Thermal conditions experienced during differentiation affect metabolic and contractile phenotypes of mouse myotubes

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Little AG, Seebacher F. Thermal conditions experienced during differentiation affect metabolic and contractile phenotypes of mouse myotubes. Am J Physiol Regul Integr Comp Physiol 311: R457–R465, 2016. First published July 6, 2016; doi:10.1152/ajpregu.00148.2016.—Central pathways regulate metabolic responses to cold in endotherms to maintain relatively stable internal core body temperatures. However, peripheral muscles routinely experience temperatures lower than core body temperature, so that it would be advantageous for peripheral tissues to respond to temperature changes independently from core body temperature regulation. Early developmental conditions can influence offspring phenotypes, and here we tested whether developing muscle can compensate locally for the effects of cold exposure independently from central regulation. Muscle myotubes originate from undifferentiated myoblasts that are laid down during embryogenesis. We show that in a murine myoblast cell line (C2C12), cold exposure (32°C) increased myoblast metabolic flux compared with 37°C control conditions. Importantly, myotubes that differentiated at 32°C compensated for the thermodynamic effects of low temperature by increasing metabolic rates, ATP production, and glycolytic flux. Myotube responses were also modulated by the temperatures experienced by “parent” myoblasts. Myotubes that differentiated under cold exposure increased activity of the AMP-stimulated protein kinase (AMPK), which may mediate metabolic changes in response to cold exposure. Moreover, cold exposure shifted myosin heavy chains from slow to fast, presumably to overcome slower contractile speeds resulting from low temperatures. Adjusting thermal sensitivities locally in peripheral tissues complements central thermoregulation and permits animals to maintain function in cold environments. Muscle also plays a major metabolic role in adults, so that developmental responses to cold are likely to influence energy expenditure later in life.

Skeletal muscle plays an essential role in animal ecology and human health by facilitating locomotion and thereby behavior and movement. Skeletal muscle also determines metabolic phenotypes, because it makes up a large proportion of body mass and represents a major site for lipid and glucose oxidation. Muscle metabolism and contractile function are sensitive to changes in temperature so that thermal responses of muscle are important in determining whole organism phenotypes and function.

Most thermal responses in mammals are mediated centrally via sympathetic output and endocrine signaling, as well as by behavioral and cardiovascular adjustments. However, despite precise regulation of core body temperatures in active endotherms, peripheral muscles routinely reach temperatures several degrees below core body temperature, particularly during inactivity and at the early stages of activity. There is some evidence that isolated cells or tissues can mount responses to changes in temperature directly. However, whether these responses compensate for the effects of local cold exposure on metabolic and contractile (in the case of muscle) phenotypes is unresolved. Our aim was therefore to determine whether metabolic phenotypes of muscle cells change in response to the thermal conditions experienced during muscle cell differentiation to compensate for negative thermodynamic effects of low temperature. Vertebrates have a finite number of muscle precursor cells (myoblasts) that differentiate into functional myotubes during growth. Epigenetic mechanisms during differentiation of “parent” myoblasts into “offspring” myotubes can modulate myotube and muscle tissue phenotypes. Hence, we tested whether temperatures experienced by myoblasts and during cell differentiation determine the metabolic phenotype of mature myotubes.

The proximate mechanisms that can alter myotube phenotypes include differences in the sensitivity to central inputs. In an individual cell, for instance, responses to thyroid hormone signals may be modulated by altering the expression levels and activities of thyroid receptors. Decreasing temperature also causes an energy deficit in the cell, which elicits a regulated response that increases mitochondrial density and ATP production in muscle via AMP-stimulated protein kinase (AMPK) activity. AMPK switches cells from an anabolic to a catabolic state and upregulates mitochondrial function. Hence, AMPK is a locally acting regulator that could induce developmental changes in muscle phenotype in response to local changes in temperature. Muscle metabolic phenotypes are determined to a large extent by differential composition of myosin heavy chains (MHC) that range from slow, oxidative type I isoforms to fast, glycolytically poised type II isoforms. Importantly, increased activity of AMPK can shift mouse muscle to a more oxidative metabolic phenotype with increased MHC Ila concentrations, albeit without changing MHC I content. Additionally, AMPK may alter expression of thyroid receptors in adipose tissue, although it is not known whether this is also the case in muscle cells.

