**2. Results**

*a) Effect of chronic developmental hypoxia on cardiomyocyte morphometrics*

Cardiomyocytes from the H10 vs. N21 cohort were significantly smaller with a reduced length, depth, and volume, despite having larger widths (Tables 1 and S2).

*(b) Effect of developmental $O_2$ and experimental treatment*

The mixed-effects, repeated-measures GLMs revealed a significant effect of developmental $O_2$ on $\Delta[Ca^{2+}]_i$ and cell-shortening efficiency; an effect of treatment on cell shortening, $\Delta[Ca^{2+}]_i$, $pH_i$, ROS production, normalized $\Delta[Ca^{2+}]_i$, systolic $[Ca^{2+}]_i$, time to rise, and time to half-decay; an interaction between normoxia and developmental $O_2$ on the $\Delta[Ca^{2+}]_i$ and cell-shortening efficiency; and an interaction between anoxia and developmental $O_2$ on cell shortening, $pH_i$, ROS production, and cell-shortening efficiency (Table S3). Test statistics for significant within-group and between-group pairwise contrasts are provided in Supplementary Tables S4 and S5.

*(c) Myofilament $Ca^{2+}$-sensitivity*

The observation that H10 cardiomyocyte shortening was either equal to, or larger, than the N21 cells despite smaller $Ca^{2+}$ transients, suggested the H10 cohort had greater myofilament $Ca^{2+}$-sensitivity. Indeed, cardiomyocyte shortening was greater in H10 cells at any given $\Delta[Ca^{2+}]_i$, when compared to N21 cells (Fig. 4A, B). Regression lines fitted to the data revealed significant, positive correlations between $\Delta[Ca^{2+}]_i$ and contractility during normoxia and reoxygenation in N21 cardiomyocytes (but not anoxia) and during normoxia, anoxia, and reoxygenation in H10 cardiomyocytes (Fig. 4A, B). A GLM analysis revealed significant effects of developmental $O_2$, period (normoxia-anoxia), and developmental $O_2$-period interactions (N21 normoxia-N21 anoxia, N21 anoxia-H10 normoxia, N21 anoxia-H10 anoxia, and N21 anoxia-H10 reoxygenation). When comparing the estimated marginal means of the GLM, cardiomyocyte shortening was equal between the H10 cohort (normoxia, anoxia, and reoxygenation) and N21 normoxia and reoxygenation, but not N21 anoxia (Fig. 4A, B). These analyses show that H10 cardiomyocytes in anoxia shorten equally to all normoxia- or reoxygenation-exposed cells. Lastly, a GLM revealed a significant effect of $[Ca^{2+}]_e$ and a significant developmental $O_2$-$[Ca^{2+}]_e$ interaction, while the post-hoc tests showed that H10 cells were more sensitive to $Ca^{2+}$ (Fig. 4C). Regression lines were fitted to the data and revealed significant, positive correlations between $[Ca^{2+}]_e$ and length of N21 and H10 cardiomyocytes (Fig. 4C). Collectively, our data suggest that the myofilaments of H10 cardiomyocytes have a higher sensitivity to $Ca^{2+}$ than N21 cells (Fig. 4A, B, and C). Test statistics for these analyses are provided in Supplementary Tables S6 and S7s.

**Table S2. Between-group test statistics, comparing the effect of developmental normoxia (N21) and chronic hypoxia (H10) on ventricular cardiomyocyte morphometrics.**

| Variable | Comparison | Test statistic | df | P-value |
|---|---|---|---|---|
| Length | N21-H10 | t = 2.3 | 66 | 0.024 |
| Depth | N21-H10 | U = 65 | | 0.033 |
| Width | N21-H10 | U = 58 | | 0.015 |
| Volume | N21-H10 | U = 266 | | 0.031 |

Test values were calculated from a Student's t-test (length) or Mann-Whitney rank-sum tests (depth, width, and volume).

