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Inherited physical capacity: Widening divergence from young to adult to old

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Abstract

Cardiorespiratory performance segregates into rat strains of inherited low- and high-capacity runners (LCRs and HCRs); during adulthood, this segregation remains stable, but widens in senescence and is followed by segregated function, health, and mortality. However, this segregation has not been investigated prior to adulthood. We, therefore, assessed cardiorespiratory performance and cardiac cell (cardiomyocyte) structure–function in 1- and 4-month-old LCRs and HCRs. Maximal oxygen uptake was 23% less in LCRs at 1-month compared to HCRs at 1-month, and 72% less at 4 months. Cardiomyocyte contractility was 37—56% decreased, and Ca^{2+} release was 34—62% decreased, in 1- and 4-month LCRs versus HCRs. This occurred because HCRs had improved contractility and Ca^{2+} release during maturation, whereas LCRs did not. In quiescent cardiomyocytes, LCRs displayed 180% and 297% more Ca^{2+} sparks and 91% and 38% more Ca^{2+} waves at 1 and 4 months versus HCRs. Cell sizes were not different between LCRs and HCRs, but LCRs showed reduced transverse-tubules versus HCRs, though no discrepant transverse-tubule generation occurred during maturation. In conclusion, LCRs show reduced scores for aerobic capacity and cardiomyocyte structure–function compared to HCRs and there is a widening divergence between LCRs and HCRs during juvenile to near-adult maturation.

KEYWORDS
adolescence, calcium, cardiomyocyte, exercise, fitness, inheritance, maturation

INTRODUCTION

Divergence for inherited or intrinsic aerobic running or exercise capacity creates cohorts that are characterized by different parameters of whole-body exercise capacity as well as properties of skeletal muscle, vasculature, and the heart. In humans, this is a complex scenario but can be delineated from studies that show aerobic exercise capacity to strongly affect physiological, health, and mortality outcomes,[1–6] albeit in isolation the evidence may appear circumstantial. In experimental animals, the evidence is clearer and comes from studies of two-way artificial selection and breeding over generations, which has created heterogeneous cohorts of laboratory rats that segregate into low- and high-capacity runners (LCRs and HCRs, respectively).[7–10]

This two-way paradigm has resulted in rat LCR and HCR strains that differ in aerobic capacity by several-fold[7–12] and also demonstrate low or high capacities for a number of cardiac,[10,12–18] vascular,[10,19]

skeletal muscle,[20] neural and cognitive,[21,22] metabolic,[10,23,24] inflammation,[10] genetic,[25,26] carcinogenesis,[27] health,[9–11,18,19,28] and mortality[18,23] indices. In particular, the cardiometabolic health indices differ widely between LCRs and HCRs, to the degree that while HCRs develop an athletic phenotype, LCRs resemble a metabolic syndrome phenotype and show signs of mild cardiac pathology, as evidenced by visceral obesity, insulin resistance, elevated triglycerides, dyslipidemia, hypertension, reduced heart function, and development of mild concentric myocardial hypertrophy.[10,14,17,18] These LCR–HCR differences are inborn and not caused by experimental genetic manipulation or other interventions such as exercise training,[7,8] albeit they may affect the outcome of exercise training interventions.[29] Thus, the phenotypic differences between LCRs and HCRs have evolved and enriched over time due to continued selective breeding over many generations and are, therefore, genetic in origin, and as such, create a useful model to investigate intrinsic characteristics in widely different genetic backgrounds.

However, the available studies of LCR and HCR differences only have been conducted after a rat has reached adulthood[7–28] or senescence and old age,[14,18] while the phenotypic segregation shortly after birth and during development from early life to adulthood has not yet been investigated. Hence, it remains unknown when in the lifecycle the observed differences first occur and how they behave during the normal biological development of the maturation that occurs from weanling to adulthood. Thus, it becomes important to assess (i) differences in juvenile or very young LCR and HCR rats, and (ii) assess the divergence of phenotype characteristics from early life to adult stages.

This study, therefore, examined LCR and HCR rats as juveniles (1 month) and approaching adult maturity (4 months), with no difference in the interim environment in which the animals matured. We assessed parameters of cardiomyocyte cell size and architecture, systolic and diastolic Ca^{2+} handling, and contractile function, as well as maximal oxygen uptake (VO_{2max}) as a measure of whole-body aerobic and global cardiorespiratory capacities. Cardiomyocyte structure and function was specifically investigated because previous studies have reported clear and profound differences between LCRs and HCRs after reaching full adulthood.[10,14–18,26]

MATERIALS AND METHODS

Animals

We used male rats of ages 1 or 4 months from generation 16 of LCRs and HCRs origin (LCRs-1 mo, $n = 5$; LCRs-4 mo, $n = 6$; HCRs-1 mo, $n = 5$; HCRs-4 mo, $n = 6$; for simplicity, 1 and 4 mo are referred to as young and adult), following artificial selection and breeding to create heterogeneous cohorts that widely segregate into low and high aerobic exercise capacity (LCRs and HCRs, respectively), from a founder population of N:NIH rats, as previously described.[7–10] Due to the low availability of subject numbers, male rats were exclusively used to enhance within-group homogeneity. All animals were housed in the same environment with a 12-h light:dark cycle (lights on 1800/off 0600) at room temperature 22±1°C and *ad libitum* water and pellet rodent chow. All procedures were approved by the Institutional Review Board (Norwegian University of Science and Technology 129/4/03) and carried out in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 1996).

VO_{2max}

An incremental running exercise test was conducted on a custom-made 25° (47%) inclined treadmill inside a metabolic chamber, with running speed increased to 0.03 m/s every 2 minutes A single rat was tested at a time. Treadmills were ∼60 cm long, and normal running pattern and behavior consisted of rats typically running as far forward as they could in the top one third of the treadmill, which was also darkened for comfort (see Figure S1 for illustration of the treadmill). When fatigue set in, they started to drop back. At the bottom end of the treadmill, we would apply a small, mild electric shock via an electric grid placed behind the treadmill belt that induced unpleasantness but not pain, which stimulated continued running unless the rats reached fatigue. If this dropping-back behavior continued and became frequent (between three to five times per minute), the exercise test was concluded. Ambient air (0.5 L/min) was fed through the chamber, and gas samples were analyzed for oxygen and carbon dioxide. To control for the effects of differing body masses (M_b), VO_{2max} was allometrically scaled to $M_b^{0.75}$.

