Early Human Pathophysiological Responses to Exertional Hypobaric Decompression Stress

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INTRODUCTION: Consistent blood biomarkers of hypobaric (altitude) decompression stress remain elusive. Recent laboratory investigation of decompression sickness risk at 25,000 ft (7620 m) enabled evaluation of early pathophysiological responses to exertional decompression stress.

METHODS: In this study, 15 healthy men, aged 20–50 yr, undertook 2 consecutive (same-day) ascents to 25,000 ft (7620 m) for 60 and 90 min, breathing 100% oxygen, each following 1 h of prior denitrogenation. Venous blood was sampled at baseline (T0), immediately after the second ascent (T8), and next morning (T24). Analyses encompassed whole blood hematology, endothelial microparticles, and soluble markers of cytokine response, endothelial function, inflammation, coagulopathy, oxidative stress, and brain insult, plus cortisol and creatine kinase.

RESULTS: Acute hematological effects on neutrophils (mean 72% increase), eosinophils (40% decrease), monocytes (37% increase), and platelets (7% increase) normalized by T24. Consistent elevation (mean five-fold) of the cytokine interleukin-6 (IL-6) at T8 was proinflammatory and associated with venous gas emboli (microbubble) load. Levels of C-reactive protein and complement peptide C5a were persistently elevated at T24, the former by 100% over baseline. Additionally, glial fibrillary acidic protein, a sensitive marker of traumatic brain injury, increased by a mean 10% at T24.

CONCLUSIONS: This complex composite environmental stress, comprising the triad of hyperoxia, decompression, and moderate exertion at altitude, provoked pathophysiological changes consistent with an IL-6 cytokine-mediated inflammatory response. Multiple persistent biomarker disturbances at T24 imply incomplete recovery the day after exposure. The elevation of glial fibrillary acidic protein similarly implies incomplete resolution following recent neurological insult.

KEYWORDS: decompression stress, hyperoxia, exertion, oxidative stress, venous gas emboli (VGE), biomarkers.

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The pathophysiological basis of hypobaric (altitude) decompression stress is poorly defined, while consistent blood biomarkers of hyperbaric (diving) decompression sickness (DCS) remain elusive. A recent altitude chamber study evaluated risk of DCS in physically active men exposed twice in quick succession to a pressure altitude of 25,000 ft (7620 m), enabling investigation of pathophysiological responses to hypobaric stress using selected biomarkers.[6]

The primary mechanism of physical and cellular injury in DCS is the generation of bubbles in tissues supersaturated with inert gas. Besides mechanical obstruction of vessels, intravascular bubbles initiate procoagulant and proinflammatory responses through "foreign body" surface interaction with blood proteins, cellular components, and vascular endothelium, triggering platelet aggregation, leukocyte activation, cytokine and chemokine release, and activation of complement, kinin, and coagulation systems.[3] Gaseous microemboli damage endothelial cells and surface glycocalyx, compromising vascular integrity, increasing permeability, and facilitating further bubble adhesion.[18] The release of

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microparticles (MPs) reflects endothelial dysfunction secondary to oxidative stress and interaction with bubbles, influencing the systemic response to decompression.[13] MPs may be aggravated by circulating bubbles and act as foci for further bubble formation.[23,27] Local initiation of inflammatory and coagulant cascades can result in persistent effects even after the bubbles have passed, e.g., small gas emboli in the cerebral microcirculation promoting endothelial disruption with focal ischemia, inflammation, and edema.

The goal of the current study was to evaluate the pathophysiological basis of nonhypoxic altitude exposure by investigating human biomarker responses to severe hypobaric decompression stress. Inconsistent outcomes from previous research leave a broad range of potential biomarkers of interest. Specific targets selected for analysis crossed six areas, comprising cytokine responses, inflammatory markers, coagulation markers, endothelial function, oxidative stress, and brain insult/stress. **Table I** lists the final choice of target proteins.

Potent inflammatory cytokines, implicated in previous studies of decompression stress, include interleukin-1β (IL-1β), IL-6, IL-8, and interferon gamma (IFN-γ).[2,8] Chosen inflammatory markers include C-reactive protein (CRP), an acute phase protein that increases oxidative stress and correlates inversely with endothelial function, and neutrophil gelatinase-associated lipocalin (NGAL), a marker of neutrophil activation that is elevated by regular daily diving. Additionally, complement peptide C5a is elevated upon complement activation by the alternative pathway, the potential mechanism of action of intravascular bubbles, and may promote oxidative injury to pulmonary endothelium.[26] Markers of coagulopathy are coagulation factor III, also known tissue factor (CFIII/TF) or thromboplastin, primary initiator of the extrinsic coagulation pathway, and Platelet Factor 4 (PF4), a marker of platelet activation that is elevated in animal studies of hyperbaric DCS.

The hallmark of endothelial activation is cell surface expression of adhesion molecules, triggering leukocyte interaction

Table I. Assay Test Kit Manufacturers/Suppliers.