We used a murine myoblast cell line (C2C12), which allowed precise control over the local thermal environments (32 and 37°C), to investigate whether responses to temperature are regulated locally and independently from central regulatory mechanisms. We tested the hypotheses 1) that myoblasts passaged at a cool temperature (32°C) enhance metabolic capacity to compensate for the thermodynamic effects of low temperature; 2) that the thermal profile of a myoblast population would influence the metabolic phenotype of subsequent myotubes, and 3) that myotubes from hypothermic myoblast populations differentiated at 32°C have greater activities of AMPK, higher...
proportions of slow MHCs, and greater densities of thyroid receptors.

**MATERIALS AND METHODS**

**Cell culture.** C2C12 murine myoblasts can be passaged in culture indefinitely but rapidly differentiate into myotubes when grown to high confluence in differentiation medium. We passaged myoblasts under optimal thermal conditions (37°C) and measured their metabolic profiles at both temperatures (Fig. 1). We chose 32°C for our cold exposure treatment because it represents relatively typical temperature for peripheral limb muscles, which can reach even lower temperatures when ambient conditions fall below 20°C (22). We then differentiated myoblasts from both treatments at each temperature to determine whether the "parental" thermal environment of myoblasts affects the metabolic phenotype of the developing myotubes in a fully factorial design (Fig. 1). All cell culture experiments were repeated four times, and samples for analysis were obtained in equal proportions from each experiment.

We grew C2C12 murine myoblasts (CRL-1772; ATCC, Manassas, VA) in flasks with growth medium [high glucose Dulbecco’s modified Eagle’s medium (DMEM) GIBCO-Life Technologies, Mulgrave, Australia] supplemented with 20% (vol/vol) fetal bovine serum and 1% (vol/vol) Penstrep at 37°C and 5% CO2. Cultures were maintained below 75% confluence through serial passaging (every 2–3 days) using 0.25% trypsin-EDTA to dislodge cells. Cultures were not used beyond 20 passages. To achieve differentiation into myotubes, cells were seeded on plates thinly coated with an extracellular matrix gel from Engelbreth-Holm-Swarm murine sarcoma (Sigma, Castle Hill, Australia) and grown to confluence. Upon confluence, growth medium was exchanged for differentiation medium [DMEM supplemented with 2% (vol/vol) horse serum and 1% (vol/vol) penstrep], which was replenished daily.

**Temperature treatments and differentiation time course.** Myoblast cultures were split and used to seed new flasks that were grown at either 37°C or 32°C (myoblast growth temperatures). Myoblasts were then passaged at these temperatures for 10 days before experimental analysis. For myotube treatments, myoblasts from each growth temperature (37°C or 32°C) were used to seed plates that were then differentiated into myotubes at either 37°C or 32°C (myotube differentiation temperatures). Because temperature influences rates of differentiation, we ran time-course experiments to track the progression of myogenic differentiation at both temperatures. Myoblasts were differentiated in XF24 cell culture microplates (Seahorse Bioscience, North Billerica, MA) that were first treated with 10 UL of Engelbreth-Holm-Swarm murine sarcoma (Sigma) to promote attachment and differentiation. Each well was seeded with 25,000 cells. We imaged the progression of myogenic differentiation daily for 6 days at ×10 magnification on a light microscope. We used morphological markers of differentiation to approximate stages of myogenesis, as previously described (4). We found that myotubes that were differentiated at 37°C for 72 h appeared to be at similar stages of