Cardiomyocyte contractility and Ca^{2+} cycling

Left ventricular cardiomyocytes were isolated after rapid excision during diethyl ether anesthesia, in a modified Krebs–Henseleit Ca^{2+}-free solution containing collagenase II (250 IU/mL, Worthington), bovine serum albumin (Sigma Aldrich), and with the stepwise introduction of $CaCl_2$ to 1.2 mM. Cardiomyocytes were then rested for 1 h in a HEPES-buffer before 20 min loading with 2 μM Fura-2/AM (Molecular Probes, Life Technologies) and placement in a cell chamber on an inverted microscope equipped with a 40×/1.3 numerical aperture oil-immersion objective (Diaphot-TMD, Nikon) and electrically 1 Hz twitch field-stimulated from platinum electrodes with 5 ms pulses. Temperature remained at 37°C. Contractility, taken as cell shortening and relaxation, was measured with video/edge-detection (Model 104, Crescent Electronics), while global cell Ca^{2+} cycling was measured after 500 Hz alternating excitation light 340/380 nm and epifluorescence emission collection at 510 nm with a photomultiplier tube (D-104, Photon Technology International), with the signal expressed as the ratio of the two excitation wavelengths. Ten steady-state consecutive contractions were analyzed in 5–10 cells/animal.

Quiescent Ca^{2+} sparks and waves

As a measure of diastolic Ca^{2+} handling, left ventricular cardiomyocytes were isolated and prepared as described above, but incubated

for 20 min with Fluo-3/AM (2 μM; Molecular Probes) and resuspended in either 1.8 or 5.0 mM extracellular Ca^{2+} concentration ($[Ca^{2+}]$) to stimulate Ca^{2+} sparks (1.8 mM) and waves (5.0 mM), respectively, before being placed onto a confocal microscope (Axioplan 100-M LSM510, Carl Zeiss) equipped with an LSM 510 laser scanner and a plan-apochromat 63×/1.4 numerical aperture oil-immersion objective. Continuous 2 ms linescan recordings were obtained along the longitudinal axis with a 1.0 airy unit pinhole diameter yielding an XYZ resolution of ~0.5×0.5×0.8 μm, after excitation with a 488 nm Argon laser and fluorescence emission collection at 505–530 nm with a photomultiplier tube. Laser intensity was minimized, while photomultiplier tube gain was increased in order to reduce photodamage and light-induced Ca^{2+} release events. This introduces some noise, but the recordings were subsequently filtered. Image processing and analysis was done by custom-made software (Delphi), whereby Ca^{2+} release events (sparks and waves) were analyzed relative to baseline (F/F_0). Ca^{2+} waves were defined by their point of origin, such that multiples originating simultaneously were categorized and analyzed separately. Five to 10 cells/animal were analyzed for each measurement.

Transverse (T)-tubules and cell dimensions

For cellular structure and architecture, left ventricular cardiomyocytes were isolated and prepared as described above and incubated for 20 min with Di-8-ANEPPS (10 μM; Molecular Probes). To quantify T-tubules, a confocal Z-stack frame scanned with 1 μm vertical steps throughout the cell was used, with the same microscope parameters described above. This generated 512×512 pixel XY images. Using custom-made applications in IDL6.0 (ITT Visual), we then analyzed relative T-tubule density normalized to cell size from 10 images/cell from the interior of the cell by counting pixels stained with dye relative to total pixels, after excluding pixels associated with surface membrane or lying outside the cell boundary. Five to 10 cells/animal were analyzed. Also, from 20 to 30 cells/animal, we measured cell length and midpoint width with a calibrated microscope caliper.

Statistics

Data are expressed as mean ± standard deviation. Because of the low sample size of animals, nonparametric statistical analysis was employed (SPSS version 27, IBM). Between-group effects were evaluated by a Kruskal–Wallis test with a Dunn's post-hoc test. Group-stimulation frequency effects were evaluated by a two-way ANOVA after data transformation to ranks and with a Games–Howell post-hoc test that does not assume equal variances or sample sizes. Significance level was $p<0.05$ and effect size (ES) was evaluated by the product-moment $r = z/\sqrt{N}$, where z is the standardized test statistic and N is the total number of observations in the pairwise comparison.

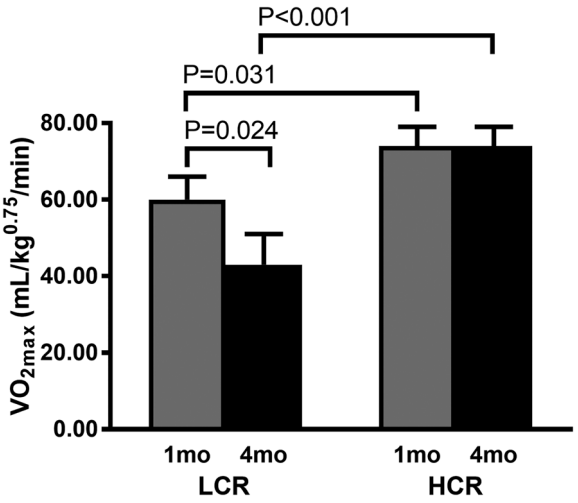

FIGURE 1 Comparison of maximal oxygen uptake (VO_{2max}) in young (1 mo) or adult (4 mo) low or high capacity runner rats (LCRs and HCRs, respectively). Statistical analysis: Kruskal–Wallis with Dunn's post-hoc test.

RESULTS

VO_{2max} and M_b

We measured whole-body aerobic cardiorespiratory capacity via VO_{2max}, measured by an incremental running exercise test. There was a significant effect of group ($H(3) = 17.789$, $p<0.001$; Figure 1). Young HCRs had 23% greater VO_{2max} compared to LCRs ($p = 0.031$, $r = 0.62$). In HCRs, VO_{2max} remained unaltered throughout maturation from young to adult ($p = 0.992$), but in LCRs, it decreased by 17 mL/kg$^{0.75}$/min (40%, $p = 0.024$, $r = 0.42$), leading to widening divergence that in adults accounted for a 72% difference ($p<0.001$, $r = 1.04$) between LCRs and HCRs.

There was a significant effect on M_b ($H(3) = 18.224$, $p<0.001$). In young, no M_b differences were present (LCRs: 80 ± 4 g and HCRs: 80 ± 6 g, $p = 1.000$). At adult age, both strains had increased M_b, but LCRs was significantly greater (LCRs: 415 ± 23 g, HCRs: 320 ± 16 g, $p = 0.017$).