BIOMARKER GROUP	TARGET	ASSAY TYPE	SUPPLIER	WEB REFERENCE
Cytokines	IL-1β	ELISA	R&D Systems	human-il-1-beta-il-1f2-quantikine-hs-elisa-kit_hslb00d
	IL-6	ELISA	R&D Systems	human-il-6-quantikine-hs-elisa-kit_hs600c
	IL-8	ELISA	R&D Systems	human-il-8-cxcl8-quantikine-elisa-kit_d8000c
	IL-10	ELISA	R&D Systems	human-il-10-quantikine-hs-elisa-kit_hs100c
	IFNγ	ELISA	R&D Systems	human-ifn-gamma-quantikine-elisa-kit_dif50c
Inflammation	CRP	ELISA	Enzo Life Sciences	ENZ-KIT102/crp-human-elisa-kit/
	C5a	ELISA	Thermo Fisher Scientific	Complement-C5a-Human-ELISA-Kit/BMS2088
	NGAL	ELISA	Thermo Fisher Scientific	NGAL-Human-ELISA-Kit/KIT036
Coagulopathy	CFIII/TF	ELISA	R&D Systems	human-coagulation-factor-iii-tissue-factor-quantikine-elisa_dcf300
	PF4	ELISA	Abcam	human-pf4-elisa-kit-cxcl4-ab100628
Endothelial function	ICAM-1	ELISA	Thermo Fisher Scientific	ICAM-1-Soluble-Human-ELISA-Kit/BMS201
	Endoglin	ELISA	Thermo Fisher Scientific	Endoglin-CD105-Human-ELISA-Kit/EHENG
	VCAM-1	ELISA	Thermo Fisher Scientific	VCAM-1-Soluble-Human-ELISA-Kit/BMS232
	eNOS	ELISA	Thermo Fisher Scientific	elisa/product/EH169RB.html
	VEGF	ELISA	R&D Systems	human-vegf-quantikine-elisa-kit_dve00
Endothelial microparticles	CD54	Flow cytometry	Bio Rad	monoclonal/human-cd54-antibody-84h10-mca532
	CD105	Flow cytometry	Bio Rad	monoclonal/human-cd105-antibody-sn6-mca1557
	CD106	Flow cytometry	Bio Rad	monoclonal/human-cd106-antibody-1-g11b1-mca907
Oxidative stress	SOD	Colorimetry	Cambridge Bioscience	product~97,995
	GSH	Colorimetry	Thermo Fisher Scientific	product/EIAGSHC
	TBARS	Colorimetry	Cambridge Bioscience	product~91,969
Brain injury	S100B	ELISA	Abcam	s100b-elisa-kit-ab234573
	NSE	ELISA	Abcam	human-neuron-specific-enolase-elisa-kit-ab217778
	GFAP	ELISA	Abcam	human-gfap-elisa-kit-ab223867
	Glu	Colorimetry	Abcam	glutamate-assay-kit-ab83389
Others	Cortisol	ELISA	Enzo Life Sciences	ADI-900-071/cortisol-elisa-kit/
	CK-M	ELISA	Abcam	human-ckm-elisa-kit-ab264617

ELISA = enzyme-linked immunosorbent assay; IL = interleukin; IFN = interferon; CRP = C-reactive protein; C5a = Complement peptide 5a; NGAL = neutrophil gelatinase-associated lipocalin; CFIII/TF = coagulation factor III/tissue factor; PF = platelet factor; ICAM = intercellular adhesion molecule; VCAM = vascular cell adhesion molecule; eNOS = endothelial nitric oxide synthase; VEGF = vascular endothelial growth factor; CD = cluster of differentiation (microparticles); SOD = superoxide dismutase; GSH = glutathione; TBARS = thiobarbituric acid reactive substances; S100B = S100 calcium-binding protein B; NSE = neuron specific enolase; GFAP = glial fibrillary acidic protein; Glu = glutamate; CK-M = muscle creatine kinase.

within minutes. Endothelium activated or damaged by decompression stress sheds endothelial microparticles (EMPs) that express these adhesion molecules and may themselves mediate further endothelial injury.[28] Another membrane glycoprotein, endoglin, is important for endothelial integrity. EMPs expressing endoglin are associated with oxidative stress and impaired endothelial function post-dive.[4,14] We have targeted soluble and microparticle-associated forms of adhesion molecules and endoglin. Endothelial nitric oxide synthase (eNOS) is specifically included as its induction may mitigate bubble formation upon decompression. We have included enzymatic (superoxide dismutase, SOD), nonenzymatic (glutathione, GSH) and lipid peroxidation (thiobarbituric acid reactive substances, TBARS) markers of oxidative stress.

The nature of the association between nonhypoxic decompression stress and brain white matter hyperintensities (WMH) remains unclear. Modest, brief, infrequent hypobaric decompressions appear harmless, but exposures sufficient to present significant risk of DCS, whether hypoxic or nonhypoxic, influence cerebral physiology.[16,17] The nonhypoxic decompression stress imposed in the current study afforded an opportunity to target biomarkers of potential brain insult implicated in diving decompression stress, including serum calcium-binding protein S100β and neuron-specific enolase (NSE). We included astrocyte-derived glial fibrillary acidic protein (GFAP), a sensitive marker of brain insult,[1] and serum glutamate (Glu). Glu is the main excitatory neurotransmitter in the central nervous system (CNS). Extracellular homeostasis is maintained by endothelial cells of the blood–brain barrier, which actively transport Glu into the blood. After CNS injury, permeability increases to avoid local neurotoxicity, elevating Glu efflux for peripheral redistribution and metabolism.[25]

Finally, samples for hematology evaluated cellular responses and enabled correction of whole blood and plasma volumes with respect to hydration status. Serum cortisol was included to assess the corticosteroid stress response, as well as muscle creatine kinase (CK-M) to evaluate any influence of muscle damage secondary to repeated bouts of asymmetric exercise (squats).

METHODS

Subjects

The underpinning study design, methodology, and nonbiomarker outcomes are described in detail elsewhere.[6] Relevant details are summarized here for convenience. The study adhered to the principles of the Declaration of Helsinki. The research was funded by the UK Ministry of Defence (MOD) and the experimental protocol was approved in advance by the MOD Research Ethics Committee, an independent body constituted and operated in accordance with national and international guidelines.

Study participants were 15 healthy, nonsmoker men, ages 20–50 yr, mean (± SD) age 38 ± 11 yr. By chance, they comprised 5 men aged under 30 yr (mean 24 yr, range 20–28) and 10 men aged over 40 yr (mean 46 yr, range 41–50). Their mean (± SD) height was 1.82 ± 0.07 m, mean weight was 82.2 ± 8.4 kg, and mean body mass index was 24.9 ± 2.4 kg · m^{-2}. Following medical examination, healthy volunteers were screened using bubble contrast echocardiography, at a clinical center of excellence, to exclude underlying right-to-left vascular shunts, either intracardiac (patent foramen ovale) or pulmonary. Those who passed were then screened using high-resolution brain magnetic resonance imaging (fluid-attenuated inversion recovery sequences), at an academic research unit, to exclude excess prior white matter hyperintensities. A maximum of five discrete punctate subcortical lesions were allowed for study entry, consistent with previous reports.