**Table S3. GLM test statistics, comparing the effects of developmental $O_2$ and experimental treatment on cardiomyocyte physiology.**

| Factor | Variable | t-value | df | P-value |
|---|---|---|---|---|
| Developmental $O_2$ | $\Delta[Ca^{2+}]_i$ | 2.9 | 18 | 0.009 |
| | Cell-shortening efficiency | -3 | 19 | 0.007 |
| Treatment | Cell shortening | 9.6 | 107 | < 0.001 |
| | $\Delta[Ca^{2+}]_i$ | 10.9 | 97 | < 0.001 |
| | $pH_i$ | 22.8 | 125 | < 0.001 |
| | ROS production | 8.4 | 83 | < 0.001 |
| | Normalized $\Delta[Ca^{2+}]_i$ | 14.8 | 103 | < 0.001 |
| | Systolic $[Ca^{2+}]_i$ | 4.2 | 105 | < 0.001 |
| | Time to rise | -6.1 | 31 | < 0.001 |
| | Time to half-decay | -2.7 | 29 | 0.011 |
| Developmental $O_2$-normoxia interaction | $\Delta[Ca^{2+}]_i$ | 4.1 | 20 | < 0.001 |
| | Cell-shortening efficiency | -3 | 19 | 0.007 |
| Developmental $O_2$-anoxia interaction | Cell shortening | -2.3 | 21 | 0.033 |
| | $pH_i$ | 2.4 | 22 | 0.028 |
| | ROS production | 2.88 | 16 | 0.011 |
| | Cell-shortening efficiency | -2.7 | 36 | 0.012 |

Test values were calculated from mixed-effects, repeated-measures GLMs.

**Table S4. Within-group test statistics of the estimated means of the interactive effects between developmental oxygen and experimental time/period on cardiomyocyte physiology**

| Variable | Developmental cohort | Time comparison (min) | t-value | df | P-value |
|---|---|---|---|---|---|
| Shortening | N21 | 5 - 15 | 4.4 | 106 | < 0.001 |
| | | 5 - 20 | 3.7 | 106 | 0.003 |
| | | 5 - 25 | 4.6 | 107 | < 0.001 |
| | | 5 - 30 | 5.0 | 107 | < 0.001 |
| | | 10 - 15 | 4.1 | 106 | 0.001 |
| | | 10 - 20 | 3.4 | 106 | 0.007 |
| | | 10 - 25 | 4.4 | 107 | < 0.001 |
| | | 10 - 30 | 4.8 | 107 | < 0.001 |
| | | 35 - 15 | 4.5 | 108 | < 0.001 |
| | | 35 - 20 | 3.8 | 108 | 0.002 |
| | | 35 - 25 | 4.8 | 107 | < 0.001 |
| | | 35 - 30 | 5.2 | 107 | < 0.001 |
| | | 40 - 15 | 4.5 | 108 | < 0.001 |
| | | 40 - 20 | 3.8 | 108 | 0.002 |
| | | 40 - 25 | 4.7 | 108 | < 0.001 |
| | | 40 - 30 | 5.1 | 107 | < 0.001 |
| | H10 | 5 - 15 | 5.3 | 106 | < 0.001 |
| | | 5 - 20 | 2.9 | 106 | 0.045 |
| | | 10 - 15 | 5.2 | 106 | < 0.001 |
| | | 10 - 20 | 2.9 | 106 | 0.050 |
| | | 25 - 15 | 3.2 | 106 | 0.042 |
| | | 30 - 15 | 3.4 | 106 | 0.024 |
| | | 35 - 15 | 3.6 | 107 | 0.006 |
| | | 40 - 15 | 3.7 | 107 | 0.004 |
| $\Delta[Ca^{2+}]_i$ | N21 | 5 - 15 | 6.5 | 96 | < 0.001 |
| | | 5 - 20 | 6.8 | 96 | < 0.001 |
| | | 5 - 25 | 6.8 | 96 | < 0.001 |
| | | 5 - 30 | 6.1 | 96 | < 0.001 |
| | | 10 - 15 | 8.4 | 96 | < 0.001 |
| | | 10 - 20 | 8.8 | 96 | < 0.001 |
| | | 10 - 25 | 8.8 | 96 | < 0.001 |
| | | 10 - 30 | 8.0 | 96 | < 0.001 |
| | | 35 - 15 | 7.6 | 96 | < 0.001 |
| | | 35 - 20 | 8.0 | 97 | < 0.001 |
| | | 35 - 25 | 8.0 | 97 | < 0.001 |
| | | 35 - 30 | 7.4 | 96 | < 0.001 |
| | | 40 - 15 | 7.9 | 96 | < 0.001 |
| | | 40 - 20 | 8.2 | 97 | < 0.001 |