Cardiomyocyte contractility

Both LCRs and HCRs presented with normal contraction–relaxation characteristics (see example traces in Figure 2A). However, contraction magnitude, measured as fractional shortening, differed between groups ($H(3) = 18.127$, $p<0.001$). It was significantly larger in HCRs than LCRs, 37% in young ($p = 0.027$, $r = 0.70$) and 56% in adult ($p = 0.002$, $r = 0.99$; Figure 2B). The expanding young-to-adult difference between LCRs and HCRs was caused by the increase from young to adult HCRs by 21% ($p = 0.041$, $r = 0.42$), whereas no age-change occurred in LCRs ($p = 0.544$).

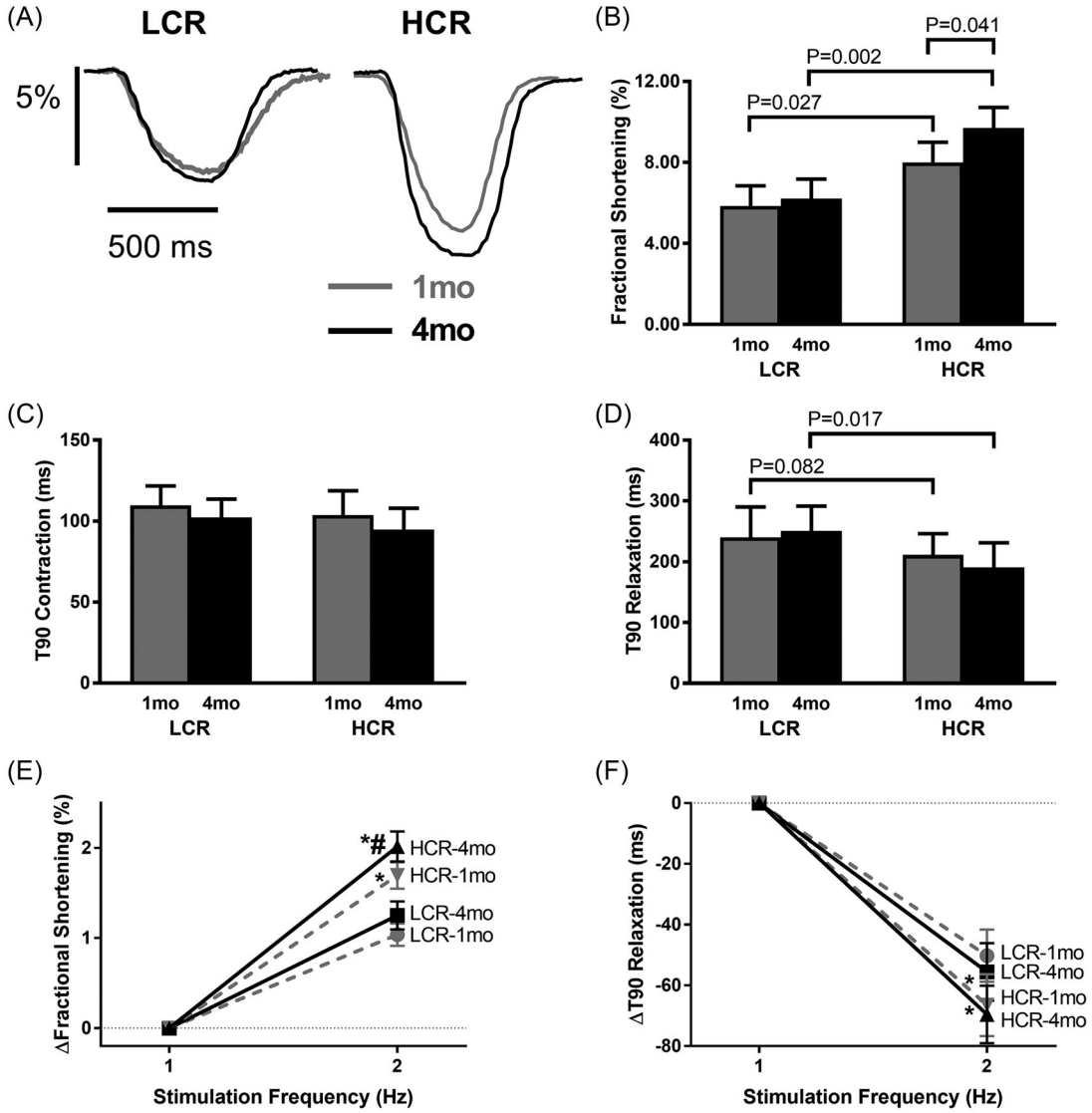

FIGURE 2 Comparison of twitch-stimulated cellular contraction–relaxation in young (1 mo) and adult (4 mo) low or high capacity runner rats (LCRs and HCRs, respectively). (A) Example traces of contraction–relaxation cycles, 1 Hz stimulation; horizontal bar indicates time scale of contraction–relaxation and vertical bar indicates magnitude of contraction–relaxation, measured as fractional (%) shortening from relaxed cell length. (B) Percent fractional shortening. (C) Time to 90% (T90) contraction. (D) T90 relaxation. (E) Change in percent fractional shortening 1–2 Hz stimulation. $^{*}p<0.05$, HCRs versus LCRs; $^{\#}p<0.05$, HCRs-4 mo versus HCRs-1 mo. (F) Change in T90 relaxation 1–2 Hz stimulation; frequency-dependent acceleration of relaxation (FDAR). $^{*}p<0.05$ HCRs versus LCRs. Statistical analysis for panels B–D: Kruskal—Wallis with Dunn's post-hoc test. Statistical analysis for panels E–F: two-way ANOVA after rank transformation with Games—Howell post-hoc test.

T90 contraction was not different between LCRs and HCRs nor did it change during maturation ($H(3) = 4.673$, $p = 0.260$; Figure 2C). However, group effects were identified for T90 relaxation ($H(3) = 11.075$, $p = 0.011$). T90 relaxation tended to be faster in HCRs compared to LCRs, 14% ($p = 0.082$, $r = 0.25$) in young and 29% ($p = 0.017$, $r = 0.93$) in adults, respectively, whereas it did not change with maturation in either HCRs ($p = 0.109$) or LCRs ($p = 0.445$; Figure 2D).

