrinsic myocardial response to dobutamine. Ageing did not affect this chronotropic response, implicating the mutation as opposed to background deterioration of maximal heart rate with age.

FIGURE 5 Mean heart rates in the 5 minute analysis window before and after dobutamine administration in young and aged WT and Pgc-1β$^{-/-}$ mice
Aged mice, independent of genotype, also showed episodes of isorhythmic AV dissociation with dobutamine challenge. During these episodes regular ventricular responses were seen, with normal, narrow QRS complexes despite the absence of a fixed PR interval, even when P-wave complexes were buried within the ventricular signal. These findings suggest that the murine SAN is vulnerable to degenerative changes with age, with appearances of supraventricular, most likely junctional, pacemaker foci intermittently dictating the ventricular rate.

Pathological bradycardic rhythms secondary to cardiac conduction system disease are known to occur with age, often necessitating permanent pacemaker implantation. Progressive fibrotic change is a recognised feature of cardiac ageing in both animal and human studies. Fibrotic change could directly disrupt gap junction function, increasing tissue resistance, or increase fibroblast-cardiomyocyte coupling, increasing effective membrane capacitance. More recent studies indeed implicate abnormal gap junction function in both SAN and AVN disease. Interestingly, oxidative stress, associated with mitochondrial impairment increases transforming growth factor (TGF)-β activity, in turn implicated in such age-related myocardial fibrosis. Conversely augmented mitochondrial anti-oxidant capacity protects against features of cardiac ageing including fibrotic change.

Mitochondrial dysfunction could also impair gap junction function through elevating intracellular [Ca^{2+}] or altering connexin phosphorylation through oxidative stress. Finally, the range of ionic currents including RyR2 channel function involved in SAN and AVN activity, are potentially modifiable by mitochondrial dysfunction. Isolated cardiomyocytes from Pgc-1β^{-/-} hearts have previously been reported to display altered diastolic Ca^{2+} transients in keeping with abnormal RyR2 function.

ECG deflections related to ventricular activation and recovery confirmed previous reports that murine ECGs lack well-defined ST segments. The murine ECG shows a R' wave deflection, immediately following the S wave not seen in the human ECG. This is followed by a further but variably reported deflection which has not been
systematically identified or formally correlated with particular action potential components. This variability may be attributed to the greater rostro-caudal anatomical alignment of the mouse heart in the thoracic cavity and variations in limb positioning during experimental recording between reports, with consequent variations in the effective positioning of the centre of the Einthoven triangle relative to the heart. Thus, although we also identified C waves, small changes in lead positioning could lead to its apparent disappearance in one or both ECG leads. This may account for the controversy concerning its inconsistent appearance.17

The onset of ventricular recovery in the murine ECG has been considered to occur from time points ranging from the S wave nadir to the R’ peak. A number of authors have suggested that the late component of the R’ wave or the R’ wave in totality is in fact part of ventricular repolarisation.16,18,44 Others suggested that inclusion of the R’ wave may lead to systematic overestimation of ventricular activation, whilst its exclusion in genetically modified mouse models, such as that of the Brugada syndrome, which displays slowed activation, whilst its exclusion in genetically modified mouse models of the R’ wave may lead to systematic overestimation of ventricular activation parameters is likely to capture activation and recovery in different areas of myocardium, reflecting the non-simultaneous nature of electrical activity in the myocardium. This increased the robustness of our analysis and permitted us to assess the possibility of early repolarisation in our genetic model. The statistical analysis of the different parameters of recovery and activation were highly concordant. $Pgc-1\beta^{-/-}$ hearts showed a prolongation of all such measures of ventricular activation with age whether before or following adrenergic stress, in an absence of independent effects of age or genotype. These findings parallel the reduced conduction velocities reported in other arrhythmic genetic models. These had accompanied either fibrotic change or reduced Na$^+$ currents resulting from Nav1.5 deficiency in Scn5a$^{-/-}$,32,33,45 and Scn5a$^{-/-} $ or secondary to Ca$^{2+}$ handling abnormalities in RyR2-P2328S hearts.44,46,47 These findings are also compatible with reports that mitochondrial abnormalities could alter $I_c$ and therefore result in current-load mismatch.6,30