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| | | 40 - 25 | 8.2 | 97 | < 0.001 |
| | | 40 - 30 | 7.6 | 96 | < 0.001 |
| | H10 | 35 - 15 | 3.2 | 95 | 0.021 |
| | | 35 - 20 | 3.5 | 95 | 0.011 |
| | | 35 - 25 | 3.1 | 95 | 0.028 |
| | | 35 - 30 | 3.2 | 95 | 0.024 |
| $pH_i$ | N21 | 5 - 15 | 6.5 | 124 | < 0.001 |
| | | 5 - 20 | 6.8 | 124 | < 0.001 |
| | | 5 - 25 | 6.8 | 124 | < 0.001 |
| | | 5 - 30 | 6.1 | 124 | < 0.001 |
| | | 10 - 15 | 8.4 | 124 | < 0.001 |
| | | 10 - 20 | 8.8 | 124 | < 0.001 |
| | | 10 - 25 | 8.8 | 124 | < 0.001 |
| | | 10 - 30 | 8.0 | 124 | < 0.001 |
| | | 35 - 15 | 7.6 | 125 | < 0.001 |
| | | 35 - 20 | 8.0 | 125 | < 0.001 |
| | | 35 - 25 | 8.0 | 125 | < 0.001 |
| | | 35 - 30 | 7.4 | 125 | < 0.001 |
| | | 40 - 15 | 7.9 | 125 | < 0.001 |
| | | 40 - 20 | 8.2 | 125 | < 0.001 |
| | | 40 - 25 | 8.2 | 125 | < 0.001 |
| | | 40 - 30 | 7.6 | 125 | < 0.001 |
| | H10 | 5 - 15 | 7.4 | 124 | < 0.001 |
| | | 5 - 20 | 7.6 | 124 | < 0.001 |
| | | 5 - 25 | 7.6 | 125 | < 0.001 |
| | | 5 - 30 | 7.3 | 125 | < 0.001 |
| | | 10 - 15 | 8.1 | 124 | < 0.001 |
| | | 10 - 20 | 8.3 | 124 | < 0.001 |
| | | 10 - 25 | 8.3 | 125 | < 0.001 |
| | | 10 - 30 | 8.0 | 125 | < 0.001 |
| | | 35 - 15 | 9.5 | 125 | < 0.001 |
| | | 35 - 20 | 9.7 | 125 | < 0.001 |
| | | 35 - 25 | 9.8 | 125 | < 0.001 |
| | | 35 - 30 | 9.5 | 125 | < 0.001 |
| | | 40 - 15 | 8.8 | 125 | < 0.001 |
| | | 40 - 20 | 9.0 | 125 | < 0.001 |
| | | 40 - 25 | 9.1 | 125 | < 0.001 |
| | | 40 - 30 | 8.8 | 125 | < 0.001 |
| ROS | N21 | 5 - 20 | 3.1 | 82 | 0.035 |
| | | 5 - 25 | 3.4 | 82 | 0.015 |
| | | 5 - 30 | 3.3 | 82 | 0.017 |

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|---|---|---|---|---|---|
| | | 10 - 25 | 3.1 | 82 | 0.035 |
| | | 10 - 30 | 3.0 | 82 | 0.038 |
| | H10 | 5 - 15 | 5.3 | 82 | < 0.001 |
| | | 5 - 20 | 5.8 | 83 | < 0.001 |
| | | 5 - 25 | 6.1 | 83 | < 0.001 |
| | | 5 - 30 | 6.4 | 83 | < 0.001 |
| | | 5 - 35 | 3.5 | 83 | 0.015 |
| | | 5 - 40 | 3.7 | 83 | 0.009 |
| | | 10 - 15 | 4.7 | 82 | < 0.001 |
| | | 10 - 20 | 5.2 | 83 | < 0.001 |
| | | 10 - 25 | 5.5 | 83 | < 0.001 |
| | | 10 - 30 | 5.8 | 83 | < 0.001 |
| | | 35 - 30 | 2.9 | 82 | 0.041 |
| Normalized $\Delta[Ca^{2+}]_i$ | N21 | 5 - 15 | 4.5 | 98 | < 0.001 |
| | | 5 - 20 | 5.4 | 98 | < 0.001 |
| | | 5 - 25 | 4.4 | 100 | < 0.001 |
| | | 5 - 30 | 5.0 | 100 | < 0.001 |
| | | 10 - 15 | 5.7 | 98 | < 0.001 |
| | | 10 - 20 | 6.6 | 98 | < 0.001 |
| | | 10 - 25 | 5.5 | 100 | < 0.001 |
| | | 10 - 30 | 6.1 | 100 | < 0.001 |
| | | 35 - 15 | 5.1 | 99 | < 0.001 |
| | | 35 - 20 | 5.9 | 101 | < 0.001 |
| | | 35 - 25 | 5.0 | 102 | < 0.001 |
| | | 35 - 30 | 5.6 | 100 | < 0.001 |
| | | 40 - 15 | 4.8 | 99 | < 0.001 |
| | | 40 - 20 | 5.6 | 101 | < 0.001 |
| | | 40 - 25 | 4.8 | 102 | < 0.001 |
| | | 40 - 30 | 5.3 | 100 | < 0.001 |
| | H10 | 5 - 15 | 4.1 | 100 | 0.001 |
| | | 5 - 20 | 4.6 | 100 | < 0.001 |
| | | 5 - 25 | 4.1 | 100 | 0.001 |
| | | 5 - 30 | 4.5 | 100 | < 0.001 |
| | | 10 - 15 | 5.3 | 100 | < 0.001 |
| | | 10 - 20 | 5.8 | 100 | < 0.001 |
| | | 10 - 25 | 5.3 | 100 | < 0.001 |
| | | 10 - 30 | 5.7 | 100 | < 0.001 |
| | | 35 - 15 | 6.6 | 97 | < 0.001 |
| | | 35 - 20 | 7.0 | 97 | < 0.001 |
| | | 35 - 25 | 6.6 | 97 | < 0.001 |
| | | 35 - 30 | 6.9 | 97 | < 0.001 |

