Lactate administration increases mRNA expression of PGC-1 α and UCP3 in mouse skeletal muscle

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Lactate administration increases mRNA expression of PGC-1 α and UCP3 in mouse skeletal muscle

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Running Head: Lactate and mitochondrial genes in skeletal muscle

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ABSTRACT

To examine the potential role of lactate as a signaling molecule in skeletal muscle, we performed global gene expression analysis of the mouse gastrocnemius muscle, 3 h after lactate administration using the Affymetrix GeneChip system. Among the top 15 genes with the largest fold change, increased expression of *Ppargc1a*, *Pdk4*, and *Ucp3* was confirmed using real-time quantitative PCR. Our findings suggest that lactate serves as a signal for upregulating genes related to mitochondrial function.

Key Words: Lactate; Skeletal muscle; PGC-1 α ; PDK4; UCP3

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INTRODUCTION

Currently, lactate is recognized not merely as an end product of glycogenolysis in skeletal muscle but also as a carbohydrate fuel source and is shuttled between cells and tissues (Bonen et al. 2006; Brooks 2009). Furthermore, recent studies suggest that lactate may act as a signaling molecule, which contributes to metabolic adaptations in various tissues (Lezi et al. 2013; Carriere et al. 2014). Intriguingly, Hashimoto et al. demonstrated that incubation of L6 cells with 20 mM lactate increased mRNA expression of peroxisome proliferator-activated receptor gamma coactivator 1 alpha, (PGC-1 α), which is known as a master regulator of mitochondrial biogenesis (Hashimoto et al. 2007). The increase in PGC-1 α mRNA levels during recovery from acute exercise is known to be dependent on exercise intensity (Egan et al. 2010). However, our group recently reported that reduced lactate accumulation following administration of dichloroacetate, an activator of pyruvate dehydrogenase, attenuated the high-intensity interval training-induced mitochondrial adaptations in mouse skeletal muscles (Hoshino et al. 2015). To address the hypothesis that lactate plays an important role in exercise-induced metabolic adaptations, possibly via activation of PGC-1 α in skeletal muscles, we administered lactate at upper physiological levels (~20 mM) that can be reached during an “all-out” maximal exercise (Goodwin et al. 2007; Abe et al. 2015). We collected muscle samples at 3 h after lactate administration, since many studies have examined exercise-induced transient gene responses in skeletal muscle after 3 h of recovery (Mahoney et al. 2005; Little et al. 2011).

MATERIALS AND METHODS

Animals

C57BL/6J male mice (8 weeks of age; CLEA Japan, Tokyo, Japan) were used in this study. The mice were housed in an air-conditioned room on a 12:12-h light–dark cycle with standard chow and water *ad libitum*. Mice (n=5/group) were randomly assigned to either control (CON) or Lactate administration (LAC). All experimental treatments were approved by the University of Tokyo committee on animal care.

Lactate Treatment

Mice in LAC group received 1 g/kg of sodium lactate by intraperitoneal injection. The lactate solution concentration was 200 mg/mL, and the pH was 7.3. Mice in CON group were administered same volume of saline. Blood samples were obtained from the tail, and lactate levels were measured using a portable blood lactate analyzer (Lactate Pro 2, Arkray, Tokyo, Japan). The gastrocnemius and tibialis anterior muscles were harvested 3 hours after the administration, immediately snap-frozen, and stored at -80°C . Whole tibialis anterior muscle was homogenized with 0.6 N HClO_4 and centrifuged at 3000 g for 5 min, and muscle lactate concentration was measured using the enzymatic method (Gutmann and Wahlefeld 1974).

RNA Isolation

Whole gastrocnemius muscle was homogenized on ice in Trizol reagent (Life Technologies, Gaithersburg, MD), and then separated into organic and aqueous phases with chloroform. Total RNA was isolated using RNeasy Mini kit (Qiagen), from the aqueous phase following precipitation with ethanol. RNA concentration was measured by spectrophotometry (Nanodrop ND1000, Thermo Scientific, Waltham, MA), and RNA integrity was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). The average RIN (RNA integrity number) value for all samples was 8.7 ± 0.1 (scale 1–10), ensuring high quality isolated RNA.

