Effects of temperature acclimation on Pacific bluefin tuna (Thunnus orientalis) cardiac transcriptome

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Jayasundara N, Gardner LD, Block BA. Effects of temperature acclimation on Pacific bluefin tuna (Thunnus orientalis) cardiac transcriptome. Am J Physiol Regul Integr Comp Physiol 305: R1010–R1020, 2013. First published September 4, 2013; doi:10.1152/ajpregu.00254.2013—Little is known about the mechanisms underpinning thermal plasticity of vertebrate hearts. Bluefin tuna hearts offer a unique model to investigate processes underlying thermal acclimation. Their hearts, while supporting an endothermic physiology, operate at ambient temperature, and are presented with a thermal challenge when migrating to different thermal regimes. Here, we examined the molecular responses in atrial and ventricular tissues of Pacific bluefin tuna acclimated to 14°C, 20°C, and 25°C. Quantitative PCR studies showed an increase in sarcoplasmic reticulum Ca2+ ATPase gene expression with cold acclimation and an induction of Na+/Ca2+ -exchanger gene at both cold and warm temperatures. These data provide evidence for thermal plasticity of excitation-contraction coupling gene expression in bluefin tunas and indicate an increased capacity for internal Ca2+ storage in cardiac myocytes at 14°C. Transcriptomic analysis showed profound changes in cardiac tissues with acclimation. A principal component analysis revealed that temperature effect was greatest on gene expression in warm-acclimated atrium. Overall data showed an increase in cardiac energy metabolism at 14°C, potentially compensating for cold temperature to optimize bluefin tuna performance in colder oceans. In contrast, metabolic enzyme activity and gene expression data suggest a decrease in ATP production at 25°C. Expression of genes involved in protein turnover and molecular chaperones was also decreased at 25°C. Expression of genes involved in oxidative stress response and programmed cell death suggest an increase in oxidative damage and apoptosis at 25°C, particularly in the atrium. These findings provide insights into molecular processes that may characterize cardiac phenotypes at upper thermal limits of teleosts.

bluefin tuna; cardiac function; excitation contraction coupling; temperature acclimation; transcriptomics

BLUEFIN TUNAS (FAMILY SCOMBRIDAE) are migratory apex predators. They are renowned for their endothermic physiology, supported by counter-current heat exchangers and elevated metabolic rates (8). Juvenile Pacific bluefin tunas (PBFT), Thunnus orientalis, sustain minimal metabolic rates of 220–235 mg·kg−1·h−1 at 20°C, a rate ~1.3-fold higher than that of yellowfin tunas at the same temperature (5), and about 1.5–4-fold higher than routine metabolic rates of salmonids (26). This high aerobic demand of bluefin tunas (BFT) is sustained by an enhanced cardiac physiology that maintains performance across large thermal gradients experienced by these fish (8). However, in contrast to their skeletal muscle, visceral organs, eyes, and brain that are buffered from the environmental temperature variations by conserving heat, BFT hearts operate at ambient temperature. They lack counter-current heat exchangers, and ventricles receive blood through coronary circulation directly from the gills. Therefore, BFT hearts have to support an endothermic physiology and maintain elevated cardiac performance at ambient temperature; hence, cardiac limitations likely define thermal boundaries for these organisms (6, 8, 19, 22, 39, 57).

PBFT predominantly migrate across sea surface temperatures ranging from 10°C to 25°C and dive to waters as cold as 5–6°C in search of prey (7). As juveniles, they migrate from the warm waters of western Pacific into the cold California current off North America’s western shores, and return to the Pacific Ocean to spawn in waters near Japan. Blank et al. (6) showed that PBFT have the capacity to retain cardiac function across a large temperature range (2°C–25°C) compared with tropical tunas, but also indicated that cardiovascular performance in PBFT is strongly temperature dependent, leading to cardiac bradycardia at cold temperatures. Biochemical studies demonstrated that Ca2+ stored in sarcoplasmic reticulum (SR) significantly contributes to maintaining excitation-contraction (EC) coupling in PBFT compared with other tunas and is thought to be crucial for sustaining cardiac performance in the cold for this species (22, 39, 57).