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|---|---|---|---|---|---|
| | | 40 - 15 | 5.0 | 98 | < 0.001 |
| | | 40 - 20 | 5.5 | 98 | < 0.001 |
| | | 40 - 25 | 5.0 | 98 | < 0.001 |
| | | 40 - 30 | 5.3 | 98 | < 0.001 |
| Diastolic $[Ca^{2+}]_i$ | N21 | 5 - 35 | -3.8 | 124 | 0.007 |
| | | 5 - 40 | -3.8 | 124 | 0.006 |
| | H10 | 5 - 35 | -3.6 | 124 | 0.013 |
| | | 5 - 40 | -4.4 | 124 | 0.001 |
| | | 10 - 35 | -3.3 | 124 | 0.036 |
| | | 10 - 40 | -4.0 | 124 | 0.002 |
| Systolic $[Ca^{2+}]_i$ | N21 | 5 - 35 | -3.9 | 104 | 0.002 |
| | | 5 - 40 | -3.3 | 104 | 0.014 |
| | | 35 - 15 | 4.0 | 104 | 0.002 |
| | | 35 - 20 | 3.9 | 104 | 0.002 |
| | | 35 - 25 | 3.2 | 104 | 0.019 |
| | | 35 - 30 | 2.9 | 104 | 0.045 |
| | | 40 - 15 | 3.4 | 104 | 0.010 |
| | | 40 - 20 | 3.3 | 104 | 0.014 |
| | H10 | 5 - 35 | -3.6 | 103 | 0.012 |
| | | 5 - 40 | -3.8 | 103 | 0.008 |
| | | 10 - 35 | -3.2 | 103 | 0.038 |
| | | 10 - 40 | -3.4 | 103 | 0.027 |
| | | 35 - 15 | 3.3 | 104 | 0.014 |
| | | 35 - 20 | 2.9 | 104 | 0.048 |
| | | 40 - 15 | 3.5 | 104 | 0.010 |
| | | 40 - 20 | 3.0 | 104 | 0.035 |

| **Variable** | **Developmental cohort** | **Time comparison (period)** | **t-value** | **df** | **P-value** |
|---|---|---|---|---|---|
| Time to rise | N21 | N - A | -3.8 | 30 | 0.001 |
| | | R - A | -3.5 | 31 | 0.002 |
| | H10 | N - A | -4.6 | 32 | < 0.001 |
| | | R - A | -3.2 | 30 | 0.003 |
| Time to half-decay | N21 | N - A | -2.3 | 29 | 0.044 |
| | H10 | N - A | -2.3 | 30 | 0.039 |

Test values were calculated from mixed-effects, repeated-measures GLMs, followed by sequential Sidak post-hoc tests. Abbreviations: N21 and H10 indicate embryonic development in 21% and 10% $O_2$, respectively; df, degrees of freedom; and N, A, and R indicate experimental periods normoxia, anoxia, and reoxygenation, respectively.