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Fig. 1. Experimental design. Myoblasts were grown in culture for at least 10 days at 32°C or 37°C (myoblast growth temperature) before analyses. Aerobic (MO2) and anaerobic (extracellular acidification rate, ECAR) metabolic flux were measured in myoblasts from both growth temperatures at 32°C and 37°C test temperatures to determine thermal sensitivity. Myoblasts from both growth temperatures were also used to differentiate into myotubes at 32°C and 37°C (myoblast differentiation temperatures). Myotubes differentiated at 32°C for 120 h reached parallel stages of differentiation to myotubes differentiated at 37°C for 72 h. These stages were verified through analyses of morphological differentiation markers, myoblast fusion index, and overall myotube density. Total protein and specific protein [AMP-stimulated protein kinase (AMPK), thyroid hormone receptor, and fast/slow myosin heavy chain isoform ratios] concentrations were also determined. Metabolic flux was measured again in myotubes from each growth-differentiation temperature treatment at both 32°C and 37°C test temperatures to determine whether myoblast growth and myotube differentiation temperatures interact to determine thermal sensitivity.
We then used May Grunwald-Giemsa staining to quantify myogenic differentiation. We calculated fusion indices and myotube densities at these respective time points (72 h for 37°C-differentiated myotubes, and 120 h for 32°C-differentiated myotubes), both of which represent measures routinely used to quantify myogenic phases of differentiation (30). For these experiments, we differentiated the myotubes on six-well plates coated in Engelbreth-Holm-Swarm murine sarcoma. Specifically, we collected myoblasts from 37°C and 32°C populations, differentiated them at either 37°C or 32°C (Fig. 1), and then stained them with 1:10 May Grunwald-Giemsa solution at 72 or 120 h, respectively. To determine the fusion index, we took images at ×10 magnification (BX53 light microscope, Olympus, Sydney) and calculated the total percentage of nuclei in myotubes (number of nuclei in myotubes/total number of nuclei × 100). The fusion index for each sample (n = 5 samples per treatment) was then averaged within treatments. To calculate myotube density, we took five images at ×4 magnification (BX53 light microscope, Olympus, Sydney, Australia) per well and used image-processing software (ImageJ, NIH, Bethesda, MD) to calculate the average myotube density per well. After staining was completed, myotube formation is depicted by pixels attributed to the darkest tones (30). Hence, we manipulated image intensity thresholds to capture the relative abundance of these pixels, thereby approximating overall myotube density (Fig. 2, A–H) (30). Myotube density for each sample (n = 9 samples per treatment) was then averaged within each treatment.

**Total protein content.** We used Bradford reagent to determine total protein content of differentiated myotubes. Myotubes grown at 37°C and 32°C were differentiated in Engelbreth-Holm-Swarm murine sarcoma-treated XF24 cell culture microplates for either 72 h at 37°C or 120 h at 32°C. Cells were lysed within each well by adding 50 µl of CelLytic (Sigma) and 5 µl of protease inhibitor cocktail (Roche, Sydney, Australia). Bradford assays were performed within each well as per the manufacturer’s instructions. Protein concentrations were determined by comparison to a standard curve based on bovine serum albumin.