Subjects avoided hypobaric or hyperbaric environments (i.e., flying, diving, parachuting, or mountaineering) in the 72 h prior to decompression and for 24 h afterwards. They also avoided alcohol and strenuous physical exertion for 48 h prior to decompression and 24 h afterwards. Otherwise, they ate and drank normally and ensured a good night's rest before and after their experiment. This comprised two consecutive ascents to an equivalent pressure altitude of 25,000 ft (7620 m) for 60 and then 90 min, each preceded by 1 h of denitrogenation, breathing 100% oxygen throughout. Exposures were separated by 1 h of breathing air normally at ground level. Subjects drank freely before and between ascents to maintain normal hydration and had a snack lunch between exposures. They simulated the duties of parachutist dispatchers, including short spells of load carriage at ground level prior to each ascent. Predominantly ambulatory activities at altitude included numerous squats, notably at 25,000 ft, where sessions of 16 squats over about 4 min were undertaken every 15 min, simulating equipment checks prior to parachutist dispatch. The response to decompression stress was evaluated using precordial "2D + Doppler" echocardiography conducted every 15 min at altitude. Apical four-chamber views enabled grading of venous gas emboli (VGE) "bubble" loads passing through the right side of the heart, consistent with an Expanded Eftedal-Brubakk scale, by an experienced investigator.[6]

Equipment

Biomarkers were analyzed at the University of Hull using bespoke high-sensitivity test kits, mostly enzyme-linked immunosorbent assays (ELISAs) with some colorimetric assays. EMP counts were analyzed by flow cytometry as previously described.[11] The specific assays used are detailed in Table I. Test kit suppliers were Abcam (Cambridge, UK), Bio-Rad AbD Serotec GmbH (Puchheim, Germany), Bio-Techne (Abingdon, UK, for R&D Systems), Cambridge Bioscience (Cambridge, UK), Enzo Life Sciences (UK) Ltd (Exeter, UK), and Life Technologies Ltd. (Paisley, UK, for Thermo Fisher Scientific). ELISAs were performed following the relevant manufacturers' instructions and plates were read at the appropriate wavelength using a BioTek Synergy HT Microplate Reader running Gen5 software (BioTek now Agilent, Santa Clara, CA, USA). For fluorescence-activated cell-sorting

(FACS), plasma samples (25 µL) were incubated with appropriate antibodies (5 µL) for 30 min in the dark at room temperature. Phosphate-buffered saline (0.2 µm filtered, 150 µL) and AccuCheck counting beads (25 µL, PCB100, Invitrogen, Waltham, MA, USA) were added prior to MP quantification by flow cytometry. Samples were analyzed using a BD FACSCalibur flow cytometer running CELLQuest Pro software (BD Biosciences, San Jose, CA, USA). The MP gate was set as described previously, using Megamix SSc beads (Biocytex, Marseille, France).[5]

Procedure

Decompressions were conducted in the high-performance hypobaric chamber of the Altitude Research Facility at MOD Boscombe Down, Wiltshire, UK. Subjects arrived at 08:00 and, following confirmation of fitness to proceed, a 20 mL pre-exposure baseline (T0) venous blood sample was obtained by antecubital venipuncture before 08:30. The first ascent typically commenced between 09:30–10:00 such that the second ascent was completed around 16:00. A second post-exposure (T8) venous sample was collected immediately after completion of the second ascent. Subjects returned the following day for a third midmorning sample, approximately 24 h after the start of their first ascent (recovery, T24). Experiments were curtailed by three instances of limb bend DCS, including two on the first ascent after 29 and 37 min at 25,000 ft (7620 m), and one after 60 min on the second ascent. All resolved with recompression and were treated with 100% oxygen for 1 h at ground level. These subjects' T8 samples were collected immediately upon completion of oxygen breathing.

Each 20 mL venous collection was divided to provide one 3.0 mL ethylene diamine tetra-acetic acid (EDTA) sample for whole blood hematology; two 2.5 mL citrated samples for plasma; and two 6.0 mL silica-coated "clotted" samples for serum. The plasma samples were immediately double-centrifuged, first at 1500 × g for 10 min and then, after careful pipetting to leave a generous residue, at 5000 × g for 20 min. A total of 2.0 mL of platelet-free plasma was derived and aliquoted into four 0.5 mL vials. After standing for 45 min, the clotted samples were centrifuged for 15 min at 2000 × g to obtain a total 6.0 mL of serum, aliquoted into four 1.5 mL vials. All samples were packaged and transported immediately to the Clinical Laboratory at Defence Science and Technology Laboratory (DSTL) Porton Down, Wiltshire, UK. A complete blood count and differential was performed on the EDTA sample. The vials of plasma and serum were labeled and frozen at -80°C, within 2 h of sampling, for later batch transfer on dry ice to the University of Hull, Hull, UK.

Analysis

Post-exposure (T8, T24) values for whole blood cellular biomarkers were corrected for minor fluctuations in circulating blood volume with respect to hemoglobin (Hb) levels, in accordance with Eq. 1, since total Hb was not expected to fluctuate significantly between samples.[15] Post-exposure plasma and serum markers were corrected for fluctuations in plasma volume to adjust for hydration state, in accordance with convention, Eq. 2.[7]

$$\Delta TBM = \frac{BM_{post}}{BM_{pre}} \text{ X } \frac{Hb_{pre}}{Hb_{post}} - 1 \qquad \text{Eq. 1}$$

Eq. 1 shows calculation of post-exposure correction for changes in total blood volume applied to whole blood cellular markers. ΔTBM = change in total blood marker; BM = blood marker value pre- or post-exposure; Hb = hemoglobin.

$$\Delta PV = \frac{PV_{post} - PV_{pre}}{PV_{pre}} = \frac{Hb_{pre}\ x\ (1 - Hct_{post})}{Hb_{post}\ x\ (1 - Hct_{pre})} - 1 \qquad \text{Eq. 2}$$

Eq. 2 shows calculation of post-exposure correction for changes in plasma volume applied to plasma/serum markers. ΔPV = change in plasma volume; Hct = hematocrit.