Under chronic temperature changes, vertebrate hearts, including teleosts, are shown to modulate their transcriptome and proteome to restore cellular homeostasis and adjust their cardiac phenotype to shift to a new thermal optimum (2, 10, 23, 57, 61). Although PBFT inhabit a wide thermal range, metabolic rate studies indicated that metabolic thermal-minimum zone of juvenile PBFT is around 16°C–18°C (5). Therefore, during seasonal migrations, PBFT might alter their phenotype (acclimatization) to compensate for temperature effects and optimize performance at different temperatures (27). Previous studies demonstrated that PBFT hearts increase the intracellular Ca2+ cycling through the SR during cold acclimation (57) and modify the role of K+ channels (21) with temperature acclimation. Castilho et al. (10) showed changes in gene expression patterns in PBFT ventricle at 15°C and 20°C, based on a heterologous hybridization with a Gillichthys mirabilis-specific microarray. However, this study did not examine PBFT at warmer acclimation temperatures. The first study to determine cardiac molecular responses to temperature acclimation utilizing a microarray approach in an active teleost—rainbow trout—examined acclimation effects at 4°C and 18°C (61). This study indicated changes in expression of genes involved in a number of biological pathways, including glycolysis, protein biosynthesis, and cytoskeletal structure, which might underlie cardiac hypertrophy detected in cold-acclimated (4°C) trout ventricle. Although several studies have provided insights into teleost transcriptomic responses to temperature (10, 42, 52, 61), very little is known about gene expression through acclimation...
changes in different cardiac tissue types (atrial, compact, and spongy tissues), at temperatures near the upper thermal limit of active pelagic fishes.

To further investigate the thermal plasticity of PBFT heart, in this study, we investigated gene expression and metabolic enzyme activity changes in cardiac tissues in fish acclimated to 14°C, 20°C, and 25°C. To examine the gene level regulation of EC coupling proteins that results in cardiac phenotype described by Shiels et al. (57), we quantified the gene expressions of sarcoplasmic reticulum Ca\(^{2+}\) ATPase (SERCA), ryanodine receptor (RyR), and Na\(^+\)/Ca\(^{2+}\)-exchanger (NCX) using gene-specific primers. Large-scale transcriptomic studies provide a powerful tool to examine species’ responses to environmental stress. Therefore, we utilized a PBFT-specific oligonucleotide microarray (24) to investigate molecular responses to cellular perturbations caused by long-term temperature changes in PBFT. To determine effects of acclimation on PBFT cardiac energy metabolism, we coupled our metabolic gene expression data with enzyme assay studies. We focused our studies on atrial (AT), ventricular compact (VC), and ventricular spongy (VS) tissues of the tuna heart, considering the anatomical differences and temperature and oxygen variations experienced by these tissues (8).

This study is the first to investigate broad-scale molecular mechanisms underpinning thermal limits of a large pelagic fish. Understanding these mechanisms in bluefin tunas has important ecological and economic implications and can be used as a tool to predict shifts in thermal niche of these species in response to global ocean temperature changes.

**MATERIALS AND METHODS**

**Fish Origin, Acclimation, and Tissue Collection**

PBFT were captured in waters off of Baja, Mexico, by hook and line and transported to the Tuna Research and Conservation Center at Hopkins Marine Station of Stanford University. They were held at 20°C under a 12:12-h light-dark photoperiod, as previously described (8). Upon 3 mo of acclimation to captive conditions, a total of 10 fish remained less than 1.3-fold.

- **A one-way ANOVA statistical analysis with a Bonferroni post hoc test was conducted across temperature groups for a given tissue to compare expression values for each gene in each tissue type. Statistical significance was accepted if \( P < 0.05 \).**

**Enzyme Assays**

Freeze-clamped atrial, ventricular, compact, and spongy tissues were homogenized using metal beads and a Tissue Lyser (Qiagen, Valencia, CA), with a homogenization buffer (50 mM KPO\(_4\), pH 6.8 at 4°C) resulting in a 9:1 dilution/l g of tissue. Twenty-five microliters of this homogenate was used per each reaction for each enzyme assay. Enzyme activities were measured for each tissue type from every acclimation temperature. Each measurement was made in triplicate, and average activity per gram of fresh tissues was calculated. All activity measurements were made in triplicate, and average activity per gram of fresh tissues was calculated. All activity measurements were made in a thermostatted Shimadzu Bio-Spec UV-visible spectrophotometer at 25°C.

**Array Platform, RNA Processing, and Hybridization**

As described by Gardner et al. (24), the array platform (BFT 4 \( \times \) 44K array) consisted of 7078 unique sequences derived from Atlantic bluefin tuna. Three probes were designed to represent each
unique sequence. The microarray design (GPL 15374) and data (GSE36886) are publically accessible at the Gene Expression Omnibus repository.