**Table S5. Between-group test statistics of the estimated means of the interactive effects between developmental oxygen, treatment, and time on cardiomyocyte physiology**

| Variable | Treatment | Time (min) | Comparison | t-value | df | P-value |
|---|---|---|---|---|---|---|
| Shortening | Anoxia | 25 | N21 - H10 | -2.6 | 55 | 0.013 |
| | | 30 | N21 - H10 | -3.1 | 59 | 0.003 |
| $\Delta[Ca^{2+}]_i$ | Normoxia | 5 | N21 - H10 | 3.5 | 30 | 0.001 |
| | | 10 | N21 - H10 | 4.3 | 30 | < 0.001 |
| | | 35 | N21 - H10 | 3.1 | 37 | 0.004 |
| | | 40 | N21 - H10 | 3.2 | 39 | 0.002 |
| $pH_i$ | Normoxia | 10 | N21 - H10 | 2.0 | 40 | 0.049 |
| | Anoxia | 30 | N21 - H10 | 2.1 | 45 | 0.042 |
| ROS | Anoxia | 15 | N21 - H10 | 2.1 | 36 | 0.043 |
| | | 20 | N21 - H10 | 2.2 | 38 | 0.031 |
| | | 25 | N21 - H10 | 2.3 | 38 | 0.028 |
| | | 30 | N21 - H10 | 2.5 | 38 | 0.016 |
| Efficiency | Normoxia | 5 | N21 - H10 | -2.4 | 124 | 0.019 |
| | Anoxia | 25 | N21 - H10 | -2.1 | 126 | 0.034 |
| | | 30 | N21 - H10 | -3.8 | 126 | < 0.001 |

Test values were calculated from mixed-effects, repeated-measures GLMs, followed by sequential Sidak post-hoc tests. Abbreviations: N21 and H10 indicate embryonic development in 21% and 10% $O_2$, respectively; df, degrees of freedom

**Table S6. Regression and GLM test statistics of the effects of developmental $O_2$ and experimental treatment on cardiomyocyte shortening, as a function of the calcium transient ($\Delta[Ca^{2+}]_i$).**

| Developmental cohort | Regression | Treatment | Test statistic | P-value | $R^2$ |
|---|---|---|---|---|---|
| N21 | Shortening vs. $\Delta[Ca^{2+}]_i$ | N | F(1, 13) = 6.19 | 0.027 | 0.323 |
| | | R | [F(1, 6) = 6.82 | 0.040 | 0.532 |
| H10 | Shortening vs. $\Delta[Ca^{2+}]_i$ | N | F(1, 16) = 7.42 | 0.015 | 0.317 |
| | | A | F(1, 29) = 5.67 | 0.024 | 0.164 |
| | | R | F(1, 13) = 4.59 | 0.05 | 0.261 |

| GLM Factor | Comparison | df | P-value |
|---|---|---|---|
| Developmental $O_2$ | N21-H10 | 1 | < 0.001 |
| Experimental period | N-A | 1 | 0.022 |
| Developmental $O_2$-period interactions | N21 N-N21 A | 1 | 0.008 |
| | N21 A-H10 N | 1 | < 0.001 |
| | N21 A-H10 A | 1 | < 0.001 |
| | N21 A-H10 R | 1 | 0.001 |

Test values were calculated from regression analyses and mixed-effects GLMs. Abbreviations: N21 and H10 indicate embryonic development in 21% and 10% $O_2$, respectively; N, A, and R indicate experimental periods normoxia, anoxia, and reoxygenation, respectively.

**Table S7. Regression and GLM test statistics of the effect of extracellular calcium ($[Ca^{2+}]_e$) on relaxed cardiomyocyte length.**

| GLM component | Test statistic | P-value |
|---|---|---|
| $[Ca^{2+}]_e$ factor | F(1, 34) = 80.93 | < 0.001 |
| Developmental $O_2$-$[Ca^{2+}]_e$ interaction | F(1, 34) = 15.98 | < 0.001 |
| N21-H10 post-hoc comparison | t = 3.19 | < 0.001 |

| Regression | Developmental cohort | Test statistic | P-value | $R^2$ |
|---|---|---|---|---|
| Length vs. $[Ca^{2+}]_e$ | N21 | F(1, 22) = 30.75 | < 0.001 | 0.583 |
| | H10 | F(1, 22) = 34.72 | < 0.001 | 0.612 |

Test values were calculated from regression analyses and mixed-effects GLMs. Abbreviations: N21 and H10 indicate embryonic development in 21% and 10% $O_2$, respectively.