Occasional extreme values in a minority of datasets were attributed to test kit technical errors. To ensure consistent analyses for all biomarkers, outliers were defined as any extreme values greater than three SD from the cohort mean at any sample time; all of that subject's data were then removed from the dataset for that marker. No more than one subject's data required removal from any dataset and no subject had their data removed from more than one dataset, supporting the impression that these were random outliers. Most datasets remained intact (45 data points). Each T0 dataset was examined for a normal distribution using the Shapiro-Wilk test ($\alpha = 0.05$). Data transforms ($\log_{10}$, square root or inverse) were applied, if necessary, to achieve this, and then extended to the entire dataset for that marker. Inferential analysis employed one-way repeated measures analysis of variance (rmANOVA) for the factor "Sample Time" (T0, T8, T24). The assumption of sphericity was checked with Mauchly's test and, if violated, Greenhouse-Geisser correction was applied automatically. If rmANOVA achieved statistical significance ($P < 0.05$), paired *t*-tests isolated pairwise differences, with Sidak correction for multiple comparisons. If the normality assumption was not satisfied, corresponding nonparametric analyses were performed. Possible associations were evaluated using simple scatter plots with linear or polynomial regression.

RESULTS

There were three occurrences of limb bend DCS that curtailed exposures, two in the first ascent and one in the second. The remaining 12 subjects completed both decompression profiles. There were 11 subjects who generated heavy and persistent loads of VGE, especially during the first ascent, with older subjects (>40 yr) consistently generating earlier and heavier bubble loads.[6] This influence of age was not reflected in any biomarker dataset. For the results that follow, outcomes of rmANOVA or Friedman's analysis are shown in the relevant figures. Results of post hoc pairwise comparisons are detailed in the text.

Table II. Total Hemoglobin and Hematocrit.

HEMOGLOBIN/HEMATOCRIT*	BASELINE (T0) (MEAN ± SD)	POST-EXPOSURE (T8) (MEAN ± SD)	RECOVERY (T24) (MEAN ± SD)
Total Hemoglobin (Hb), $g \cdot dL^{-1}$	15.5 ± 0.7	15.3 ± 1.2	15.4 ± 0.8
Hematocrit (Hct), %	0.465 ± 0.03	0.458 ± 0.04	0.463 ± 0.02

*$N = 15$.

Hemoglobin and hematocrit data are summarized in **Table II**. Hematocrit data are also represented in **Fig. 1A**, omitting a single aberrant (impossibly low) T8 value, attributed to technical error during measurement, which lowered the cohort mean T8 value in Table II. Fig. 1A illustrates that hematocrits were consistent throughout, reflecting well-maintained normal hydration states across the cohort.

Hematological responses were highly consistent between subjects (Fig. 1). **Fig. 1B** illustrates a consistent (mean 40%) increase in circulating leukocyte count at T8 that recovered to baseline by T24. Only one subject, whose first ascent was curtailed by DCS after only 29 min at 25,000 ft (7620 m), failed to show an elevated white cell count at T8. There was a mean 70% increase in circulating neutrophils at T8 (**Fig. 1C**), with every subject exhibiting an increase relative to T0. Total lymphocyte counts (mean ± SD) were: T0, 1.69 ± 0.41; T8, 1.75 ± 0.3; T24, $1.59 \pm 0.3 \times 10^9 \cdot L^{-1}$. The slight variation is consistent with diurnal variation in healthy young men.[21] A modest but statistically

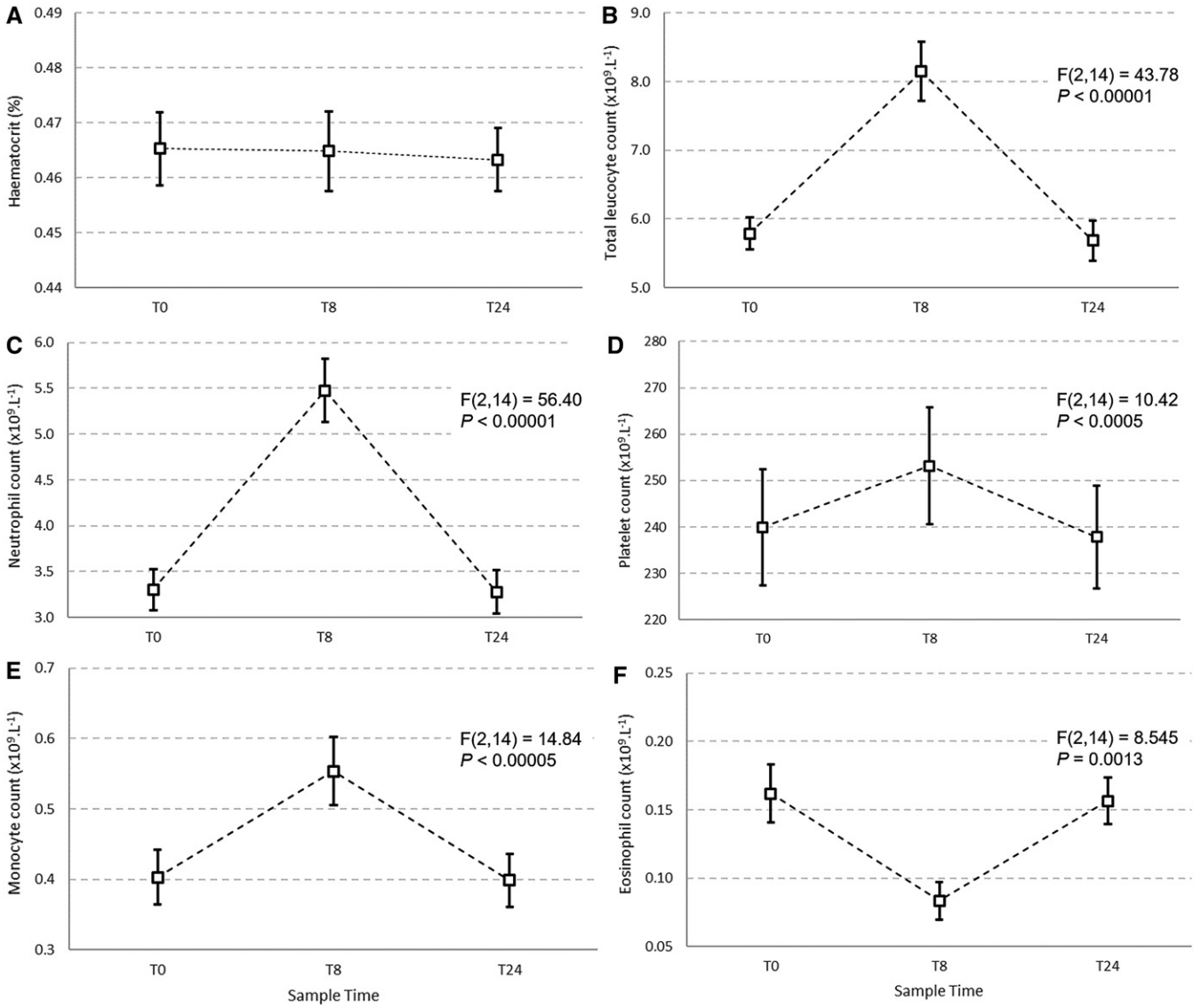

Fig. 1. Hematological parameters (mean ± SE). The statistically significant cell count changes at T8, recovering to baseline by T24, reflect highly consistent responses across the cohort.