A total of 36 extracted RNA samples from atrial, ventricular compact, and spongy tissues from fish acclimated to 14°C (n = 4), 20°C (n = 4), and 25°C (n = 4) were used in this study. One microgram of RNA from each tissue sample was reverse transcribed and amplified with the Ambion AminoAllyl MessageAmp II kit (Applied Biosystems, Carlsbad, CA) following manufacturers’ instructions. To accurately compare across different samples with a minimal number of hybridizations, we employed a reference design. The reference RNA sample consisted of 1 μg of pooled RNA from every experimental sample. RNA samples were reverse transcribed with T7 Oligo (dT) primer and Arrayscript reverse transcriptase, independently across each temperature group. Twenty micrograms of purified aRNAs from reference and experimental samples were separately labeled with Cy3 or Cy5 RNA (aRNA) from every experimental and reference sample. Twenty-five micrograms of purified aRNAs from reference and experimental samples were separately labeled with Cy3 or Cy5 N-Hydroxysuccinimide ester dyes followed by column purification. Dye incorporation was assessed with a NanoDrop spectrophotometer (ND-1000, NanoDrop Technologies, Wilmington, DE). Hybridization steps were performed on the basis of the hybridization protocol of the two-color microarray-based gene expression analysis protocol with reagents from the gene expression hybridization kit (Agilent Technologies, Santa Clara, CA). Then, 825 ng of each Cy3- and Cy5-labeled aRNA was competitively hybridized to the array. Array slides were hybridized at 65°C for 17 h in a rotating hybridization oven. All washing steps were also performed on the basis of the two-color microarray-based gene expression analysis protocol (Agilent Technologies). Each array on 4 × 44K slides was scanned separately using an Axon GenePix 4000B microarray scanner (Axon Instruments, Molecular Devices, Sunnyvale, CA).

Data Extraction, Normalization, and Analysis

Array images were analyzed using Feature Extraction 4.0 software (Agilent Technologies) Spots that Feature Extraction software identified as “good” among all samples were included in further analysis. Lowess normalization was performed to correct for dye biases. Log2 ratios were calculated on the basis of experimental and reference samples and were analyzed using GeneSpring GX 11.0 software (Agilent Technologies). Log2 ratio values for each sample were averaged across experimental replicates. The statistical significance was determined using a one-way ANOVA test for each tissue type independently across each temperature group (P < 0.001). P values were adjusted for false discovery rate (FDR), with a Benjamini-Hochberg multiple-testing correction followed by a Tukey’s post hoc analysis. Entities with a minimum of three-fold expression difference (determined using a volcano plot) when compared against two conditions from the same tissue type (e.g., 14°C atrium vs. 25°C atrium) were selected. Since there were three probes representing each gene, if any one of the probes showed opposite directionality in expression, all three probes were removed from the analysis. The resulting list of microarray entities was hierarchically clustered with the Euclidean distance metric and visualized as a heat map plot. Using the same entity list, we performed a principal component analysis (PCA) on experimental conditions. PCA can determine overall variation among transcriptomic profiles from each condition; conditions with similar scores for one or more PCA components can be considered similar in their transcriptomic expression profile. Statistically significant genes were assigned to a functional group based on Blast2GO software (16) or previously published literature. Differentially expressed probes from each functional category are presented as heat maps with each row representing a unique probe and each column representing a tissue type acclimated to a particular temperature.

RESULTS AND DISCUSSION

SERCA, NCX, and RyR Gene Expression

Excitation-contraction coupling proteins, such as SERCA, RyR, and NCX, play a vital role in regulating cytosolic Ca2+ concentration in PBFT myocytes (11, 39, 57, 59). In the current study, SERCA gene expression in atrial and ventricular tissues was significantly increased with cold acclimation (CA) (Fig. 1A). Compared with 25°C fish, cold-acclimated atrial tissues showed a 5.9-fold increase, and VC and VS tissues showed a 5.4- and 4-fold increase in SERCA gene expression, respectively. Compared with 20°C, NCX gene expression increased at both 14°C and 25°C in atrial and ventricular tissues (Fig. 1B). Warm acclimation (WA) had a profound effect on NCX gene expression particularly on ventricular tissues, showing a three-fold difference in expression compared with 20°C. Temperature acclimation had little or no effect on RyR gene expression, except in warm-acclimated VS tissues, where RyR expression increased by 1.8-fold compared with other acclimation groups (Fig. 1C).

SERCA protein actively transports cytoplasmic Ca2+ into the SR, facilitating relaxation, which plays a key role in maintaining PBFT cardiac function. Previous studies show that compared with other tunas, PBFT cardiac myocytes have a large Ca2+ release transient (22), the highest rates of SR Ca2+ uptake across a range of temperatures (11), and an increased SERCA protein expression (39). Given the similarities in the temperature sensitivity of SERCA protein in enzyme assays across tuna species (11), the species-specific Ca2+ uptake...
differences detected in previous studies are considered to be a result of varying SERCA protein expression in the SR. Two previous studies on acclimated PBFT demonstrated the overall thermal plasticity of PBFT cardiac contractile mechanism (21, 57). In addition to bluefin tunas, several other teleosts have also demonstrated increases in SR function in the cold (1, 34, 36, 37, 62). Increased internal Ca\(^{2+}\) regulation by SR in rainbow trout in the cold is also associated with elevated SERCA gene and protein expression (36). The current data on increased SERCA mRNA expression in PBFT hearts complement these findings and suggest that upregulation of SERCA gene during CA may underlie the increase in Ca\(^{2+}\) uptake by SR in the cold.