Cardiomyocyte Ca^{2+} cycling

Similar to contractility, both LCRs and HCRs presented with normal Ca^{2+} cycling characteristics (see example traces in Figure 3A); however, twitch-stimulated transient Ca^{2+} release amplitude, which assesses the amount of Ca^{2+} release, differed between groups and was larger in HCRs than LCRs ($H(3) = 19.241$, $p<0.001$; Figure 3B). Specifically, Ca^{2+} transient amplitude was 34% larger in young HCRs compared to young LCRs ($p = 0.039$, $r = 0.82$) and 62% larger in adult HCRs compared to adult LCRs ($p = 0.003$, $r = 0.87$). Similar to contractility, the expanding young-to-adult difference between LCRs and HCRs was caused by Ca^{2+} transient amplitude increasing 36% from young to adult HCRs ($p = 0.026$, $r = 0.42$), whereas no age-change was observed in LCRs ($p = 0.325$).

Ninety percent rise time (T90) was not significantly different between LCRs and HCRs nor did it change during maturation ($H(3) = 4.836$, $p = 0.204$; Figure 3C). There was a significant effect on T90

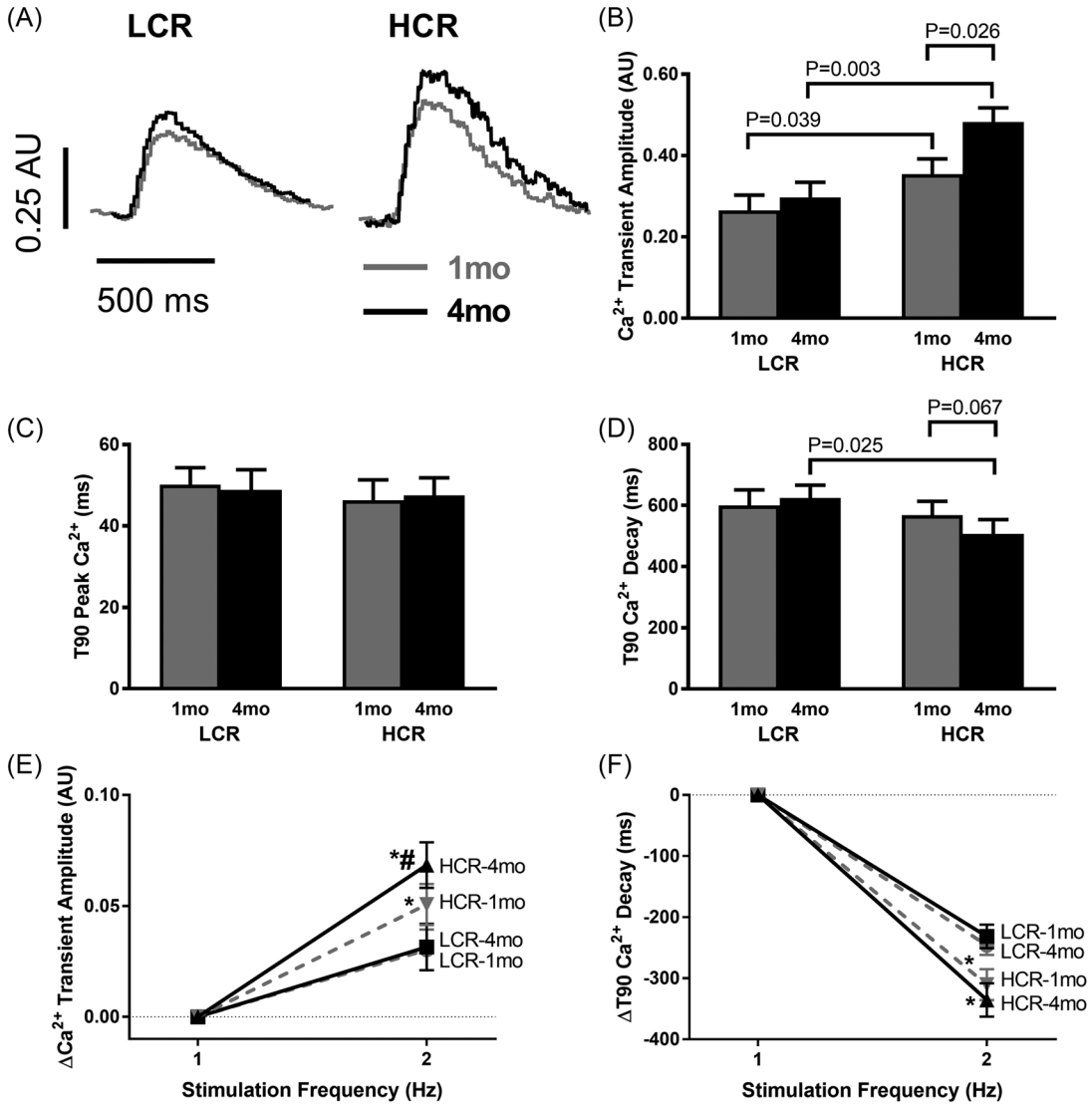

FIGURE 3 Comparison of twitch-stimulated intracellular Ca^{2+} handling in young (1 mo) and adult (4 mo) low or high capacity runner rats (LCRs and HCRs, respectively). (A) Example traces of intracellular Ca^{2+} transients, 1 Hz stimulation; horizontal bar indicates time scale of Ca^{2+} handling and vertical bar indicates magnitude of Ca^{2+} release, measured as arbitrary units (AU) of Ca^{2+}-sensitive fluorescence. (B) Ca^{2+} transient amplitude. (C) Time to 90% (T90) peak Ca^{2+}. (D) T90 Ca^{2+} decay. (E) Change in Ca^{2+} transient amplitude 1–2 Hz stimulation. $^{*}p<0.05$, HCRs versus LCRs; $^{\#}p<0.05$, HCRs-4 mo versus HCRs-1 mo. HCRs-1 mo versus LCRs-1 mo, $p = 0.041$; HCRs-4 mo versus LCRs-4 mo, $p = 0.016$. (F) Change in T90 Ca^{2+} decay 1–2 Hz stimulation; frequency-dependent acceleration of Ca^{2+} transient decay. $^{*}p<0.05$, HCRs versus LCRs. HCRs-1 mo versus LCRs-1 mo, $p = 0.042$; HCRs-4 mo versus LCRs-4 mo, $p = 0.027$. Statistical analysis for panels B–D: Kruskal—Wallis with Dunn's post-hoc test. Statistical analysis for panels E–F: two-way ANOVA after rank transformation with Games—Howell post-hoc test.

decay time ($H(3) = 9.456$, $p = 0.016$; Figure 3D). T90 in adult HCRs displayed a 19% ($p = 0.025$, $r = 1.07$) faster Ca^{2+} transient decay compared to adult LCRs. T90 did not change in young compared to adult LCRs ($p = 0.635$), but there was a trend toward a decrease from young to adult HCRs (11%, $p = 0.067$, $r = 0.55$).