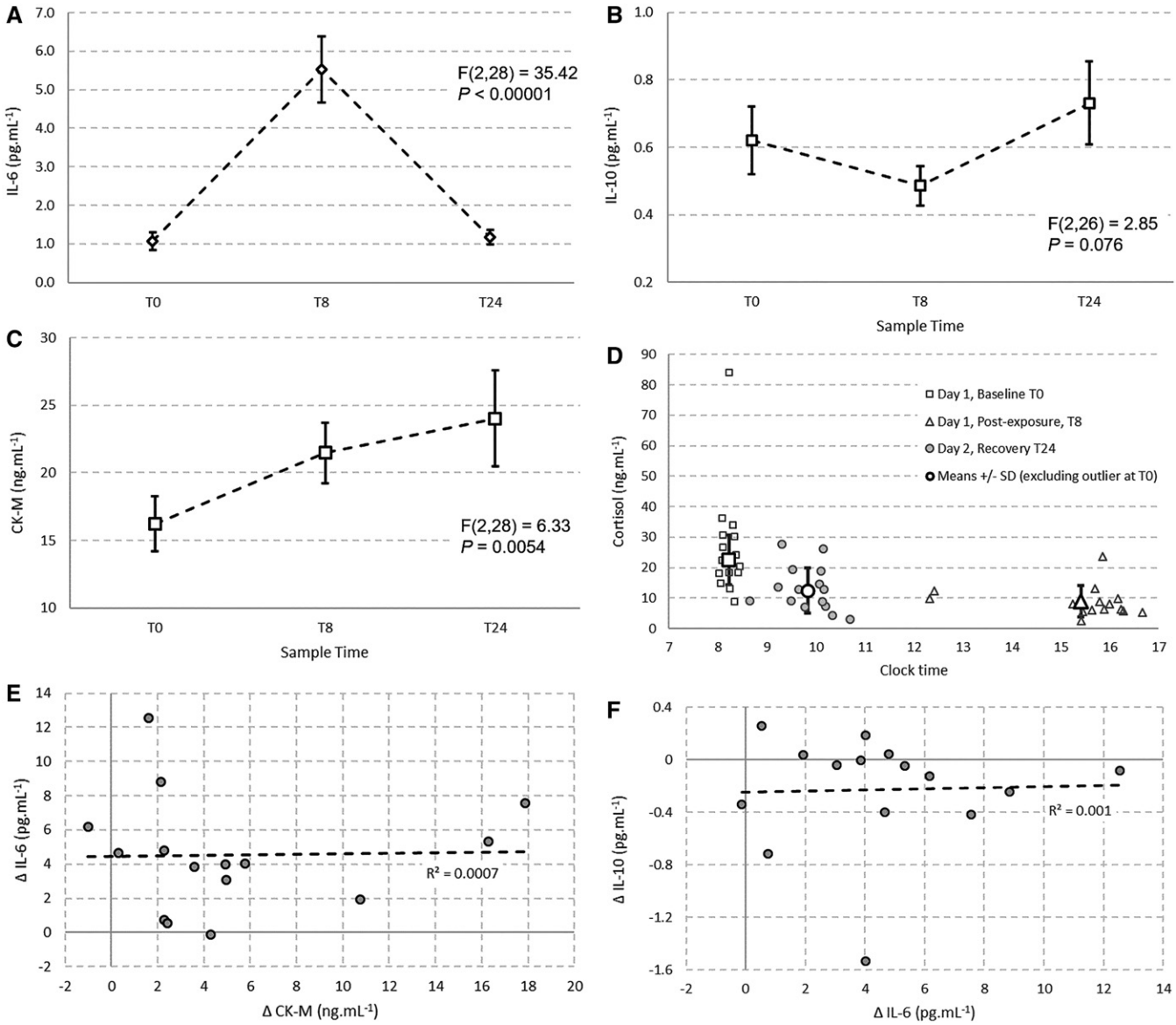

Fig. 2. Cytokine, creatine kinase, and cortisol responses. A) Mean (± SE) IL-6; B) mean (± SE) IL-10; C) mean (± SE) CK-M; D) mean (± SD) cortisol; E) absence of association between CK-M and IL-6 responses at T8; and F) absence of association between IL-6 and IL-10 responses at T8. For abbreviations, see caption under Table I.

significant (mean 6%) increase in circulating platelets at T8 (**Fig. 1D**) reflected an increase over baseline in 14 subjects (range 1–14%), with just 1 decreasing by 10%. This slight elevation is also consistent with diurnal variation.[21] At T8, there was also a mean 37% increase in circulating monocytes (**Fig. 1E**) and a 40% reduction in eosinophils (**Fig. 1F**), both normalizing by T24.

There was a substantial, highly statistically significant, mean fivefold elevation of IL-6 immediately post-exposure at T8, relative to the expected normal background levels of ~1.0 pg · mL^{-1} seen at T0 and T24 (**Fig. 2A**). IL-6 may be generated as an anti-inflammatory myokine in working skeletal muscle, upregulating the anti-inflammatory IL-10, so IL-10 was also evaluated.[22] Modest fluctuations of IL-10 were not statistically significant (**Fig. 2B**). IL-1β, IL-8, and IFN-γ were undetectable throughout, remaining below the highly sensitive detection thresholds of the respective test kits (IL-1β < 0.1 pg · mL^{-1}; IL-8 < 31.2 pg · mL^{-1}; IFN-γ < 15.6 pg · mL^{-1}). Modest post-exposure elevation of CK-M was statistically significant at T8 ($P < 0.0005$) but not at T24 ($P = 0.039$; Sidak $\alpha = 0.017$) (**Fig. 2C**). Baseline (T0) cortisol samples were taken within the 30 min preceding diurnal acrophase (0830); subsequent post-exposure (T8) and recovery (T24) samples were proportionately lower and consistent with expectation for diurnal variation (**Fig. 2D**). Scatter plots of T8 increments over T0 for CK-M relative to IL-6 (**Fig. 2E**) and for IL-6 with respect to IL-10 (**Fig. 2F**) do not suggest any association.