In contrast to cold-acclimated tissues, the lack of a SERCA transcriptional response at 25°C in all tissues implies a reduced role for this protein under WA. This could be due to faster rates of Ca\(^{2+}\) ion diffusion with increased temperatures offsetting the need for elevated SR Ca\(^{2+}\) uptake and loading capacity. It is also possible that this decrease in SERCA expression might be a thermal compensatory strategy to counter accelerated SERCA enzyme activity under warm temperatures. However, Shiels et al. (57) demonstrated that inhibition of SR in 24°C acclimated myocytes had little or no impact on amplitude or decay of Ca\(^{2+}\) current, confirming that warm temperatures reduce the need for Ca\(^{2+}\) cycling through the SR in PBFT cardiac myocytes. SERCA mRNA expression data under WA from the current study suggest that this reduced role of SR at warm temperatures is potentially regulated at the transcriptional level. Overall, these data suggest that changes in SERCA gene expression contribute to thermal malleability of Ca\(^{2+}\) uptake to the SR in the bluefin tuna cardiomyocytes.

Ryanodine receptors function as SR Ca\(^{2+}\) release channels (4). RyR mRNA expression showed little or no effect of temperature acclimation in PBFT, except for in VS at 25°C. Previous studies on trout also showed no effect of temperature acclimation on RyR protein expression (4), suggesting very little regulation of RyR activity at the transcriptional level in teleosts during thermal acclimation. The marginal increase in RyR expression in 25°C VS tissues suggests that these tissues may exhibit increased RyR activity under warm temperatures, potentially leading to more efficient Ca\(^{2+}\) release from the SR. Further studies are necessary to better understand the role of RyR under thermal acclimation in teleosts.

NCX is primarily involved in Ca\(^{2+}\) efflux, but also mediates Ca\(^{2+}\) influx under certain conditions (59). Previous studies indicate that NCX plays an important role in maintaining cardiac contractility in fish as they experience large ambient temperature gradients (22, 30, 63). In trout atrium, Ca\(^{2+}\) influx via reverse-mode NCX appears to trigger SR Ca\(^{2+}\) release (29, 30) and shows a lower temperature sensitivity compared with mammals. Galli et al. (22) showed that in bonito, NCX plays a more pivotal role in Ca\(^{2+}\) entry into cardiomyocytes compared with related scombrid fish. While the precise mechanisms require further investigation, these existing studies imply that NCX is a key protein in maintaining cardiac contractility in fish, particularly when they are exposed to temperatures outside of their normal thermal range. The increase in NCX expression detected in both cold- and warm-acclimated fish in the present study supports this hypothesis. Furthermore, the increased NCX gene expression at 14°C compared with 20°C may also possibly offset Q\(_{10}\) effects of temperature to maintain efficient Ca\(^{2+}\) cycling from the cytoplasm. The elevated NCX gene expression at 25°C may potentially contribute to elevated sarcolemmal Ca\(^{2+}\) flux, to compensate for reduced SR Ca\(^{2+}\) involvement under 25°C, as discussed previously. Further studies involving NCX functional studies are necessary to better understand the role of this protein in PBFT hearts.

It should be noted that quantitative PCR data presented here may indicate expression profiles of only one isoform or multiple isoforms of each of the EC coupling genes (SERCA, NCX, and RyR) in PBFT. A recent study on eurythermal crucian carp showed two cardiac isoforms of SERCA proteins, while burbot and trout appear to have only one SERCA isoform (36, 37). Previous studies characterizing SERCA protein in PBFT using Western blots identified only one protein for this species (39). Furthermore, only one cardiac isoform for RyR has been previously described in fish (48). Thus, PBFT may only express one isoform of SERCA and RyR genes, as characterized in our current study. However, alternative splicing is thought to generate multiple variants of RyR (60), as well as NCX (54) in mammalian hearts. In addition, four genes coding for NCX are described in teleosts (50). Therefore, it is possible that in fish, including in tunas, different isoforms and splice variants of EC coupling genes are potentially expressed, particularly during changes in environmental factors, such as temperature.

Global Transcriptomic Analysis

Overall transcriptomic data showed an increase in energy metabolism and protein turnover in CA hearts. In contrast, during WA, a decrease in expression of genes involved in molecular chaperoning activity and an increase in genes associated with oxidative damage and programmed cell death is apparent, particularly in the atrium. Here, we discuss these findings and overall changes in gene expression patterns in detail.