Microarray analysis

Gene expression analysis was performed using GeneChip Mouse Genome 430 2.0 Array (Affymetrix, Santa Clara, CA). Briefly, 100 ng of total RNA ($n=3/\text{group}$) was used to prepare Biotin-labeled cRNA, followed by fragmentation and hybridization to the array. The array was incubated for 16 hours, washed and stained, then scanned using a GeneChip Scanner 3000 7G. The data was analyzed with the Affymetrix GeneChip Command Console Software and Affymetrix Expression Console, using MAS5 algorithm. Further analyses were carried out on the 45101 probes (Mouse430_2, 2014.3.21; Affymetrix) using the GeneSpring GX software version 13.1.1 (Agilent Technologies). Raw intensity values were normalized to the 75 percentile shift and then submitted to a Bayesian

correction based on the median of the control samples. Probe sets with 1) p-value less than 0.05 with Welch's t-test, and 2) the change of at least two-fold, were retained for gene ontology analysis. The significance was assessed by Fisher's exact test with multiple comparison correction (Benjamini–Yekutieli method), and the ontologies with corrected p-value smaller than 0.05 were considered significant.

Real-time quantitative PCR

First-strand cDNA synthesis from 1 µg of total RNA was performed with random hexamer primers using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). The expression of *Ppargc1a* (*Pgc-1α*), *Pdk4* (pyruvate dehydrogenase kinase 4), *Ucp3* (uncoupling protein 3), *Mt1* (metallothionein 1), and *Mt2* were quantified using the Thermal Cycler Dice Real-Time System and SYBR Premix Ex taq II (Takara Bio, Shiga, Japan). All samples were run in duplicate simultaneously with a negative control that contained no cDNA. TBP (TATA box binding protein) was used as a control housekeeping gene, the expression of which did not alter between groups. The following primers were used for real-time PCR analysis: *Pgc-1α*, forward: 5'-TTCCACCAAGAGCAAGTAT-3', reverse: 5'-CGCTGTCCCATGAGGTATT-3'; *Pdk4*, forward: 5'-AAGATGCTCTGCGACCAGTAT-3', reverse: 5'-GAAGGTGTGAAGGAACGTACA-3'; *Ucp3*, forward: 5'-CCTACGACATCATCAAGGAGAAGTT-3', reverse: 5'-TCCAAAGGCAGAGACAAAGTGA-3'; *Mt1*, Forward: 5'-ACCTCCTGCAAGAAGAGCTG-3', reverse: 5'-GCTGGGTTGGTCCGATACTA-3'; *Mt2*, Forward: 5'-CAAACCGATCTCTCGTCGAT-3', reverse: 5'-AGGAGCAGCAGCTTTTCTTG-3'; *Tbp*, forward: 5'-CTGCCACACCAGCTTCTGA-3', reverse: 5'-TGCAGCAAATCGCTTGGG-3'.

Statistical analysis

Data were expressed as mean ± SEM. Statistical analysis of data, except microarray analysis, was performed using GraphPad Prism 6.0 (La Jolla, CA). Unpaired t-tests were used to test for differences between groups. Values of $P < 0.05$ were considered significant.

RESULTS

The time course of changes in blood lactate concentration after i.p. injection of lactate is shown in Figure 1A. Blood lactate concentration reached 18.6 mM at 5 min after the injection and returned to the baseline level after 3 h. We observed no difference in muscle lactate concentration between CON (13.5 ± 0.6 mmol/kg) and LAC (14.7 ± 0.9 mmol/kg) group. Gene expression analysis revealed that 44 genes were differentially expressed (fold change ≥ 2.0 , $P < 0.05$), with 35 genes up-regulated (supplementary table S1) and 9 genes down-regulated (supplementary table S2), at 3 h after lactate administration in skeletal muscle. Table 1A listed the top 15 upregulated genes with the largest fold change. GO functional groups associated with upregulated genes were listed in Table 1B. Altered expression of *Pgc-1 α* , *Pdk4*, *Ucp3*, *Mt1*, and *Mt2* was confirmed by real-time quantitative PCR (Figure 1B).

DISCUSSION

In the current study, we investigated the effect of lactate administration on global gene expression in the skeletal muscle of mice to examine the potential of lactate as a signal. Here, we observed an increase in mRNA expression of PGC-1 α , which is considered the most important gene for exercise-induced mitochondrial biogenesis, at 3 h after lactate administration. Furthermore, we found increased mRNA expression of PDK4 and UCP3, which are known to be upregulated after exercise and regulated by PGC-1 α (St-Pierre et al. 2003; Wende et al. 2005). Additionally, elevated expression of metallothionein genes was also reported in human skeletal muscle at 3 h after an exhaustive bout of high-intensity cycling (Mahoney et al. 2005). In recent years, there is a growing understanding that high-intensity interval training is a time-efficient strategy to induce mitochondrial biogenesis in skeletal muscle (Burgomaster et al. 2008; Little et al. 2011). When trained and untrained subjects exercised at the same absolute workload, the increase in PGC-1 α mRNA expression and plasma lactate concentration in trained subjects was reported to be less than that in untrained subjects (Nordsborg et al. 2010). Our results are consistent with the hypothesis that the intensity-dependent expression of PGC-1 α following exercise is dependent, at least in part, on lactate accumulation. Supporting this concept, a recent study reported that ingestion of sodium bicarbonate, which elevates

