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Transcriptional Activity in the Peripheral Blood Leukocytes of Military Divers Following Trimix Dives

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Introduction: Recent studies suggest repeated bouts of decompression stress due to venous gas emboli promote biomolecular alterations leading to adaptive immunoinflammatory responses that may in-part account for the "work-up" effect prior to diving. Using a combination of flow cytometry and microarray analysis, we recently demonstrated significant differences between military clearance divers and non-divers in the expression of several key immunoinflammatory mediators and associated peripheral blood leukocyte (PBL) mRNAs following air dives to 45 msw for 30 minutes bottom time. We broadened our study to examine transcriptional activity (significantly altered gene transcriptions post-dive) in the PBLs of military clearance divers during decompression stress following Trimix table validation dives for 20 minutes to 60, 69 and 81 msw.

Methods: Whole blood was collected into Paxgene blood RNA tubes (PreAnalytiX) from a group of naval clearance divers pre- and post-75 min after surfacing from Trimix hyperbaric exposures for 20 minutes at 60 (*n*=5), 69 (*n*=7) and 81 (*n*=6) msw. mRNA from peripheral blood leukocytes (PBLs) was purified using PAXgene blood RNA kits (QIAGEN) and stored at -70°C. Significantly altered (paired *t*-test) transcriptional activity (calculated as a negative or positive fold change in gene transcription) between the Pre and P75 samples was determined using Agilent two-colour microarray analysis normalized to transcriptional activity in a human PBL reference library. Arrays were quantified and analyzed using Feature Extraction v9.5 (Agilent Technologies) and GeneSpring, respectively. GeneSpring was used to generate heat map displays of genes with significant alterations (indicated as fold change; red = increased gene expression, green = decreased gene expression) after the dive. Genes were categorized by biological function within the immuno-inflammatory paradigm. Venous gas emboli were measured (K-M scoring) using Doppler ultrasound.

Results: The heat map configuration of 3910 genes significantly expressed following a dive at 69 msw dive showed the clearest distinction pattern of gene expression, followed by the 60 msw dive that resulted in 2386 significantly altered genes. In contrast, the distinction pattern of the 81 msw dive resulting in 1320 significantly altered genes was relatively disrupted. Similarly, the 69 msw dive produced the highest number of significantly altered genes closely related to immunoinflammatory response, followed by the 60 and 81 msw dives. Whereas, common gene comparison between dives yielded a degree of repetitious gene expression, a small number of common genes were significantly expressed in the same function groups in all dives including, CD99, SPON2, EMR1, SELL, PXN, GPR56, TSPAN2, KIR2DS2, LAT2, TEGT, IHPK2, LGALS1, AKTIP and NCR3. Interestingly, MYD88 was heavily expressed in all dives.

Summary: Similar to that in earlier studies using Doppler ultrasound, the results of the present study indicated that of the three Trimix diving protocols for 20 minute bottom times, the 69 msw dive represented the highest level of decompression stress, followed by the 60 and 81 msw dives, respectively. Although no correlation existed between individual PBL transcriptional activities and Doppler-detected VGE scores, the 69 msw dive for 20 minutes resulted in the highest level of significant transcriptional activity in PBLs and the deeper 81 msw dive resulted in the lowest level of transcriptional activity. We are presently repeating these diving protocols to confirm their relationships to transcriptional activities in the PBLs of military clearance divers breathing Trimix. To determine the effects decompression stress on transcriptional activity, we also plan to reduce the decompression stress of the 69 msw dive by 1) extending the decompression period (before scheduled oxygen decompression); and 2) extend the period of oxygen decompression (following normal scheduled decompression) to that of the 81 msw dive. Although Doppler ultrasound continues to perform a valuable research role as the most reliable metric of multifaceted decompression stress, it cannot practically be used for diagnosis. Similarly, diagnosis of nebulous symptoms usually leads to HBOT by default. Adaptive immuno-inflammatory responses that may in part account for the "work-up" effect prior to diving could reveal pathways leading to characterization of diagnostic markers for DCS.

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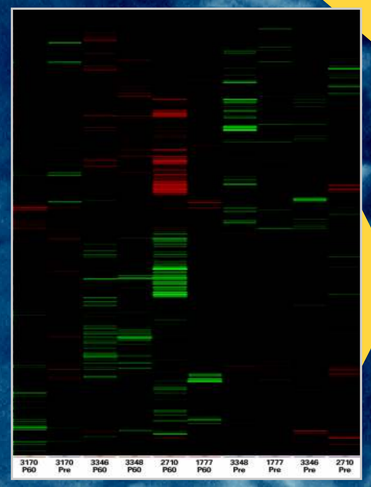
Gene counts of biological function groups closely related to immunoinflammatory response significantly different following a Trimix dive at 60 msw for 20 minutes. Total number of genes significantly different following the dive = 2386.