Tissue-Specific Gene Expression Variation

The 4,730 probes representing 3,250 unique genes (46% of all genes) showed a 3-fold or greater expression change (ANOVA, FDR corrected, \(P < 0.001\) for temperature effect) in PBFT atrial, VC, and VS tissues at 14°C, 20°C, and 25°C. They are hierarchically clustered and presented as a heat map in Fig. 2A. It is possible that some of the transcriptomic changes detected here result from cold-induced cardiac hypertrophy detected in teleosts (61). However, it should be noted that, although cardiac cell volume increased with CA, as demonstrated on the basis of histological analysis by Shiels et al. (57), overall cardiac mass remained constant across acclimation temperatures in the current study and in previous PBFT acclimation experiments (57). Additionally, the greatest transcriptomic changes—both upregulation and downregulation of genes—were detected in warm-acclimated atrial tissues. Therefore, considering hypertrophy is primarily detected in teleost ventricular tissues, it is unlikely that overall transcriptomic responses detected here are simply due to changes in cell size or volume.

Overall trends in transcriptomic profiles across treatments, visualized using PCA (Fig. 2B), illustrated that the variation in gene expression was primarily due to effects of temperature; 46.92% of the variation, explained by principal component 1
oxygenation may lead to the marked gene expression differ-
ences detected between WA atrial and ventricular tissues. These significantly differentially expressed genes from the transcriptomic analysis were assigned to different functional
categories and are discussed below in detail.

Effect of Acclimation on Different Biological Processes

Cardiac energy metabolism. To sustain an elevated cardiac
performance and to maintain cellular homeostasis (38) across a
thermal gradient, PBFT have to sustain an adequate energy
production. Transcriptomic analysis of PBFT hearts revealed
significant changes in expression of 52 genes involved in
cellular metabolism in cold- and warm-acclimated tissues
(Supplemental Fig. S1 and S2). Here, coupled with metabolic
enzyme activity data, we discuss eight genes involved in lipid
metabolism (Fig. 3A) and six genes pertaining to carbohydrate
metabolism (Fig. 3B). These 14 genes were selected from the
52 genes due to their direct involvement with carbohydrate and
lipid metabolism. Overall trends indicated a global downregu-
lation of genes involved in lipid and carbohydrate metabolism
under WA, particularly in the atrium.

Lipid metabolism. Genes involved in lipid metabolism are
upregulated in cold-acclimated PBFT hearts (Fig. 3A). The gene
coding for HOAD, a key enzyme involved in β-oxidation of
fatty acids that is frequently used as a quantitative index for lipid
metabolism (18) is upregulated in both VC and VS tissues at
14°C. Genes coding for fatty acid and sphingolipid-metabolizing
enzymes—isoamyl acetate-hydrolyzing esterase (20), glycer-
ophosphodiester phosphodiesterase (EC 3.1.4.46), n-acetylphos-
ganosine amidohydrolase (EC 3.5.1.23), and proactivator polypep-
tide precursor (EC 3.2.1.45)—were also upregulated under CA.
These data provide strong evidence for temperature-induced
changes in membrane lipid composition and a metabolic shift
toward lipid synthesis during CA.

Increase in lipid metabolism under CA is further demon-
strated by enzyme assay studies based on HOAD enzyme
activity (Fig. 4A). Both atrial and VS tissues showed a 1.5-fold
increase in HOAD activity at 14°C compared with 25°C. However,
in contrast to gene expression analysis, VC tissue showed
no difference with acclimation, demonstrating the
capacity to metabolize lipids at 25°C in this tissue. Overall, our
results are indicative of an increase in lipid metabolism with
CA in PBFT hearts.

Genes encoding three proteins involved in fatty acid trans-
port-solute carrier family 27 (SLC 27), apolipoprotein a-i and
c-i (Apo a-i and c-i) were downregulated under CA. These
proteins participate in the reverse transport of cholesterol from
tissues to the liver for excretion, by promoting cholesterol
eflux from tissues (13). Decrease in gene expression of such
glyceride-carrying proteins at 14°C suggests that cold-accli-
matized cardiac tissues may reduce lipid efflux to utilize them as
local energy stores.

Carbohydrate metabolism. Warm acclimation resulted in
significant downregulation of genes involved in glycolysis and
associated pathways (Fig. 3B). Decrease in expression of genes
coding glycolytic enzymes—pyruvate kinase (PK) (EC: 2.7.1.40),
pyruvate dehydrogenase (PDHA) (EC 1.2.4.1), and
phosphoglycerate mutase (PGAM) (EC: 5.4.2.1)—suggests an
overall depression of glycolysis under WA. Downregulation of
the gene for transaldolase 1 (EC:2.2.1.2), an enzyme linking
the pentose phosphate pathway to glycolysis, at 25°C further

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and 25°C. Values are presented as means ± SE (n = 5). †Significant difference compared with 14°C within a tissue (P < 0.05).