blood and muscle lactate concentration during exercise (Hollidge-Horvat et al. 2000), prior to acute high-intensity interval exercise induced greater increase in PGC-1 α mRNA expression after 3 h of recovery as compared to that seen for a placebo (Percival et al. 2015). Another group has reported that lactate regulates gene transcription through the inhibition of histone deacetylase (Latham et al. 2012); however, further studies are required to clarify the molecular mechanisms.

Although the skeletal muscle is the primary site of lactate production, the effects of lactate may not be limited to the skeletal muscle. A previous study reported that treadmill running increased mRNA levels of PGC-1 α and PDK4 in the liver, and similar adaptations were observed with lactate injection (Lezi et al. 2013). Furthermore, lactate injection increased the UCP1 mRNA level and induced browning of the white adipose tissue (Carriere et al. 2014). There is considerable evidence that several circulating factors secreted from the skeletal muscle (myokines) in response to exercise mediate physiological adaptations in other organs (Pedersen et al. 2008). For example, IL-15 partially regulates exercise-induced mitochondrial adaptations in the skin and skeletal muscle (Crane et al. 2015). Our results suggest that lactate, which is well known to be increased during exercise, also acts as a signal for upregulating genes related to mitochondrial function.

Conflict of interest statement

The authors have no conflicts of interest to disclose.

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Table 1A. Top 15 genes up-regulated with the largest fold change in skeletal muscle at 3 h after lactate administration (n=3/group)

Entrez Gene ID	Gene Symbol	Gene Name	Fold Change	p-value
22229	Ucp3	uncoupling protein 3 (mitochondrial, proton carrier)	4.37	0.0008
17748	Mt1	metallothionein 1	3.14	0.0009
225372	Apbb3	amyloid beta (A4) precursor protein-binding, family B, member 3	3.09	0.0004
15200	Hbegf	heparin-binding EGF-like growth factor	2.90	0.0159
17750	Mt2	metallothionein 2	2.90	0.0066
17993	Ndufs4	NADH dehydrogenase (ubiquinone) Fe-S protein 4	2.88	0.0090
19219	Ptger4	prostaglandin E receptor 4 (subtype EP4)	2.82	0.0214
27273	Pdk4	pyruvate dehydrogenase kinase, isoenzyme 4	2.69	0.0169
59079	ErbB2ip	ErbB2 interacting protein	2.69	0.0051
20975	Synj2	synaptojanin 2	2.50	0.0029
12983	Csf2rb	colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)	2.47	0.0054
83814	Nedd4l	neural precursor cell expressed, developmentally down-regulated gene 4-like	2.45	0.0405
16190	Il4ra	interleukin 4 receptor, alpha	2.41	0.0336
101401	Adamts9	a disintegrin-like and metalloproteinase (reprolysin type) with thrombospondin type 1 motif, 9	2.38	0.0011
19017	Ppargc1a	peroxisome proliferative activated receptor, gamma, coactivator 1 alpha	2.38	0.0410

Table 1B. GO categories up-regulated in skeletal muscle at 3 h after lactate administration (n=3/group)

GO ACCESSION	GO Term	Number of Genes	p-value	corrected p-value
GO:0042221	response to chemical	17	0.000011	0.033
GO:0070887	cellular response to chemical stimulus	13	0.000006	0.033
GO:0033993	response to lipid	10	0.000004	0.033
GO:0048661	positive regulation of smooth muscle cell proliferation	4	0.000007	0.033
GO:0046320	regulation of fatty acid oxidation	3	0.000015	0.033
GO:0010273	detoxification of copper ion	2	0.000013	0.033
GO:1990169	stress response to copper ion	2	0.000013	0.033

Figure Legend.

Figure 1. Lactate regulates the expression of genes related to mitochondrial biogenesis and antioxidant in skeletal muscle. A) The time course of changes in blood lactate concentration after i.p. injection of lactate. B) Real-time PCR analysis for mRNA expression of mitochondrial and antioxidant genes in the skeletal muscle at 3 h after lactate administration. Data are presented as mean $\pm$ S.E.M. $n = 5$ in each group. **Significantly different from control subjects ($P < 0.01$) and *Significantly different from control subjects ($P < 0.05$).

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