After post hoc Sidak correction ($\alpha = 0.017$), complement peptide C5a was statistically significantly elevated over baseline values at T24 ($P = 0.008$) but not at T8 ($P = 0.04$) (**Fig. 3A**). After removing one outlier with an exceptionally high T0 baseline value, CRP was significantly elevated at T24 relative to both

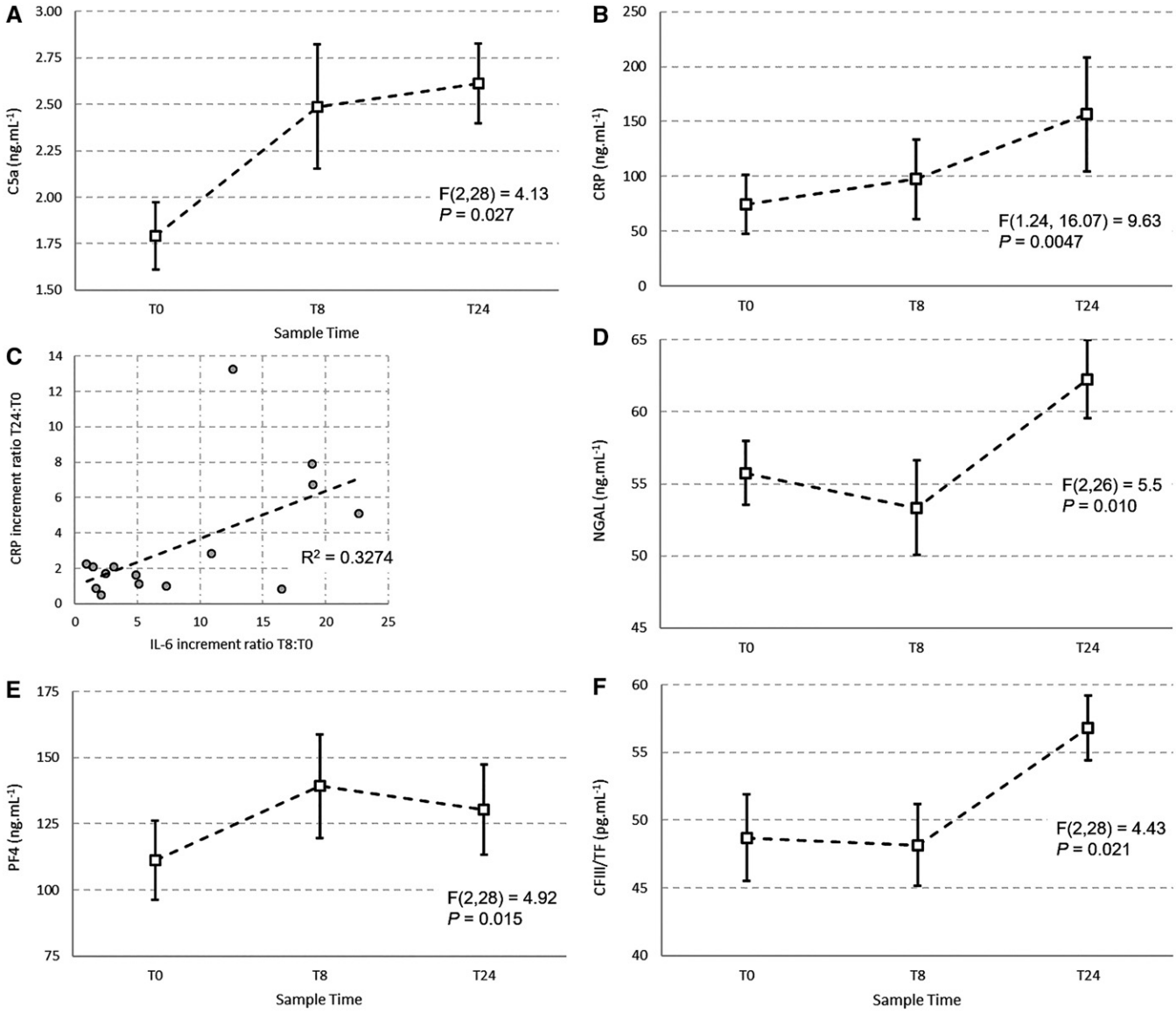

Fig. 3. Mean (± SE) biomarker data for: A) complement activation (peptide C5a); B) acute phase response (CRP); D) neutrophil activation (NGAL); E) platelet activation (PF4); and F) circulating tissue factor (CFIII/TF). C) Relationship between IL-6 increment at T8 and CRP response at T24. For abbreviations, see caption under Table I.

T0 ($P = 0.006$) and T8 ($P = 0.008$) (**Fig. 3B**). The IL-6 increment ratio at T8, relative to baseline, may have influenced the magnitude of the CRP response at T24 (**Fig. 3C**). NGAL levels (**Fig. 3D**) increased significantly at T24 relative to a slight post-exposure dip ($P < 0.005$). Relative to T0, PF4 was elevated at T8 ($P = 0.010$) but not at T24 ($P = 0.079$) (**Fig. 3E**). CFIII/TF was elevated at T24 relative to T8 ($P = 0.015$) but not with respect to T0 after Sidak correction ($P = 0.04$) (**Fig. 3F**).

The data for all endothelial biomarkers are summarized in **Table III**. Within-subject responses of all three EMP types were highly consistent, exemplified when comparing the T24:T0 increment ratios for CD54 (intercellular adhesion molecule-1, ICAM-1) and CD105 (endoglin), shown in **Fig. 4A**. CD106 (vascular cell adhesion molecule-1, VCAM-1) responded similarly. Accordingly, rather than differentiate between their responses, we have considered EMPs collectively by evaluating subject total EMP counts; these are represented in **Fig. 4B** omitting one subject with a grossly elevated total EMP count at T8 that is disproportionate to the rest of the cohort. The collective responses of the remaining subjects are better characterized by the boxplots of EMP counts in **Fig. 4C**, suggesting elevated levels at T24 driven by around half the subjects. On this basis, nonparametric comparison ($N = 15$) supports an increase at T24 relative to T0 (one-tailed Wilcoxon $Z = 1.851$, $P = 0.032$). Of the soluble endothelial markers listed in Table III, only ICAM-1 exhibited a notable response (**Fig. 4D**), with T24 levels significantly lower than T8 ($P = 0.002$).