Fig. 4. Maximum enzyme activity rates of 3-hydroxyacyl CoA dehydrogenase (HOAD; A), lactate dehydrogenase (LDH; B), and citrate synthase (CS; C), measured as international units per gram fresh weight of tissue in atrial, ventricular compact, and spongy tissues from fish acclimated to 14°C, 20°C, and 25°C. Values are presented as means ± SE (n = 5). †Significant difference compared with 14°C within a tissue (P < 0.05).

implies a decrease in glycolysis during WA. Another downregulated gene during WA encodes 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2/FBPase-2). This enzyme catalyzes the synthesis and degradation of fructose 2,6-bisphosphate, the most potent stimulator of 6-phosphofructo-1-kinase (49). Marsin et al. (44) showed that the heart type 6-phosphofructo-1-kinase isoform stimulates glycolysis under energy-deprived conditions in monocytes, suggesting that the decrease in PFK-2/FBPase-2 at 25°C in PBFT might result in diminished ability to stimulate glycolysis under WA.

Because of perfusion limitations that occur during increases in temperature (17), cardiac cells may rely more on anaerobic metabolism to meet the increased ATP demand. However, during severe cardiac ischemia, glycolysis is arrested (47). It is possible that the decrease in glycolytic gene expression in warm-acclimated PBFT hearts is to reduce glycolysis, thus decreasing accumulation of glycolytic products (lactate, NADH, and H⁺ ions) (28) in myocytes as a result of low-perfusion capacity (oxygenation). Alternatively, given the high glycolytic capacity of cardiac muscle tissues, it is possible that a decrease in glycolytic gene expression at 25°C might be a thermal compensatory mechanism by PBFT hearts.

LDH enzyme activity was measured to further examine effects of acclimation on anaerobic metabolism. LDH activity was increased (~1.5-fold) in the atrial and VC tissues at 14°C compared with 25°C (Fig. 4B). Results from both the atrial and VC LDH enzyme activity are consistent with the observed gene expression patterns, validating the conjecture that reduced glycolysis and anaerobic metabolism occurs in these tissues at 25°C. Cardiac cells also use plasma lactate as a metabolic fuel to generate pyruvate, particularly during exercise (18). Thus, although LDH is an indicator of anaerobic metabolism, the increase in in vitro LDH activity at 14°C may suggest that lactate oxidation is also potentially increased with elevated LDH activity. The VS layer showed no effect of acclimation on LDH enzyme activity and was elevated under both CA and WA compared with VC tissues, suggesting that VS tissues may depend more on glycolysis for ATP production. Considering the potential differences in tissue oxygenation of VC and VS layers, it is possible that VS myocytes have evolved better mechanisms to sequester glycolytic products resulting from increased glycolysis. This hypothesis is supported by Gemelli et al. (25) describing a higher lactate accumulation and a greater capacity to oxidize and incorporate lactate into the total cardiac lipids by VS compared with VC tissues in PBFT. The relative increase in glycolytic gene expression and LDH activity at 14°C in all tissues suggests an increased glycolytic capacity at this temperature. This might be a result of increased glycogen stores in cardiac myocytes, as previously shown in cold-acclimated PBFT (57) to maintain an adequate energy supply at cold temperatures (18).

**Overall aerobic metabolism.** We quantified CS, an enzyme involved in tricarboxylic acid cycle activity, to measure overall aerobic metabolic capacity in PBFT hearts (Fig. 4C). Acclimation temperature had no effect on CS activity in the atrium, but CS activity decreased at 25°C in both VC (1.3-fold) and VS tissues (2-fold) compared with cold-acclimated hearts. These changes in CS activity could be a thermal compensatory response to offset Q₁₀ effects. However, the marked decrease in CS activity in warm-acclimated VS tissues suggests an overall decrease in aerobic metabolic capacity and reduced ATP production in these tissues. Furthermore, the gene for cytosolic malate dehydrogenase (E.C. 1.1.1.37), an oxidoreductase enzyme that interconverts malate and oxaloacetate is also downregulated during warm acclimation (Fig. 3B). These data and the increase in LDH activity in warm-acclimated VS tissues further suggest a potential oxygen delivery limitation to the VS layer compared with VC layer. The overall increase in mitochondrial density in cold-acclimated PBFT demonstrated by Shiels et al. (57) might contribute to this increase in aerobic metabolism.

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Fig. 3. Annotated heat maps showing the differentially expressed probes at 14°C, 20°C, and 25°C in each tissue type, representing unique genes from each functional category (GO database, UniProt Knowledge database and published literature search): lipid metabolism (A), carbohydrate metabolism (B), protein turnover (C), cellular stress response (D), oxidative stress (E), and apoptosis (F). Each row represents a unique probe and each column represents a tissue type at a given temperature. The color scale bar indicates three-fold or greater change in expression, with blue denoting downregulation, yellow denoting upregulation, and black denoting no change in expression. Individual probe names for genes involved in these categories are available in the electronic supplemental material (Supplemental Figs. S1–S6).