$Pgc-1\beta^{-/-}$ hearts also showed shorter recovery parameters than WT after dobutamine administration, without effects of age whether acting independently or interacting with genotype. Such shortened repolarisation intervals have also been implicated in arrhythmic risk. Human short QT syndrome is diagnosed using the J point to T peak interval that may represent the interval between the end of the ventricular complex to the peak of the repolarisation wave.48 Short QT syndrome has been traced to HERG and other K$^+$ channel mutations and more recently, Ca$^{2+}$ channel function.59 The present findings are thus consistent with reported alterations in K$^+$ conductance properties in the $Pgc-1\beta^{-/-}$ system that would also modify current-load matching.15 These changes appeared to result in shortened QTc intervals for mutant mice with adrenergic stress. Although the mechanisms underlying these changes remain unclear, increased expression of Kcnq1 was reported in the latter study and may contribute to the increased K$^+$ conductance observed. Additionally, the opening and K$^+$ conductance of the sarcKATP is linked to rising cellular ADP levels, therefore correlating its activity to cellular metabolic status. Its activity is known to reduce the action potential duration and is thought to contribute to increased arrhythmic risk.50 Oxidative stress is also known to enhance sarcKATP activity; however the cellular mechanism are yet to be established but may occur through depletion of cellular ATP. Nevertheless, the effects of ROS upon sarcKATP activity could be attenuated through inhibition of protein kinase C, protein kinase G and calcium-calmodulin kinase II but not protein kinase A, providing some insights into the pathways involved.51

Finally, these findings prompted us to measure QTc intervals reflecting the total activation times of the ventricular myocardium. $Pgc-1\beta^{-/-}$ mice showed shorter QTc intervals than their WT counterparts. Most of this effect seemed to arise in the young mutant mice, though this was not significant. This is in contrast to the shortened repolarisation parameters in both young and aged $Pgc-1\beta^{-/-}$. This likely reflects the additional, prolonged, depolarisation parameters in aged $Pgc-1\beta^{-/-}$ mice, offsetting to some degree the shortening in the repolarisation parameters.

In summary, ECG analysis demonstrates a range of electrocardiographic abnormalities associated with the $Pgc-1\beta^{-/-}$ genotype and those features particularly vulnerable to advanced age. Thus,
**Methods**

**4.1 Animals**

This research has been regulated under the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 following ethical review by the University of Cambridge Animal Welfare and Ethical Review Body (AWERB). The experiments also conformed to the Guide for the Care and Use of Laboratory Animals, US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Mice from a consistent C57/B6 background to avoid possible strain-related confounds, were housed in an animal facility at 21°C with 12-hour light/dark cycles. Animals were fed sterile chow (RM3 Maintenance Diet, SDS, Witham, Essex, UK) and had free access to water. Wild Type C57/B6 and Pgc-1β−/− adult mice were bred for the experimental protocols. Mice were divided into four groups: groups 1 and 2 consisted of mice aged between 12 and 16 weeks, and consisted respectively of littermate WT controls (n = 5) and Pgc-1β−/− mice (n = 9). Group 3 was composed of aged (greater than 52 weeks) littermate WT controls (n = 8). Group 4 consisted of Pgc-1β−/− mice of age similarly greater than 52 weeks (n = 6). These timings parallel those used in previous studies in transgenic Scn5a+/− mice which reported increased fibrotic and electrophysiological alterations at ages above 12 months.45