The data for oxidative stress biomarkers are included in Table III. The response pattern for SOD suggested initial upregulation at T8, followed by downregulation at T24, but did not

Table III. Selected Biomarker Data.

BIOMARKER	T0 (MEAN ± SE)	T8 (MEAN ± SE)	T24 (MEAN ± SE)	NOTES*
Endothelial Markers				
EMP counts				
CD54 (ICAM-1), μL^{-1}	635 ± 108	990 ± 416	1004 ± 231	
CD105 (Endoglin), μL^{-1}	470 ± 72	764 ± 222	850 ± 211	
CD106 (VCAM-1), μL^{-1}	536 ± 116	736 ± 243	689 ± 157	
Total EMPs, μL^{-1}	1641 ± 247	2491 ± 864	2543 ± 585	
Soluble membrane glycoproteins				
ICAM-1, ng · mL^{-1}	408 ± 15	424 ± 19	374 ± 16	
Endoglin, ng · mL^{-1}	10.99 ± 1.8	9.85 ± 1.5	10.41 ± 2.1	
VCAM-1, ng · mL^{-1}	800 ± 52	784 ± 34	868 ± 87	
Endothelial function				
eNOS, ng · mL^{-1}	268 ± 57	240 ± 59	245 ± 62	$N = 12^{\dagger}$
VEGF, pg · mL^{-1}	448 ± 73	389 ± 73	464 ± 69	
Oxidative Stress Markers				
SOD, U.mL^{-1}	71 ± 5	78 ± 4	65 ± 4	$N = 14$
Total GSH, μM	1.96 ± 0.25	1.61 ± 0.15	3.98 ± 0.72	
TBARS (MDA), μM	4.98 ± 0.5	5.81 ± 0.8	6.36 ± 1.0	$N = 14$
Brain Insult Markers				
S100B, pg · mL^{-1}	–	–	–	Undetectable (<139 pg · mL^{-1})
NSE, ng · mL^{-1}	2.35 ± 0.29	2.42 ± 0.34	2.49 ± 0.36	
GFAP, ng · mL^{-1}	7.9 ± 3.9	8.2 ± 4.1	9.2 ± 4.9	
Glu, nmol	8.9 ± 0.4	10.1 ± 0.7	8.9 ± 0.7	

MDA = malondialdehyde activity; SE = standard error.
*$N = 15$ except where stated.
†Sample size decreased due to undetectable levels throughout in some subjects.

achieve statistical significance after sphericity and Sidak correction. Total glutathione data could not be normalized. Friedman analysis supports a significant increase in total glutathione at T24 (χ^2_r [2, $N = 15$] = 15.6, $P < 0.0005$). Due to technical difficulty, oxidized glutathione was not available. After removal of one outlier, TBARS data indicate no effect on malondialdehyde activity (lipid peroxidation).

Brain biomarker outcomes are also summarized in Table III. S100β was not detected in any sample. Minor fluctuations of NSE were inconsistent between individuals, with no clear response pattern across the cohort. In contrast, despite widely varying baseline values between individuals, across two orders of magnitude, there was a consistent mean 10% (SE ± 3.7%) increase in GFAP at T24 relative to baseline (Friedman's χ^2_r [2, $N = 15$] = 8.93, $P = 0.015$), with no change at T8 (0 ± 2%). A suggestive but statistically nonsignificant increase in Glu at T8 recovered to baseline at T24 (F[2,14] = 2.69, $P = 0.085$).

DISCUSSION

Approximately 90% of the increase in total leukocyte count at T8 is attributable to neutrophils, with the remainder attributable to monocytes, suggesting an innate immune response (**Fig. 5**). Two instances of limb pain DCS curtailed initial ascents, thereby probably limiting their neutrophil responses (Fig. 5A).

Neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), and Systemic Immune-inflammation Index (SII) are detailed in **Table IV**. The cohort NLR and SII are elevated at T8, recovering to baseline by T24. The normal mean NLR in healthy young adults is 1.65, range (± 1.96 SD) is 0.78–3.53.[9] Subjects' NLR at T8 ranged from 2.07–4.41, with 5 exceeding the upper limit of the normal range. PLR remained steady throughout, with minor fluctuations in platelets and lymphocytes consistent with diurnal variation. The elevated SII suggests a proinflammatory response or possibly a corticosteroid-mediated stress response. However, lymphocyte counts were not suppressed and cortisol data do not support a stress response, with T8 values proportionate for diurnal variation relative to acrophase (Fig. 2D). The fall in eosinophils at T8 is consistent with an acute phase response and contrasts with the expected morning nadir at cortisol acrophase and elevation toward evening (Fig. 1F). Overall, hematological data suggest an acute phase response rather than a stress response.

The headline finding is mean fivefold elevation in IL-6 at T8 that recovers to baseline by T24 (Fig. 2A). This was highly consistent between subjects (Fig. 5B) and greatly exceeded normal diurnal variation.[20] In contrast, IL-1β, IL-8, and IFN-γ were undetectable. IL-6 is a proinflammatory cytokine generated by neutrophils and endothelial cells, but it can also be produced as an anti-inflammatory myokine in working skeletal muscle. A proinflammatory cytokine response is most likely in the current study. The low intensity exertion during decompression promoted modest elevation of CK-M (Fig. 2C), unrelated to IL-6 increments (Fig. 2E), whereas myokine responses are associated with high intensity exercise to volitional fatigue or endurance activities (e.g., marathon running). Further, the half-life of circulating IL-6 myokine is short (only a few minutes), so grossly disproportionate myokine release would be necessary to generate the observed IL-6 peaks at T8, around 20–25 min following