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Protein turnover. Transcriptomic analysis revealed 60 genes involved in protein turnover, and these genes were grouped into proteolysis (cathpsin, metallloproteases, proteins involved in ubiquitination, and sumoylation) and protein biosynthesis (Fig. 3C) (Supplemental Fig. S3, A and B). The most striking pattern in this gene set is the downregulation of genes involved in protein biosynthesis and proteolysis under WA, particularly in the atrium and their marked increase under CA.

Temperature stress is known to cause protein damage (27). Unrepaired, damaged proteins are degraded and removed from the cell through proteolysis, and new proteins are synthesized to maintain adequate protein levels. Current data infer an increase in total protein turnover during CA. Increase in protein biosynthesis was also previously detected in cold-acclimated PBFT ventricles (10) and rainbow trout hearts (61), which was attributed to cold-induced cardiac hypertrophy. These data suggest that PBFT hearts are able to induce the necessary genes to sustain an apparently higher level of protein turnover than hearts of warm-acclimated specimens.

Decreased expression of genes involved in protein turnover machinery at 25°C is rather surprising, considering protein denaturation is likely to be exacerbated at warm temperatures. Previous studies also documented a similar decrease in protein degradation in two highly eurythermal species, Gillichthys mirabilis and Austrofundulus limnaeus acclimated to warm temperatures, but also showed a concurrent increase in protein synthesis (42, 52). Podrabsky and Somero (52) described this as an attempt by the organisms to maintain an adequate protein level at the warm temperatures that these fish experience in highly fluctuating intertidal environments. Considering PBFT experience temperatures as high as 25°C only for a period of time during their migrations to the western Pacific Ocean, it is possible that these fish do not have the capacity to upregulate genes involved in protein turnover machinery during constant exposure to 25°C.

Molecular chaperones and oxidative stress response. Cellular response to temperature includes an increase in expression of molecular chaperones to repair and refold damaged proteins (38). Fifty-four genes involved in overall cellular stress response showed significantly different expression patterns across treatments (Supplemental Fig. S4). Fifteen of these genes are directly involved in molecular chaperoning activity (Fig. 3D) and are discussed below in detail.

Heat shock proteins (HSP) play a critical role as chaperones during stress-induced protein unfolding (38) and are involved in protective mechanisms against apoptosis (3) and oxidative stress (33). Genes coding for HSP 10, 14, 70, 90, and the constitutive form of HSP (HSC70) were decreased at 25°C and increased at 14°C. HSP10 is shown to protect cardiac myocytes against apoptosis during ischemia/reperfusion injury (40). In contrast to other HSP genes, gene expression of HSP 70 binding protein (HSP-70-bp 1), which inhibits ATP-binding domain of HSP 70 interfering with its function (56), was increased at 25°C, potentially resulting in further repression of HSP70 chaperoning activity under WA.

The increase in HSP gene expression at 14°C implies a protective role played by these proteins during cold stress. The downregulation of HSPs in PBFT cardiac tissues at 25°C, particularly in the atrium, is surprising, considering the crucial role of HSPs as a repair protein during temperature stress. However, goby fish Gillichthys mirabilis also showed a lack of pronounced HSP gene expression with WA (42), inferring that eurythermal species can overcome acute temperature stress effects through acclimation. Similarly the decrease in HSP gene expression in warm-acclimated PBFT could also be due to the efficiency with which PBFT heart muscles were able to acclimate to warm temperatures and restore its cellular homeostasis. However, increased expression of HSP70-bp-1 and significant decrease in expression of constitutive heat shock proteins (HSC70) that are involved in protection against oxidative stress and apoptosis under WA, suggests decreased capacity for molecular chaperoning activity in PBFT at 25°C (38).

Eukaryotic cells constantly cope with reactive oxygen species (ROS). The imbalance between ROS production and elimination results in oxidative stress and can cause damage to cellular macromolecules. Cold and warm temperatures induce oxidative stress in various organisms, including fish (40). Transcriptomic data recognized 18 differentially expressed genes that are involved in cellular redox signaling and control (Fig. 3E; Supplemental Fig. S5). Eight isoforms of genes coding for tartrate-resistant acid phosphatase (TRAP), a protein catalyzed by formation of ROS (55), were detected in the current study. Most of these isoforms (six in atrial tissues and five in ventricular tissues) were increased during WA. Eight genes coding for proteins that are involved in cellular protection against oxidative stress—peroxiredoxins, glutathione peroxidases, superoxide dismutase, glutathione transferases, thioredoxin, and several other selenoproteins, (45, 58) are upregulated in atrial and VS tissues under WA. Eighteen of these proteins remain constant in expression at 14°C.