GO ACCESSION	GO TERM	GENE COUNT
GO:0007249	I-κB kinase/NF-κB cascade	19
GO:0043122	regulation of I-κB kinase/NF-κB cascade	20
GO:0043123	positive regulation of I-κB kinase/NF-κB cascade	23
GO:0043124	negative regulation of I-κB kinase/NF-κB cascade	6
GO:0006954	inflammatory response	43
GO:0050727	regulation of inflammatory response	7
GO:0050729	positive regulation of inflammatory response	2
GO:0050728/GO:0050728	negative regulation of inflammatory response	2
GO:0006915	apoptosis	92
GO:0042981	regulation of apoptosis	70
GO:0043065	positive regulation of apoptosis	41
GO:0043066	negative regulation of apoptosis	27
GO:0006916	anti-apoptosis	24
GO:0045767	regulation of anti-apoptosis	2
GO:0045768	positive regulation of anti-apoptosis	2
GO:0016987	negative regulation of anti-apoptosis	0
GO:0042035/GO:0042032	cytokine biosynthetic process	3
GO:0042035	regulation of cytokine biosynthetic process	17
GO:0042106	positive regulation of cytokine biosynthetic process	13
GO:0042036	negative regulation of cytokine biosynthetic process	3
GO:0045321	leukocyte activation	23
GO:0002694	regulation of leukocyte activation	10
GO:0002696	positive regulation of leukocyte activation	14
GO:0002695	negative regulation of leukocyte activation	5
GO:0050817	coagulation	15
GO:0050818	regulation of coagulation	0
GO:0050819	negative regulation of coagulation	0
GO:0030168	platelet activation	0
GO:0006935	chemotaxis	16
GO:0050920	regulation of chemotaxis	0
GO:0050927	positive regulation of chemotaxis	0
GO:0050922	negative regulation of chemotaxis	0
GO:0007155	cell adhesion	67
GO:0030155	regulation of cell adhesion	11
GO:0045785	positive regulation of cell adhesion	6
GO:0007162	negative regulation of cell adhesion	7



Heat map configuration of 2386 significantly altered genes following a dive to 60 msw for 20 minutes.

Common genes significantly different following Trimix dives at 60 and 69 msw for 20 minutes from biological function groups closely related to immunoinflammatory response. Total number of common significantly different genes following the dives = 357.

GO ACCESSION	GO TERM	GENES
GO:0007249	I-κB kinase/NF-κB cascade	STAT1
GO:0043122	regulation of I-κB kinase/NF-κB cascade	MYD88, LGALS9, LGALS1, RHOA, CFLAR
GO:0043123	positive regulation of I-κB kinase/NF-κB cascade	MYD88, LGALS9, LGALS1, RHOA, CFLAR
GO:0043124	negative regulation of I-κB kinase/NF-κB cascade	———
GO:0006954	inflammatory response	MYD88, NCR3, CD55, MIF, FOS, CD48, IL18RB
GO:0050727	regulation of inflammatory response	ZFP36, BCL6, SPN
GO:0050729	positive regulation of inflammatory response	———
GO:0050728/GO:0050728	negative regulation of inflammatory response	ZFP36, SPN
GO:0006915	apoptosis	AKTIP, DDIT3, STAT1, BIRC5, GZMB, PLEKHF1, TEGT, RAN1, FASDS
GO:0042981	regulation of apoptosis	TUBB, MX1, BCL6, STAT1, BIRC5, PLEKHF1, LGALS1, MIF, CFLAR, TEGT, IHPK2, SPN, CALR, YWHAZ
GO:0043065	positive regulation of apoptosis	TUBB, MX1, BCL6, PLEKHF1, CFLAR, IHPK2, SPN
GO:0043066	negative regulation of apoptosis	BIRC5, MIF, CFLAR, TEGT, YWHAZ
GO:0006916	anti-apoptosis	BIRC5, CFLAR, YWHAZ
GO:0045767	regulation of anti-apoptosis	———
GO:0045768	positive regulation of anti-apoptosis	———
GO:0016987	negative regulation of anti-apoptosis	———
GO:0042035/GO:0042032	cytokine biosynthetic process	MYD88
GO:0042035	regulation of cytokine biosynthetic process	MYD88, SFTPD, SPN
GO:0042106	positive regulation of cytokine biosynthetic process	MYD88, SPN
GO:0042036	negative regulation of cytokine biosynthetic process	SFTPD
GO:0045321	leukocyte activation	KIR2DS2, BCL6, CD7, IL21R, LAT2, SPN
GO:0002694	regulation of leukocyte activation	SFTPD, BCL6, MIF, SPN
GO:0002696	positive regulation of leukocyte activation	BCL6, SPN
GO:0002695	negative regulation of leukocyte activation	SFTPD, BCL6, SPN
GO:0050817	coagulation	PF4, IL18RB, VWF
GO:0050818	regulation of coagulation	———
GO:0050819	negative regulation of coagulation	———
GO:0030168	platelet activation	———
GO:0006935	chemotaxis	CXCR3, SFTPD, PF4, CMTM6, SPN
GO:0050920	regulation of chemotaxis	———
GO:0050927	positive regulation of chemotaxis	———
GO:0050922	negative regulation of chemotaxis	———
GO:0007155	cell adhesion	BJU, ITGB7, CXCR3, PK01, ICAM2, CD99, SPON2, EMR1, PXN, GPR56, SELL, TSPAN2
GO:0030155	regulation of cell adhesion	BCL6, SPN
GO:0045785	positive regulation of cell adhesion	———
GO:0007162	negative regulation of cell adhesion	BCL6, SPN