Frequency-dependent contractility and Ca^{2+} cycling

We found a significant effect on frequency-dependent gains ($F(3,18) = 17.414$, $p<0.001$) by increasing twitch-stimulations from 1 to 2 Hz. There were significantly larger frequency-dependent gains in HCRs compared to LCRs and also significant improvements from young to adult HCRs, but not LCRs. Specifically, we observed a gain in fractional shortening during the 1–2 Hz step, with 0.66 percentage points more in young HCRs compared to young LCRs and 0.76 percentage points more in adult HCRs compared to adult LCRs. This frequency-dependent gain was also statistically significant from young to adult HCRs, but not LCRs ($p = 0.556$; Figure 2E). Frequency-dependent acceleration of relaxation was also more pronounced in HCRs compared to LCRs ($F(3,18) = 11.016$, $p = 0.031$) by 15 ms, but no maturation effects occurred (Figure 2F).

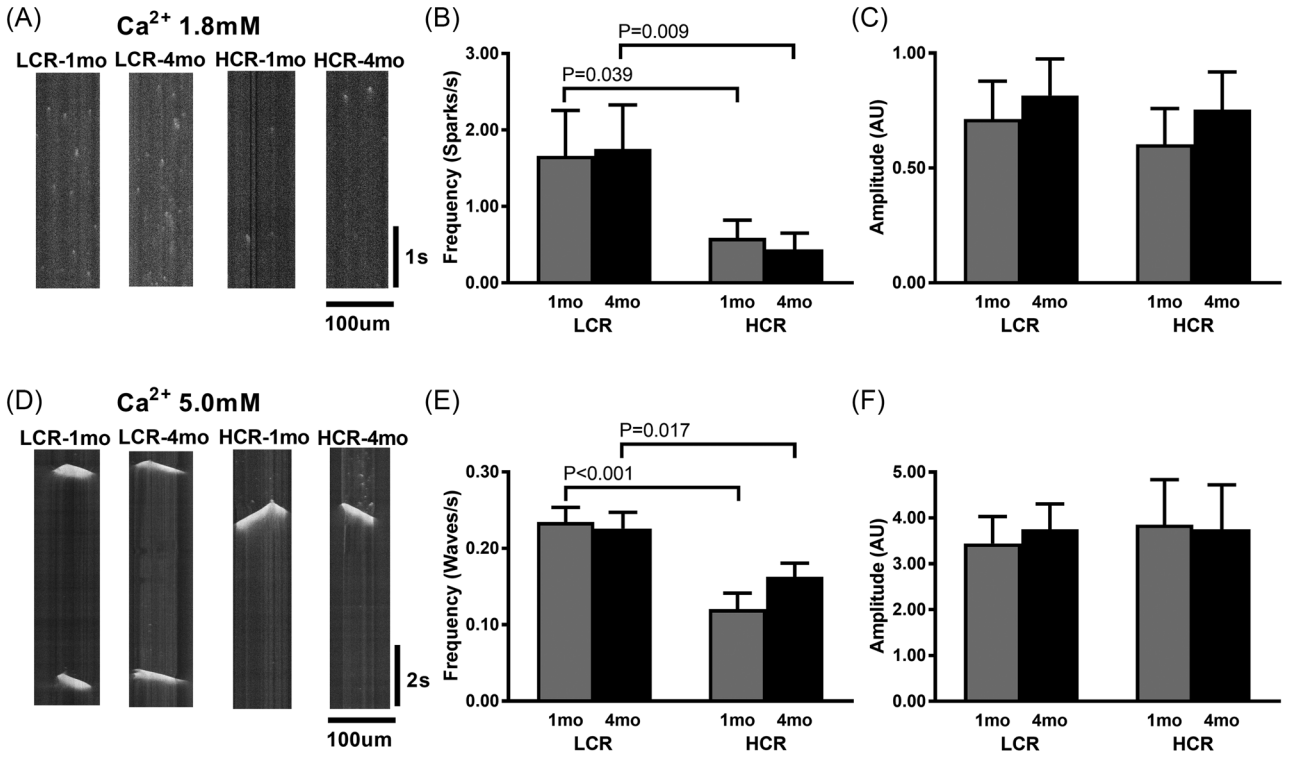

FIGURE 4 Comparison of quiescent Ca^{2+} release events in young (1 mo) and adult (4 mo) low or high capacity runner rats (LCRs and HCRs, respectively). (A) Example traces of confocal linescan recordings displaying Ca^{2+} sparks. (B) Ca^{2+} spark frequency. (C) Ca^{2+} spark amplitude. (D) Example traces of confocal linescan recordings displaying Ca^{2+} waves. (E) Ca^{2+} wave frequency. (F) Ca^{2+} wave amplitude. Statistical analysis: Kruskal–Wallis with Dunn's post-hoc test. Abbreviation: AU, arbitrary units of Ca^{2+}-sensitive fluorescence.

Ca^{2+} transient amplitude also increased during the 1–2 Hz step ($F(3,18) = 13.374$, $p = 0.008$). Ca^{2+} transient amplitude was 0.02 arbitrary units (AU) more in young HCRs compared to young LCRs and 0.04 AU more in adult HCRs compared to adult LCRs. This frequency-dependent gain from young to adult reached statistical significance in HCRs but not in LCRs ($p = 0.944$; Figure 3E). Frequency-dependent acceleration of Ca^{2+} transient decay was also more pronounced in HCRs compared to LCRs ($F(3,18) = 12.554$, $p = 0.022$) by 70–100 ms, while no maturation effects occurred (Figure 3F).