**Metabolic flux.** Rates of oxygen consumption of myoblasts and myotubes were measured at 37°C and 32°C acute measurement temperatures (test temperature) in a Seahorse XF24 analyzer (part no. 101122-100, Seahorse Bioscience) according to the manufacturer’s instructions. Myoblasts were differentiated on XF24 cell culture microplates first treated with 10 µl of Engelbreth-Holm-Swarm murine sarcoma to promote attachment and myotube differentiation. Metabolic flux assays were run with myoblasts 24 h after attachment. Myotubes were differentiated for 72 h when incubated at 37°C and 120 h when incubated at 32°C as described above. Differentiation medium was replenished in each well daily. Immediately before analysis, growth medium or differentiation medium was replaced with metabolic flux running buffer, which consisted of DMEM adjusted to contain 1 mM Na pyruvate. The assay protocol consisted of repeated cycles (loops) of fluid mixing in the wells, followed by a delay period and a measurement period. The chamber is sealed only during the measurement period to allow measurement of decreasing oxygen concentration resulting from tissue respiration. We ran pilot experiments to optimize the program parameters to ensure that the media was adequately mixed and the chamber did not become hypoxic. In each experimental run, we measured four loops to ensure that there was a stable baseline; each loop consisted of a 3-min mixing period, a 2-min delay, and a 3-min measurement period. Oligomycin (5 µM) was injected after loop 3, followed by 2 µM of FCCP after loop 6, followed by 10 µM rotenone after loop 9, and 10 µM antimycin A after loop 12. Oligomycin blocks ATP synthase and was used to estimate the rate of oxygen consumption dedicated to ATP production (the oxygen consumption rate subtracted from the basal O2 consumption rate) and proton leak (the O2 consumption rate after oligomycin). FCCP perforates the mitochondrial membrane and thereby elicits maximal substrate oxidation rates. Rotenone and antimycin A block complex I and complex III of electron transport, respectively. They were used here to measure the rate of nonmitochondrial oxygen consumption. Additionally, anaerobic glycolytic metabolic rates were estimated from the extracellular acidification rates (ECAR) according to the manufacturers instructions (Seahorse Biosciences).

**Concentrations of AMPK, MHC, and thyroid receptor proteins.** Myoblasts derived from myoblasts grown at 37°C were differentiated in six-well plates for 72 h at 37°C or 120 h at 32°C. Cells were lysed within each well by adding 50 µl of CelLytic (Sigma) and 5 µl of protease and phosphatase inhibitor cocktail (Roche). Protein concentrations were determined by capillary electrophoresis in a “Wes” Simple Western system (Protein Simple, Santa Clara, CA) according to the manufacturer’s instructions. Myoblasts were differentiated for 72 h when incubated at 37°C and 120 h when incubated at 32°C as described above. Differentiation medium was replenished in each well daily. Immediately before analysis, growth medium or differentiation medium was replaced with metabolic flux running buffer, which consisted of DMEM adjusted to contain 1 mM Na pyruvate. The assay protocol consisted of repeated cycles (loops) of fluid mixing in the wells, followed by a delay period and a measurement period. The chamber is sealed only during the measurement period to allow measurement of decreasing oxygen concentration resulting from tissue respiration. We ran pilot experiments to optimize the program parameters to ensure that the media was adequately mixed and the chamber did not become hypoxic. In each experimental run, we measured four loops to ensure that there was a stable baseline; each loop consisted of a 3-min mixing period, a 2-min delay, and a 3-min measurement period. Oligomycin (5 µM) was injected after loop 3, followed by 2 µM of FCCP after loop 6, followed by 10 µM rotenone after loop 9, and 10 µM antimycin A after loop 12. Oligomycin blocks ATP synthase and was used to estimate the rate of oxygen consumption dedicated to ATP production (the oxygen consumption rate subtracted from the basal O2 consumption rate) and proton leak (the O2 consumption rate after oligomycin). FCCP perforates the mitochondrial membrane and thereby elicits maximal substrate oxidation rates. Rotenone and antimycin A block complex I and complex III of electron transport, respectively. They were used here to measure the rate of nonmitochondrial oxygen consumption. Additionally, anaerobic glycolytic metabolic rates were estimated from the extracellular acidification rates (ECAR) according to the manufacturers instructions (Seahorse Biosciences).

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Myotubes that differentiated at 37°C for 72 h were at parallel stages of differentiation as myotubes that differentiated at 32°C for 120 h (Fig. 2, A, C, E, G). There were no significant differences in fusion index (main effects and interactions $P > 0.4$) or myotube density ($P > 0.4$ for main effects of myoblast growth temperature and myotube differentiation temperature, as well as their interaction; Fig. 3, A and B) between the treatment groups.