### Table 3: Electrocardiographic activation intervals

|                      | WT                      | Pgc-1β−/−               |
|----------------------|-------------------------|-------------------------|
|                      | Young | Aged               | Young | Aged               |
| QR duration before dobutamine challenge (ms) | 6.85 ± 0.67 | 5.89 ± 0.63 | 6.20 ± 0.48 | 8.35 ± 0.52 |
| QR duration following dobutamine challenge (ms) | 7.14 ± 0.75 | 6.12 ± 0.60 | 6.12 ± 0.48 | 8.56 ± 0.56 |
| QS duration before dobutamine challenge (ms) | 10.19 ± 0.47 | 9.43 ± 0.45 | 9.67 ± 0.45 | 11.78 ± 0.7 |
| QS duration following dobutamine challenge (ms) | 10.60 ± 0.62 | 9.76 ± 0.40 | 9.91 ± 0.43 | 12.07 ± 0.77 |
| QR' duration before dobutamine challenge (ms) | 14.24 ± 0.60 | 14.22 ± 0.40 | 13.82 ± 0.34 | 16.20 ± 0.92 |
| QR' duration following dobutamine challenge (ms) | 14.95 ± 0.41 | 14.39 ± 0.55 | 14.15 ± 0.38 | 16.72 ± 0.89 |

Electrocardiographic measurements made in QR, QS and QR' durations before and following dobutamine challenge in young WT (n = 5), aged WT (n = 8), young Pgc-1β−/− (n = 9) and aged Pgc-1β−/− mice (n = 6).

### Table 4: Electrocardiographic recovery intervals

|                      | WT                      | Pgc-1β−/−               |
|----------------------|-------------------------|-------------------------|
|                      | Young | Aged               | Young | Aged               |
| RTc duration before dobutamine challenge (ms) | 29.00 ± 0.54 | 30.60 ± 0.87 | 28.51 ± 0.05 | 28.89 ± 0.87 |
| RTc duration following dobutamine challenge (ms) | 33.43 ± 0.77 | 33.75 ± 0.44 | 31.41 ± 0.86 | 31.79 ± 0.41 |
| RTc duration before dobutamine challenge (ms) | 23.15 ± 0.45 | 23.64 ± 0.71 | 22.55 ± 0.91 | 22.55 ± 0.56 |
| RTc duration following dobutamine challenge (ms) | 25.97 ± 0.43 | 25.88 ± 0.55 | 24.16 ± 0.66 | 24.40 ± 0.29 |
| STc duration before dobutamine challenge (ms) | 26.35 ± 0.38 | 27.65 ± 0.77 | 25.79 ± 0.92 | 26.06 ± 0.55 |
| STc duration following dobutamine challenge (ms) | 30.13 ± 0.68 | 30.31 ± 0.50 | 28.00 ± 0.71 | 28.40 ± 0.30 |

Electrocardiographic measurements made in RTc, RTc and STc durations before and following dobutamine challenge in young WT (n = 5), aged WT (n = 8), young Pgc-1β−/− (n = 9) and aged Pgc-1β−/− mice (n = 6). One young and one aged Pgc-1β−/− mouse were excluded as these showed paradoxical dromotropic effects that lead to prolonged PR intervals and P waves that interfered with determinations of the end of the C wave to give the following n values: young WT (n = 5), aged WT (n = 8), young Pgc-1β−/− (n = 8) and aged Pgc-1β−/− (n = 5).
latter two groups used here accordingly consisted of animals above this age to investigate the effects of age on electrocardiographic parameters in these mice.

4.2 | Electrocardiography

Mice were anaesthetised with tribromoethanol (avertin: 2,2,2 trimethylethanol, Sigma Aldrich, Poole, UK) administered into the intraperitoneal space with a 27G hypodermic needle. They were then weighed, placed supine on a warmed (37°C) platform, and their limbs attached in anaesthetised animals. These were continued for 5 minutes to permit preparations to reach a steady state, and for a further 5 minutes to obtain traces for quantitative analysis. Dobutamine hydrochloride (3 mg/kg; Sigma Aldrich) was then administered into the intraperitoneal space. ECG recording was then continued until a new steady state was reached. A further 5 minute recording period provided traces for quantitative ECG analysis following pharmacological challenge.