Ca^{2+} sparks and waves

Ca^{2+} release events were also assessed in quiescent cardiomyocytes residing in either 1.8 or 5.0 mM extracellular [Ca^{2+}]. 1.8 mM [Ca^{2+}] generated frequent sparks and only minimal waves (see example recordings Figure 4A), whereas 5.0 mM [Ca^{2+}] generated minimal sparks and frequent waves (see example recordings Figure 4D). There was a significant effect on Ca^{2+} spark frequency ($H(3) = 17.020$, $p = 0.004$; Figure 4B). Young LCRs displayed 180% more sparks compared to young HCRs ($p = 0.039$, $r = 0.62$), and adult LCRs displayed 297% more Ca^{2+} sparks compared to adult HCRs ($p = 0.009$, $r = 1.03$). There was no significant effect on spark amplitude ($H(3) = 5.037$, $p = 0.167$; Figure 4C). There was a significant effect on Ca^{2+} waves ($H(3) = 18.237$, $p = 0.002$; Figure 4E). Young LCRs displayed 91% more Ca^{2+} waves compared to young HCRs ($p<0.001$, $r = 1.19$) and adult LCRs displayed 38% more Ca^{2+} waves compared to adult HCRs ($p = 0.017$, $r = 0.57$). There was no significant effect on wave amplitude ($H(3) = 3.717$, $p = 0.294$; Figure 4F). Albeit the LCR–HCR differences were more pronounced in adult versus young, no further significant discrepancies in maturation from young to adult within either LCRs or HCRs were observed for either Ca^{2+} sparks or waves (all $p>0.05$). Half-width and half-duration of Ca^{2+} sparks as well as velocity, half-rise, and half-decay of Ca^{2+} waves were also analyzed, but did not differ between LCRs and HCRs or between young and adult (all $p>0.05$).

Cell morphology

Cardiomyocyte sizes were assessed. There was a weak, general trend toward shorter and wider cardiomyocytes in LCRs compared to HCRs, and a trend indicating that young cardiomyocytes had reached full or near-full development (Figure 5A,B). However, LCRs and HCRs were not different, and although there was a general trend for increased size from young to adult, this also did not reach statistical significance (cell length: $H(3) = 5.887$, $p = 0.190$; cell width: $H(3) = 4.077$, $p = 0.253$).

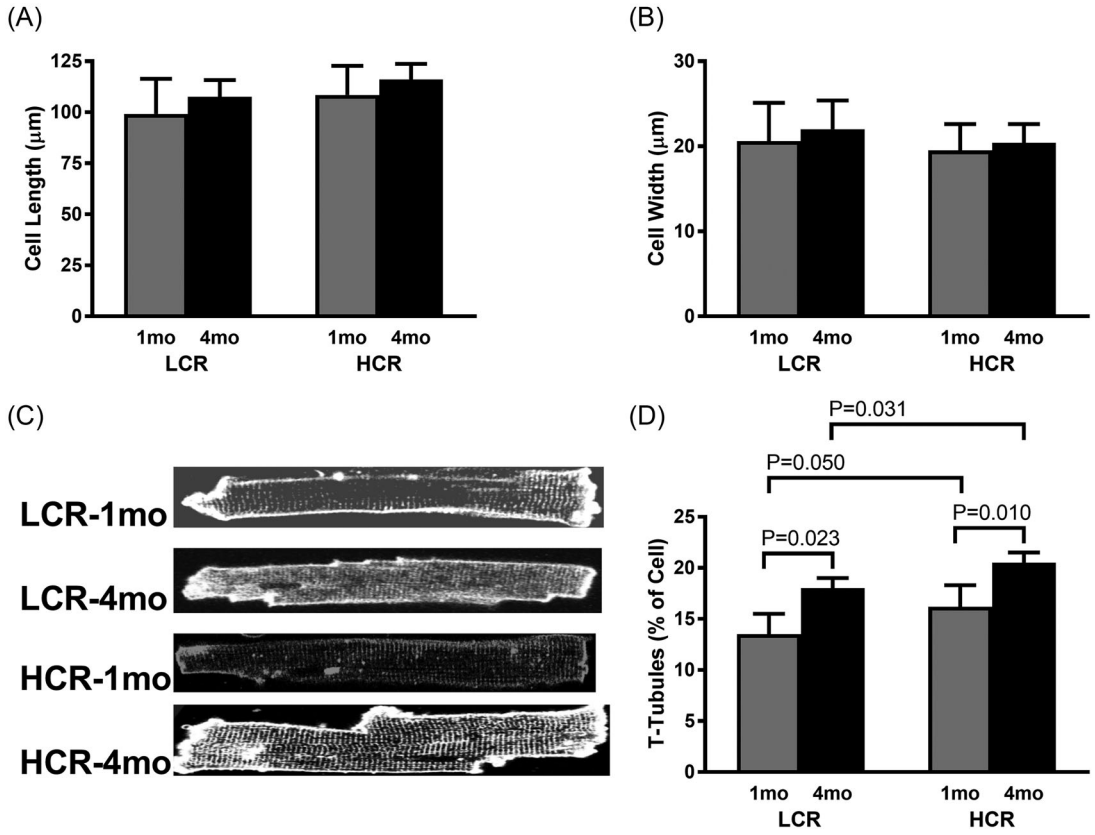

FIGURE 5 Comparison of cardiomyocyte morphology and transverse (T)-tubule structure in young (1 mo) and adult (4 mo) low or high capacity runner rats (LCRs and HCRs, respectively). (A) Cardiomyocyte length. (B) Cardiomyocyte width. (C) Example of confocal images of cardiomyocytes displaying T-tubules. (D) Relative T-tubule density. Statistical analysis: Kruskal–Wallis with Dunn's post-hoc test.

T-tubules

Finally, we assessed T-tubules (see example recordings in Figure 5C). We found a significant effect on T-tubules ($H(3) = 17.882$, $p = 0.007$; Figure 5D). Young LCRs presented with 20% reduced T-tubule density compared to young HCRs ($p = 0.05$, $r = 0.48$), and adult LCRs presented with 14% reduced T-tubules compared to adult HCRs ($p = 0.031$, $r = 0.56$). In both LCRs and HCRs, T-tubules were not yet fully formed in young, with a 33% increase from young to adult LCRs ($p = 0.023$, $r = 0.69$) and a 27% increase from young to adult HCRs ($p = 0.01$, $r = 0.77$).