**Myoblast metabolic flux.** Myoblasts grown at 32°C had significantly increased rates of basal oxygen consumption, maximal oxygen consumption, proton leak, net ATP production, and anaerobic metabolic rates (ECAR; main effects of myoblast growth temperature, Table 1; Fig. 4) compared with cells grown at 37°C. Basal metabolic rates and net ATP production were greater at 37°C compared with 32°C acute test temperature, but there was no effect of test temperature on proton leak or maximal metabolic rates; ECAR decreased at the higher test temperature (Table 1; Fig. 4). There were no interactions between myoblast growth temperature and acute test temperatures for any of the responses (Table 1).

**Myotube total protein content.** Myoblast growth temperature had no effect on the total protein content of subsequent myotubes ($P = 0.52$; Fig. 5). However, myotubes differentiated at 32°C had significantly increased total protein content ($P < 0.001$), and there was no interaction between myoblast growth temperature and myotube differentiation temperature ($P = 0.51$).

**Myotube metabolic flux.** Myoblast differentiation temperature had a significant effect on all metabolic responses, and myoblast growth temperature modulated these responses except for ECAR (Table 2; Figs. 6 and 7). There were statistically significant interactions between myoblast growth temperature and acute test temperature (Growth*Test, Table 2), and myoblast growth temperature and myotube differentiation temperature (Growth*Diff interaction; Table 2) in determining basal oxygen consumption of myotubes (Fig. 6, A and B, Table 2). Myotubes differentiated at 32°C had significantly increased rates of oxygen consumption when tested at 32°C. When tested at 37°C, however, differentiation at 32°C increased basal oxygen consumption only in myotubes that were derived from myoblasts that were grown at 37°C.

The result for basal oxygen consumption was mirrored in net ATP production (Growth*Test and Growth*Diff interactions, Table 2; Fig. 6, C and D), which was increased in myotubes differentiated and tested at 32°C. When tested at 37°C, however, exposure to 32°C increased ATP production only in those myotubes that were derived from myoblasts grown at 37°C.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Basal</th>
<th>Leak</th>
<th>ATP</th>
<th>Max</th>
<th>ECAR</th>
</tr>
</thead>
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<tr>
<td>Growth</td>
<td>$&lt;0.0001$</td>
<td>$&lt;0.0001$</td>
<td>$&lt;0.01$</td>
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<td>0.11</td>
<td>0.23</td>
<td>0.12</td>
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Metabolic parameters measured were basal metabolic rate (Basal), proton leak (Leak), net ATP production (ATP), maximal metabolic rate (Max), and an estimate of glycolytic metabolism (extracellular acidification rate, ECAR). The factors were growth temperature (Growth) and acute test temperature (Test), as well as their interaction; $N = 19$ samples per treatment.
Proton leak and maximal rates of oxygen consumption increased significantly in myotubes that differentiated at 32°C and which were measured at a test temperature of 32°C, but this was not the case when measured at 37°C test temperature (Fig. 6, E–H; Diff*Test interaction, Table 2). Again, myoblast growth temperature modulated these responses, and myotubes derived from myoblasts grown at 37°C had greater proton leak and maximal oxygen consumption rates when differentiated at 32°C (Growth*Diff interaction, Table 2).

Myotubes that differentiated at 32°C had significantly higher glycolytic rates (ECAR) when tested at 32°C but not at 37°C (Fig. 7, A and B; Diff*Test interaction, Table 2).

Myotube AMPK, MHC, and thyroid receptor protein concentrations. The ratio between phosphorylated and total AMPK, which is indicative of AMPK activity, was significantly greater in myotubes differentiated at 32°C (P = 0.005; Table 2).