No relationship existed between Doppler ultrasound scores and transcriptional activity.

Common genes significantly different following Trimix dives at 60, 69 and 81 msw for 20 minutes from biological function groups closely related to immunoinflammatory response. Total number of common significantly different genes following the dives = 71.

GO ACCESSION	GO TERM	GENES
GO:0007249	I-κB kinase/NF-κB cascade	———
GO:0043122	regulation of I-κB kinase/NF-κB cascade	MYD88, LGALS1
GO:0043123	positive regulation of I-κB kinase/NF-κB cascade	MYD88, LGALS1
GO:0043124	negative regulation of I-κB kinase/NF-κB cascade	———
GO:0006954	inflammatory response	MYD88, NCR3
GO:0050727	regulation of inflammatory response	———
GO:0050729	positive regulation of inflammatory response	———
GO:0050728/GO:0050728	negative regulation of inflammatory response	———
GO:0006915	apoptosis	TEGT
GO:0042981	regulation of apoptosis	LGALS1, TEGT, IHPK2
GO:0043065	positive regulation of apoptosis	IHPK2
GO:0043066	negative regulation of apoptosis	TEGT
GO:0006916	anti-apoptosis	———
GO:0045767	regulation of anti-apoptosis	———
GO:0045768	positive regulation of anti-apoptosis	———
GO:0016987	negative regulation of anti-apoptosis	———
GO:0042035/GO:0042032	cytokine biosynthetic process	MYD88
GO:0042035	regulation of cytokine biosynthetic process	MYD88
GO:0042106	positive regulation of cytokine biosynthetic process	MYD88
GO:0042036	negative regulation of cytokine biosynthetic process	———
GO:0045321	leukocyte activation	KIR2DS2, LAT2
GO:0002694	regulation of leukocyte activation	———
GO:0002696	positive regulation of leukocyte activation	———
GO:0002695	negative regulation of leukocyte activation	———
GO:0050817	coagulation	———
GO:0050818	regulation of coagulation	———
GO:0050819	negative regulation of coagulation	———
GO:0030168	platelet activation	———
GO:0006935	chemotaxis	———
GO:0050920	regulation of chemotaxis	———
GO:0050927	positive regulation of chemotaxis	———
GO:0050922	negative regulation of chemotaxis	———
GO:0007155	cell adhesion	CD99, SPON2, EMR1, PXN, GPR56, SELL, TSPAN2
GO:0030155	regulation of cell adhesion	———
GO:0045785	positive regulation of cell adhesion	———
GO:0007162	negative regulation of cell adhesion	———

Heat map configuration of 3910 significantly altered genes following a dive to 69 msw for 20 minutes.

Common genes significantly different following Trimix dives at 69 and 81 msw for 20 minutes from biological function groups closely related to immunoinflammatory response. Total number of common significantly different genes following the dives = 331.