DISCUSSION

It has been well established that high aerobic exercise capacity is physiologically characterized by higher function of, among others, cardiac contractile parameters both in the cell and the whole organ.[30–32] Although debate continues as to how much of this is inherited or acquired (nature versus nurture), human[33,34] and experimental[26,35,36] genetic association studies and the current LCR–HCR model system[7–10,26,29] have convincingly shown that a robust inherited component prevails that also at least partly explains the phenotype segregation between those that score for low and those that score for high exercise capacity. This segregation extends to senescence and aging,[14,18] but has as of yet not been shown in individuals of younger age. Here, we now show for the first time that inherited cardiac phenotypic segregation in the perspective of exercise capacity is already observable shortly after birth and widens from young to adult.

Divergence across lifespan

We have previously published an account of segregation observed in the same parameters as in the current study, in comparable generations of the LCR–HCR model system: previously in generations 14, 15, 17, 21,[14] and 22,[18] as well as in generation 16 in the current study. The overlap between generations renders compatibility between studies. These observations showed that HCRs, in contrast to LCRs, present with superior cardiac characteristics that overall lead to better functionality, exercise capacity, health, and even a 45% longer lifespan.[18] Data from adult animals in the current study are in agreement with these and other[10,12–17] findings at a cardiomyocyte structure–function level; specifically, cell sizes indicated the beginnings of mild concentric hypertrophy in LCRs, which was absent in HCRs, and LCRs and HCRs segregated for T-tubules, intracellular Ca^{2+} cycling and handling, and contractility, all suggesting an inherited cardiac phenotypic segregation. However, so far, phenotypic segregation has only

been characterized in adult or older animals, but with the current study, we extend this perspective into the realm of adolescent maturation from juvenile (1 month old) to near-full maturity and adulthood (4 months old), and thus we are in a position to theorize across the full lifespan. Based upon the current and previous[18] observations, we note:

(i) The current study shows that the phenomenon of inherited cardiac phenotypic segregation in the LCR–HCR model system already manifests itself shortly after birth.
(ii) The systematic increase in ES (and relative differences) between LCRs and HCRs when comparing first young and then adults suggests that there is a widening divergence between LCRs and HCRs occurring during maturation from young to adult. This has not previously been observed. Thus, the magnitude of LCR–HCR phenotypic differences, at least in the heart, establish themselves during adolescent maturation where they are subject to widening divergence.
(iii) Our data also indicate that LCRs, in contrast to HCRs, have a limited capability to improve inborn function while maturing from young to adult, whereas HCRs undergo significant improvements in the same parameters during the same maturation period.
(iv) The combined characterizations suggest that, in individual animals, functional parameters of LCRs and HCRs substantially do not diverge any further during the course of adulthood.
(v) Once in senescence and old age, deterioration of function (and health) follows different paths where widening divergence between LCRs and HCRs again reoccurs, with the decline in cardiac function in LCRs quantitatively amounting to twice that of HCRs, and which ultimately closely associates with earlier death and shorter lifespan in LCRs.

These points of a lifelong divergence between LCRs and HCRs, obtained from the current study and previous comparable observations,[18] indicate different paths of divergence between LCRs and HCRs across different stages of the lifecycle. These paths are summarized in Figure 6, with points i—v illustrating the corresponding points made above, while the smaller panels indicate that the majority of, but not all, parameters measured in the current study show widening divergence from young to adult maturation.

Mechanisms and implications of divergence

Taken together, the available evidence from this and previous studies[18] suggests that functional segregation and divergence in the heart and heart muscle cells is a life-long and ever-present feature in the LCR–HCR model; inherited, but subject to change throughout different phases of life: widening through adolescent maturation, stably divergent through adulthood, and yet again widening in senescence and old age. In the phases of widening divergence, this appears to be primarily caused by LCRs not improving function at all or improving at a lower rate than HCRs during adolescence and worsening of function at a greater rate than HCRs during senescence. During the latter, HCRs also reduce function, but less and at a lower rate than LCRs. A biological causal mechanism for the different paths that LCRs and HCRs display has not yet been identified, but in addition to inherited factors, a contribution may also come from self-governed physical activity. We have previously shown that LCRs and HCRs do not show a statistically significant difference in physical activity levels, but measurements in 24 h-metabolic chambers indicated a difference, showing that HCRs displayed increased activity (ES 2.20) and increased energy expenditure (ES 0.76).[18]

The majority, but not all, of the measured parameters indicated widening divergence between LCRs and HCRs during maturation from young to adult. Exceptions include, first, spontaneous Ca^{2+} waves where widening divergence does not occur, but these were measured at experimental conditions of high extracellular [Ca^{2+}] (5.0 mM), which predictably provoke Ca^{2+} waves to allow for measurements but do not occur in vivo. Importantly, differences in frequency of Ca^{2+} release events (i.e., sparks and waves) were not offset by different characteristics of individual events that might otherwise have canceled the increased Ca^{2+} leak in quiescence or diastole, which is in line with previous observations.[14,18] Second, T-tubules are structurally underdeveloped in LCRs, but form at nearly the same rate as HCRs during maturation. Third, LCRs show across the lifespan signs of pathologic cardiomyocyte remodeling. In old age, this is obvious,[18] but in 15- to 20-month-olds and thus toward the end of adulthood, the pathology is only starting to emerge with signs of mild concentric hypertrophy in the cell.[17,18] In 4-month-olds, this is even less obvious. Hence, much of the morphological and structural abnormalities in LCRs emerge in later life, whereas functional Ca^{2+} and contractile impairments already manifest at a young age. In contrast, HCRs do not develop overt cellular pathology at all, as observed here and previously.[14,18]

These findings of divergent cardiac phenotypes across the lifespan may have repercussions for heart health, especially in senescence, and as we hypothesize may be translationally linked to aerobic capacity. If so, this would be expected as such links have already been observed in other scenarios[32] and underscore the previously reported importance of oxygen metabolism.[1–6] Appreciation of widening divergence, therefore, also has potentially important implications for the timing of interventions to reduce heart impairments or abnormalities. Such interventions have tended to address adult and older populations,[37] but since impairments or abnormalities may start in early life, targeting younger populations may also be appropriate. One such intervention is physical activity and exercise. In the current scenario of aerobic capacity-linked dysfunctions, regular physical activity and exercise has been demonstrated to be a very effective intervention for improving physiologic and health parameters[1–6,30–33,37] and ameliorating low fitness-related impairments,[10,15,17,19,26] and it may be beneficial to introduce regular physical activity to a greater extent at a young age. This has also been advocated for by clinical trials evidencing both long-term benefit and safety of introducing added levels of physical activity to children.[30,38]