Table 2. Result of permutational analyses (P values) of myotube metabolic rates

<table>
<thead>
<tr>
<th>Factor</th>
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<th>ATP</th>
<th>Max</th>
<th>ECAR</th>
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<td>0.12</td>
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<tr>
<td>Diff</td>
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<td>&lt;0.02</td>
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<tr>
<td>Test</td>
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<td>0.16</td>
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<tr>
<td>Growth*Diff</td>
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<td>&lt;0.005</td>
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<td>0.48</td>
</tr>
<tr>
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<td>&lt;0.04</td>
<td>0.11</td>
<td>0.28</td>
</tr>
<tr>
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<td>0.84</td>
<td>0.58</td>
<td>0.78</td>
<td>0.74</td>
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Metabolic parameters measured were basal metabolic rate (Basal), proton leak (Leak), net ATP production (ATP), maximal metabolic rate (Max), and an estimate of glycolytic metabolism (ECAR). The factors were myoblast growth temperature (Growth), myotube differentiation temperature (Diff), and acute test temperature (Test), as well as their interactions; N = 19 samples per treatment. The highest significant interactions relevant for interpretation are highlighted in bold.
There were no differences in the concentrations of thyroid receptor \( /H9251\) between myotube differentiation temperatures (\( P = 0.51; \) Fig. 8B). However, myotubes differentiated at 32°C had significantly lower concentrations of slow MHCs (normalized to \( /H9251\)-tubulin) compared with those differentiated at 37°C (\( P = 0.005; \) Fig. 8C). As above, a representative example of protein bands is shown next to the summary panel (Fig. 8C). There was no difference in fast MHC concentrations between myotubes from the different differentiation temperatures (\( P = 0.53, \) Fig. 8D). As a result, the ratio between fast and slow MHCs was significantly lower in myotubes differentiated at 37°C (\( P < 0.0001; \) Fig. 8E).

**DISCUSSION**

We have shown that temperatures experienced during myoblast differentiation directly influence the phenotype of the mature myotubes. Locomotor muscles in the limbs of mammals and other animals routinely experience temperatures sev-
eral degrees below core body temperature (2, 7, 14, 24). Hence, the implications of our findings are that local temperatures experienced during differentiation during postnatal growth or muscle regeneration directly influence metabolic and contractile phenotypes of subsequent myotubes. These results are important because they suggest that thermal responses of endotherms comprise centrally controlled core body temperature regulation as well as thermal plasticity in skeletal muscle cells. However, whether or not the thermal plasticity we show in isolated cells is sufficiently persistent to affect mature muscle needs to be confirmed experimentally. Central regulation mediated by a combination of sympathetic output and endocrine signaling has played an increasingly important role in homeostatic maintenance throughout the evolution of endothermy (23). Regulation of thermal sensitivities in cells of peripheral tissues is more reminiscent of ectothermic responses (10, 19, 28), and it is possible that it represents an ancestral response in endotherms. This more direct peripheral mode of regulation may be especially important early in development when central regulatory pathways are not fully functional (29).

Mammalian cells typically increase protein content when cultured at low temperatures (1). In our myoblasts, we found this thermal response was linked with compensatory changes in metabolic and contractile phenotypes. The 40–50% drop in

Fig. 8. Protein concentrations determined by capillary electrophoresis. Mean concentrations of phosphorylated AMPK (pAMPK) relative to total AMPK, which is indicative of AMPK activity, were greater in myotubes differentiated at 32°C (A). Representative protein bands of pAMPK and AMPK are shown (B). Thyroid receptor α (C) concentrations did not differ between myotubes differentiated at different temperatures. Slow myosin heavy chain (MHC) isoform (D) concentrations were lower in 32°C differentiated myotubes, and representative protein bands are shown (E). Fast myosin heavy chain isoform (F) did not differ between myotubes, so that the ratio of fast:slow MHC was greater in myotubes differentiated at 32°C (G). All data are means ± SE; N = 6 samples per treatment; *significant differences.
metabolic flux suffered by 37°C-differentiated myotubes measured at 32°C test temperature represents the acute thermodynamic effect of cold exposure on metabolic rate. In contrast, the 32°C-differentiated myotubes had significantly increased metabolic rates, which almost entirely compensated for the thermodynamic effects of cold exposure. Glycolytic rates were also increased in 32°C-differentiated myotubes exposed to cold conditions, relative to their 37°C-differentiated counterparts. This increase suggests that myotubes respond to low temperatures by upregulating overall metabolic capacity.