GO ACCESSION	GO TERM	GENES
GO:0007249	I-κB kinase/NF-κB cascade	NLRP12
GO:0043122	regulation of I-κB kinase/NF-κB cascade	MYD88, TNFRSF1A, SLC44A2, CARD9, NLRP12, LGALS1
GO:0043123	positive regulation of I-κB kinase/NF-κB cascade	MYD88, TNFRSF1A, SLC44A2, CARD9, LGALS1
GO:0043124	negative regulation of I-κB kinase/NF-κB cascade	NLRP12
GO:0006954	inflammatory response	MYD88, NCR3, CCL4, STAT3, TNFRSF1A, ADORA2A, PTAFR, LY96, IL1RN
GO:0050727	regulation of inflammatory response	TNFRSF1A, NLRP12
GO:0050729	positive regulation of inflammatory response	TNFRSF1A, NLRP12
GO:0050728/GO:0050728	negative regulation of inflammatory response	———
GO:0006915	apoptosis	CRCP, TNFRSF1A, ADORA2A, AKTIP, NLRP12, DAPK2, LY96, STEAP3, TEGT, CZNF6
GO:0042981	regulation of apoptosis	ALOX15B, FAIM3, SOCS3, CARD9, NLRP12, LGALS1, DAPK2, TEGT, IHPK2
GO:0043065	positive regulation of apoptosis	ALOX15B, DAPK2, IHPK2
GO:0043066	negative regulation of apoptosis	FAIM3, SOCS3, TEGT
GO:0006916	anti-apoptosis	FAIM3, SOCS3
GO:0045767	regulation of anti-apoptosis	———
GO:0045768	positive regulation of anti-apoptosis	———
GO:0016987	negative regulation of anti-apoptosis	———
GO:0042035/GO:0042032	cytokine biosynthetic process	MYD88
GO:0042035	regulation of cytokine biosynthetic process	MYD88, NLRP12
GO:0042106	positive regulation of cytokine biosynthetic process	MYD88
GO:0042036	negative regulation of cytokine biosynthetic process	NLRP12
GO:0045321	leukocyte activation	KIR2DS2, LAT2, ELF4
GO:0002694	regulation of leukocyte activation	———
GO:0002696	positive regulation of leukocyte activation	———
GO:0002695	negative regulation of leukocyte activation	———
GO:0050817	coagulation	CD99, ADORA2A
GO:0050818	regulation of coagulation	———
GO:0050819	negative regulation of coagulation	———
GO:0030168	platelet activation	———
GO:0006935	chemotaxis	CCL4, MAPK14, PTAFR, IL16
GO:0050920	regulation of chemotaxis	———
GO:0050927	positive regulation of chemotaxis	———
GO:0050922	negative regulation of chemotaxis	———
GO:0007155	cell adhesion	PCDHGA7, CEACAM1, CD99, CD3-E, SPON2, CD33, SIRPA, NRN1, EMR1, PXN, GPR56, SELL, TSPAN2
GO:0030155	regulation of cell adhesion	DLC1
GO:0045785	positive regulation of cell adhesion	———
GO:0007162	negative regulation of cell adhesion	———

Gene counts of biological function groups closely related to immunoinflammatory response significantly different following a Trimix dive at 69 msw for 20 minutes. Total number of genes significantly different following the dive = 3910.

GO ACCESSION	GO TERM	GENE COUNT
GO:0007249	I-κB kinase/NF-κB cascade	4
GO:0043122	regulation of I-κB kinase/NF-κB cascade	25
GO:0043123	positive regulation of I-κB kinase/NF-κB cascade	4
GO:0043124	negative regulation of I-κB kinase/NF-κB cascade	1
GO:0006954	inflammatory response	46
GO:0050727	regulation of inflammatory response	5
GO:0050729	positive regulation of inflammatory response	2
GO:0050728/ GO:0050728	negative regulation of inflammatory response	2
GO:0006915	apoptosis	73
GO:0042981	regulation of apoptosis	70
GO:0043065	positive regulation of apoptosis	38
GO:0043066	negative regulation of apoptosis	33
GO:0006916	anti-apoptosis	24
GO:0045767	regulation of anti-apoptosis	0
GO:0045768	positive regulation of anti-apoptosis	0
GO:0016987	negative regulation of anti-apoptosis	0
GO:0042035/GO:0042032	cytokine biosynthetic process	1
GO:0042035	regulation of cytokine biosynthetic process	9
GO:0042106	positive regulation of cytokine biosynthetic process	5
GO:0042036	negative regulation of cytokine biosynthetic process	3
GO:0045321	leukocyte activation	25
GO:0002694	regulation of leukocyte activation	13
GO:0002696	positive regulation of leukocyte activation	14
GO:0002695	negative regulation of leukocyte activation	5
GO:0050817	coagulation	17
GO:0050818	regulation of coagulation	6
GO:0050819	negative regulation of coagulation	6
GO:0030168	platelet activation	0
GO:0006935	chemotaxis	22
GO:0050920	regulation of chemotaxis	1
GO:0050927	positive regulation of chemotaxis	1
GO:0050922	negative regulation of chemotaxis	0
GO:0007155	cell adhesion	91
GO:0030155	regulation of cell adhesion	11
GO:0045785	positive regulation of cell adhesion	2
GO:0007162	negative regulation of cell adhesion	6

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