These data indicate that during CA, PBFT hearts are able to upregulate genes involving antioxidant mechanisms to protect against cold-induced oxidative stress. However, under WA, particularly in the atrium, ROS generation is potentially increased with the increase in TRAP gene expression and the protective mechanisms against oxidative damage, including HSP response, appear to be decreased.

Apoptosis. Apoptosis is a complex cell-death program that is inherent in multicellular organisms. Gene ontology analysis across treatments identified 16 significantly differentially expressed genes involved in apoptotic pathways (Fig. 3F; Supplemental Fig. S6). Five of those genes showed an increase in expression under WA. Ribosomal protein 4S (RpS3), which activates caspase-8/caspase-3 to induce apoptosis (31), was significantly upregulated in all three tissues types at 25°C. Genes coding for cofilin 1, an initiator or apoptosis, and rad9-hus1-rad1 complex (9-1-1C), involved in triggering apoptosis (35, 51), were upregulated in atrial and VS tissues under WA. High-mobility-group-box 1 (HMG1) gene was downregulated under WA and upregulated in the cold. Podrabsky and Somero (52) described this protein as a potential global gene expression temperature sensor. Increased expression of HMG1 inhibits apoptosis via several mechanisms (41). Two other genes (APE1 and APIP) that are downregulated at 25°C and upregulated at 14°C are involved in positive regulation of antiapoptotic pathways (9, 64). Bcl2-x, which is involved in prevention of hypoxia-induced apoptosis (14), is also downregulated under WA. These data suggest that tuna hearts, particularly the atrium, are upregulating genes involved in inducing apoptosis at 25°C. Inhibition of glycolysis—as evident in warm-acclimated PBFT tis-
houses—is also shown to result in induction of apoptosis (53). Increase in programmed cell death can contribute to the development and progression of cardiac dysfunction in PBFT, as demonstrated in mammalian hearts (12).

**Summary.** Overall qPCR data elucidate how cold-acclimated PBFT alters EC coupling gene expression levels. In particular, SERCA gene expression is increased at 14°C, potentially optimizing the role of SR in cardiac myocytes. The metabolic enzyme activity data and transcriptomic data in 14°C-acclimated PBFT showed an increase in overall ATP production and an induction of genes involved in compensating for effects of cold temperatures. These changes are potentially associated with increased cell size detected in cold-acclimated PBFT myocytes (57). In contrast, transcriptomic data and metabolic enzyme activity data at 25°C, particularly in the atrium, indicated a decrease in overall ATP production; reduced expression of genes involved in molecular chaperones and oxidative stress responses; induction of genes potentially leading to increased ROS production; and an increase in expression of apoptotic genes. The interplay between apoptosis and cellular stress response is thought to prevent further damage or facilitate recovery to maintain cell survival (2). Assayag et al. (2) demonstrated an antiapoptotic cardiac phenotype to heat stress developed by long-term temperature acclimation in mammals. Induction of molecular chaperoning genes was demonstrated to be critical in maintaining this phenotype. Therefore, on the basis of our current data, we hypothesize that diminished cellular stress response and increased oxidative damage may mediate apoptosis in PBFT cardiac cells, particularly in the atrium. This is likely due to perfusion limitations that may occur with increasing temperatures (17) and may lead to reduced cardiac performance in bluefin tunas during long-term exposure to warm temperatures.

**Perspectives and Significance**

The current study provides insights into molecular physiologival underpinnings of cardiac thermal responses in PBFT. The cold acclimation response detected here suggests that PBFT has the capacity to maintain or improve cardiac performance at cold temperatures; this perhaps underlies this species’ ability to expand its vertical and horizontal thermal niche and migrate to colder oceans. In contrast, warm acclimation responses indicate a reduced capacity to restore cellular homeostasis at warm temperatures. This may affect cardiac performance and overall fitness of PBFT when inhabiting warm ocean habitats. To further investigate this hypothesis, experiments are currently under way to quantify blood Po2 levels, as well as several cardiac parameters and aerobic scope in PBFT acclimated to cold and warm temperatures. With increasing ocean temperatures, thermal effects on cardiac function, as described in the current study, particularly during spawning seasons in subtropical seas, may lead to reduced reproductive fitness or shifts in PBFT spawning grounds.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

Author contributions: N.J. and B.A.B. conception and design of research; N.J. and L.D.G. performed experiments; N.J. analyzed data; N.J. and L.D.G. interpreted results of experiments; N.J. prepared figures; N.J. drafted manuscript; N.J., L.D.G., and B.A.B. edited and revised manuscript; N.J., L.D.G., and B.A.B. approved final version of manuscript.

**REFERENCES**


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