In addition to metabolic responses, the 32°C-differentiated myotubes had significantly lower concentrations of slow MHC isoforms (MHC I), with no significant change in the expression of the fast isoform (MHC II). The increase in MHC II/MHC I indicates that myotubes shift to a faster, more glycolytically poised phenotype when exposed to low temperature, which parallels the increase in glycolytic flux discussed above. Previous work in mice suggests that cold-induced shifts to faster muscle types help overcome the slower contractile speeds characteristic of cold exposure (33). A more glycolytic phenotype would also support burst activity in peripheral muscles, which is more likely to be initiated from a hypothermic state than during sustained aerobic activity, where increased blood flow warms peripheral muscles relatively quickly. Faster fiber types enhance contractile properties during cold exposure (3), and their relatively high energetic cost (12) could underlie the need to upregulate overall metabolic flux during cold exposure.

We found that cold exposure significantly increased the activity of AMPK by enhancing the proportion of phosphorylated AMPK in myotubes. This is important because phosphorylated AMPK promotes mitochondrial biogenesis and increases metabolic capacity under low-energy conditions (11). Energy deficiency is an inherent characteristic of cold exposure (9), and AMPK may act as the signal that remodels local metabolic phenotypes in response to cold-induced energy deficit (25). Moreover, AMPK promotes shifts toward more oxidative muscle phenotypes, while at the same time increasing concentrations of oxidative MHC IIa without affecting slow MHC I concentrations (22). Our results support these findings, except that we saw a decrease in slow MHC I. In either case, however, increased oxidative capacity was not accompanied by increased MHC I expression, and AMPK does not seem to play a consistent role in fiber type transformation. Temperature per se can alter muscle MHC composition (31) and the interaction between temperature and AMPK activity may drive expression profiles, particularly considering that faster muscles are advantageous at colder temperatures (15).

Tissues can respond uniquely to a common central input in vivo by altering their sensitivities to the central signal through changes in expression and function of endocrine receptors. Thyroid hormone is a central regulator of cold acclimation and thermogenesis, where it increases metabolic flux and transition to fast myosin isoforms (13). We therefore predicted that cold exposure would increase the sensitivity of myotubes to thyroid hormone by upregulating thyroid receptor expression. However, thyroid hormone receptor α protein concentrations remained unchanged between our treatments. This finding may be explained because circulating levels of thyroid hormone are very low in embryonic and newborn rodents and do not reach peak levels until later in development (13).

Perspectives and Significance

We showed that growth temperatures of myoblast populations had interactive effects on the metabolic phenotype of myotubes. This finding indicates that there is plasticity even at the myoblast stage, which can extend to affect metabolic phenotypes throughout differentiation. Hence, temperature experienced during early development may have direct effects on muscle phenotype. These dynamics may be important in influencing whole organism metabolic phenotypes, but need to be confirmed in mature muscles. Thermal responses of myoblasts may also have important implications for mature individuals. Mammals retain resident populations of myoblasts in skeletal muscle tissue until well into maturity, and while their physiological significance is debatable, there is growing evidence that they continue to differentiate for routine maintenance and regeneration (17, 27). It is therefore possible that local temperatures have continued effects on muscle phenotypes into adulthood.

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GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

A.G.L. and F.S. conception and design of research; A.G.L. and F.S. performed experiments; A.G.L. and F.S. interpreted results of experiments; A.G.L. and F.S. analyzed data; A.G.L. and F.S. prepared figures; A.G.L. and F.S. drafted manuscript; A.G.L. and F.S. edited and revised manuscript; A.G.L. and F.S. approved final version of manuscript.

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