As indicated above, a mechanism that explains the blunted cardiomyocyte development and maturation in LCRs versus HCRs and the

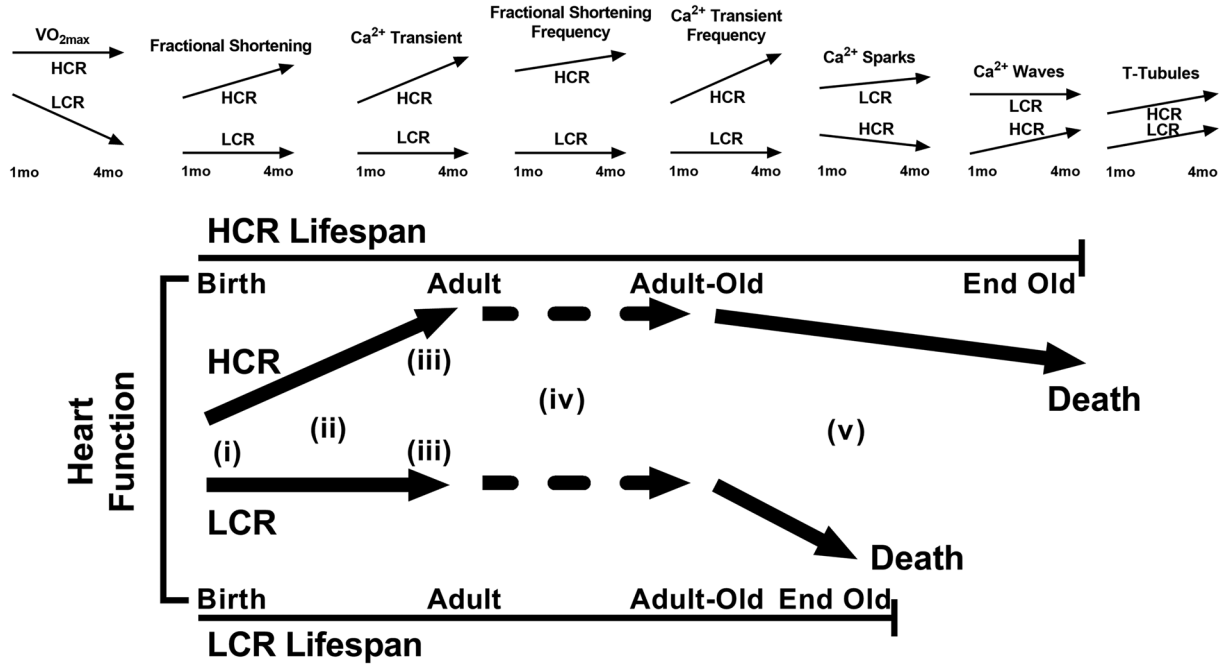

FIGURE 6 Illustration of divergence in cardiac cellular function between low or high capacity runner rats (LCRs and HCRs, respectively) across lifespan, as suggested by observations in current and previous study (see Ref. 18). The observations indicate divergence appearing at or shortly after birth, widening divergence through adolescence, statically maintained divergence through adulthood, and widening divergence reappearing through decline in senescence and old age. Roman numerals refer to points made in discussion. Panels above show widening LCR–HCR divergence in the majority, but not all, individually measured parameters during young (1 mo) to adult (4 mo) maturation.

widening divergence remains unknown, but it appears to be genetic in origin.[7–9,28] Identifying this genetic mechanism would be desirable. As such, genome-wide sequencing or screens may potentially find genotypic targets that are linked to the observed phenotype, and this may generate testable hypotheses for further study. However, there is also an obvious and potential role for mitochondria in the maturational divide between LCRs and HCRs. Mitochondria are primarily responsible for the transfer of energy by adenosine triphosphate, but besides energy transfer, mitochondria also mediate a myriad of biological functions including control of DNA, reproduction, apoptosis, and numerous pathological disorders ranging from neurodegeneration to cancer.[7–9] Mitochondria have already been linked to life-long health in this model system.[23] Moreover, since widening divergence is inherited, it is also a cause for the corollary and continuing widening divergence that is also observed between LCR and HCR cohorts across increasing generations following continued two-way artificial selection and breeding.[7,9,11,12,39] This explains why LCRs and HCRs displayed similar aerobic and functional capacities at the origin of divergence (generation 0), whereas after ~40 generations of two-way artificial selection and breeding, this has expanded to a ~10-fold difference.[39,40] This supports the notion of an overarching principle in biology that the availability of oxygen may also dictate evolution of life.[7,39,40]

CONCLUSIONS

Previous studies in the LCR–HCR model system have established that HCRs, distinguished by inherited high aerobic capacity, display superior cardiac function and performance in both the whole heart and the heart muscle cell (cardiomyocyte). During senescence and old age, this discrepancy between LCRs and HCRs widens, with the consequence that LCRs experience poorer health and shorter lives than HCRs. In this study, we expanded this perspective of segregated cardiomyocyte structure–function parameters into the realm of weanling to adulthood and the maturation that occurs in between. We found that already at 1-month old HCRs show superior characteristics of cardiomyocyte structure–function parameters as well as whole-body aerobic capacity and that these characteristics continue to improve and remain high during maturation, whereas LCRs in comparison display inferior characteristics at the same time points and impaired development during adolescence, and thus we observed a widening divergence occurring between young and adult LCRs and HCRs. This overall points to a robust cardiac function in HCRs and a comparatively frail cardiac function in LCRs, which has potentially important implications for lifelong health in those individuals with inherited high or low aerobic capacity, as well as for our understanding of inherited aerobic capacity and associated traits, including when in life these may manifest themselves. This, therefore, also partly lends support to the notion of introducing regular physical activity and exercise in early life. Effectively, it could be part of the foundation for lifelong health.

AUTHOR CONTRIBUTIONS

O.J.K., O.E., L.G.K., S.L.B., and U.W. conceived and designed the study. O.J.K., M.A.H., P.M.H., and U.W. performed experiments. O.J.K. and U.W. analyzed the data. All authors interpreted the data, O.J.K. wrote the

manuscript, all authors edited the manuscript, and all authors gave final approval and agreed to the submission of the manuscript.

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COMPETING INTERESTS

No competing interests exist.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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