### TABLE 5 Electrocardiographic recovery intervals: WT and Pgc-1β−/− compared

|                  | WT                  | Pgc-1β−/−          |
|------------------|---------------------|--------------------|
| RTc duration     |                     |                    |
| before dobutamine| 29.99 ± 0.60        | 28.65 ± 0.70       |
| following        | 33.63 ± 0.38        | 31.53 ± 0.57       |
| dobutamine       | 23.45 ± 0.46        | 22.55 ± 0.58       |
| following dobutamine | 25.91 ± 0.37        | 24.24 ± 0.44       |
| STc duration     |                     |                    |
| before dobutamine| 27.15 ± 0.51        | 25.89 ± 0.59       |
| following dobutamine | 30.24 ± 0.39        | 28.13 ± 0.48       |

Electrocardiographic measurements made in RTc, RTc and STc durations before and following dobutamine challenge in young WT (n = 5), aged WT (n = 8), young Pgc-1β−/− (n = 9) and aged Pgc-1β−/− mouse (n = 6). One young and one aged Pgc-1β−/− mouse were excluded as these showed paradoxical dromotropic effects that led to prolonged PR intervals and P waves that interfered with determinations of the end of the C wave to give the following n values: young WT (n = 5), aged WT (n = 8), young Pgc-1β−/− (n = 8) and aged Pgc-1β−/− (n = 5). This gave total n values of WT and Pgc-1β−/− of 13 in both cases.

### TABLE 6 Mean electrocardiographic QTc durations

|                  | WT                  | Pgc-1β−/−          |
|------------------|---------------------|--------------------|
| Mean QTc         |                     |                    |
| before dobutamine| 34.43 ± 0.20        | 35.60 ± 0.79       |
| following        | 40.23 ± 0.45        | 39.64 ± 0.52       |

Electrocardiographic measurements made in QTc durations before and following dobutamine challenge in young WT (n = 5), aged WT (n = 8), young Pgc-1β−/− (n = 9) and aged Pgc-1β−/− (n = 6) mice. One young and one aged Pgc-1β−/− mouse were excluded as these showed paradoxical dromotropic effects that led to prolonged PR intervals and P waves that interfered with determinations of the end of the C wave to give the following n values: young WT (n = 5), aged WT (n = 8), young Pgc-1β−/− (n = 8) and aged Pgc-1β−/− (n = 5). This gave total n values of WT and Pgc-1β−/− of 13 in both cases.
were visually verified. P-waves were analysed independently of QRS complexes by deleting the QRS complexes and subsequent isolation of the P-wave parameters. This analysis thus made no assumption of QRS complexes being preceded by P-waves. Analysis was performed on 300 second periods of ECG data immediately prior to and following administration of dobutamine hydrochloride. The effect of dobutamine was judged from observed ECG parameter changes. Figure 1 depicts a typical murine ECG recording with parameters calculated based upon this archetypal signal. Intervals were corrected using the formula previously described.52

4.4 Statistical analysis

Statistical analysis used the R programming language. Data sets were first tested for normality with the Shapiro-Wilk test before statistical analysis using two way factorial multivariate analysis of variance, i.e. MANOVA with Pillai trace. The data sets analysed were the steady state heart rates, P-wave durations, PR intervals, activation parameters of QR, QS and QR’ durations, recovery parameters of RTc, RT, and ST, durations, as well as the QTc interval. Each of these were measured from ECG records of young and aged, WT and Pgc-1β−/− mice respectively, both before and following dobutamine challenge. The initial MANOVA tests examined each parameter for significant effects of age, genotype or interactive effects of age and genotype either prior to or following dobutamine challenge. Where MANOVA testing indicated existence of significant differences prior to dobutamine administration, further ANOVA analyses were conducted on pre-drug parameters testing for effects of genotype, age or interacting effects of the two. The presence of significant effects then prompted pairwise Tukey honest significant difference testing of differences between pairs of individual parameters. Similarly, where significant differences were indicated post-dobutamine, a similar procedure of significance testing was performed examining for significant effects post drug challenge. Peak heart rates that were obtained following dobutamine challenge, were analysed by a two way factorial ANOVA: there was no meaningful peak heart rate pre-dobutamine. These were then also followed by post hoc Tukey tests for differences between individual parameters if prompted by the significance levels.

A P < .05 following Bonferroni correction where appropriate was considered to indicate a significant difference. Murine ECGs which demonstrated P-wave dissociation in the analysis period were discarded for P-wave dependent parameter analysis. All diagrams were produced with the R-program of graphics package.

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