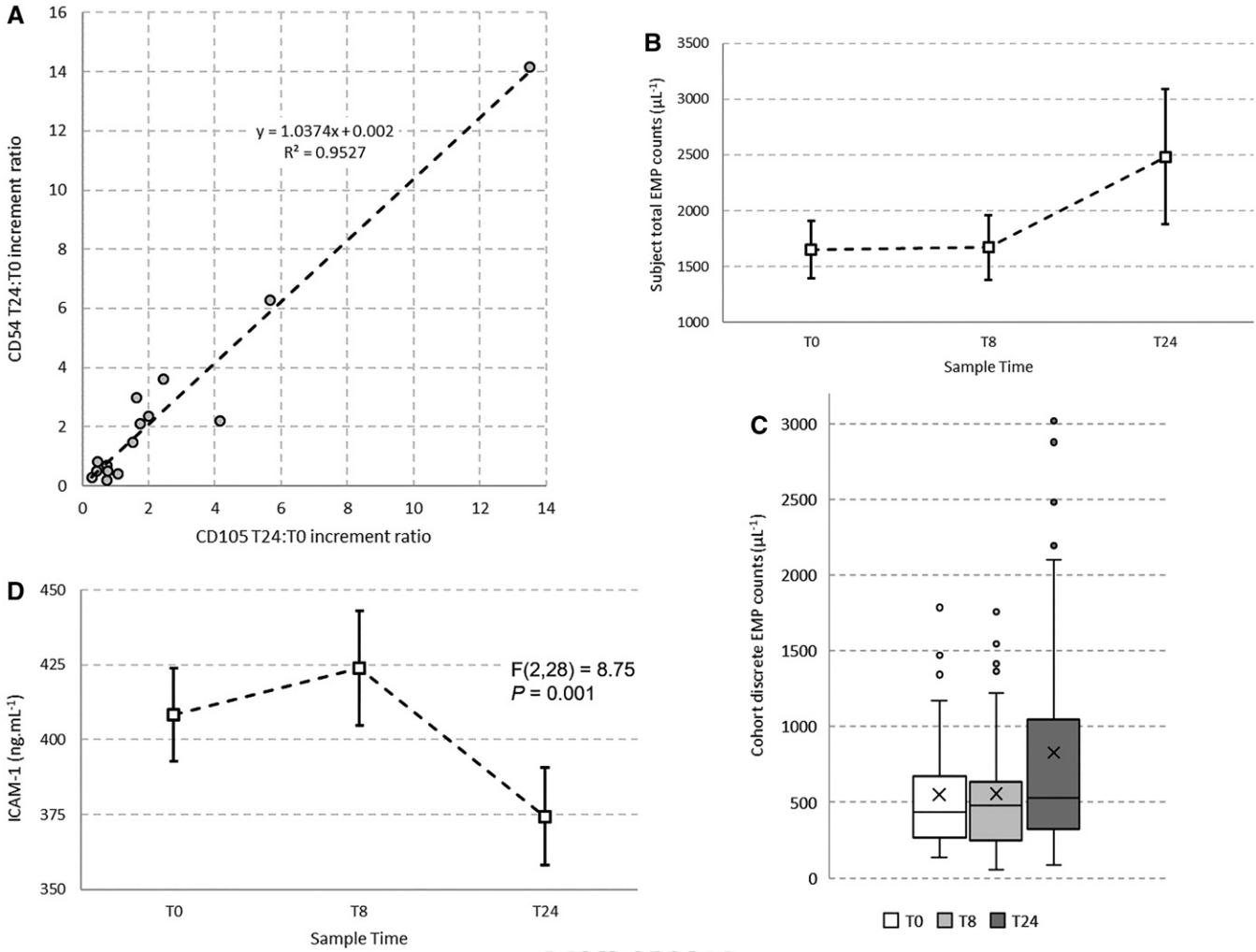

Fig. 4. Endothelial microparticle and adhesion molecule responses. A) Example of within-subjects correlation of all three measured EMP responses, here showing CD54 with respect to CD105; B) mean (± SE) individual subject total EMP counts at each sample time ($N = 14$, excluding one gross outlier with almost 10-fold elevation of EMP counts at T8 and which would misrepresent the T8 data of the cohort as a whole); C) box plot of all EMP counts, by quartiles with outliers shown, representing 42 measures at each sample time, mean represented by X ($N = 14$ subjects, excluding the same outlier as B); and D) soluble (circulating) ICAM-1 ($N = 15$). For abbreviations, see caption under Table I.

the last activity at 25,000 ft (7620 m).[24] Also, myokine responses elevate IL-10 and cortisol, whereas cohort (Fig. 2B) and individual (Fig. 2F) IL-10 responses were unrelated to IL-6 increments, and cortisol levels were unaffected.[22] Finally, IL-6 responses may be associated with severity of VGE loading (Fig. 5C).[6]

Increased C5a and CRP at T24 (Figs. 3A and 3B) provide additional indirect evidence of an inflammatory response that does not resolve fully by the following day. In sum, a proinflammatory IL-6 cytokine peak appears related to severity of decompression stress as measured by VGE bubble load, in turn influencing the magnitude of the acute phase response, as reflected by CRP levels (Fig. 3C).

The modest elevation of PF4 at T8 is attributable to diurnal variation in platelet levels and does not suggest activation.[10] However, increased NGAL (Fig. 3D) and CFIII/TF (Fig. 3F) at T24 provide indirect evidence, respectively, of persistent neutrophil activation and prior vascular endothelial disruption with exposure of subendothelial TF to the circulation. The latter may indicate a tendency toward coagulopathy, although TF expression is upregulated by CRP, C5a, and cytokines, among others. Post-exposure elevation of total EMPs, continuing in over half the subjects at T24 (Fig. 4C), indicate prior endothelial dysfunction and altered vascular responsivity, the latter implied also by elevation of CRP.

The consistent between-subjects mean 10% elevation of GFAP at T24 (Fig. 5E) is disconcerting as this is a sensitive biomarker that strongly implies specific CNS insult.[1] In this context, it is arguably inappropriate to dismiss lightly the elevation of serum Glu at T8 that reverts to baseline by T24 (Fig. 5D), but without quite achieving statistical significance. This change was the basis for including Glu as a target and it may be that a study with greater power would demonstrate a significant effect. Notably, recent reports link inflammatory indices (specifically the SII) with WMH burden in the context of cerebral small vessel disease.[12,19] Future work should explore these and additional brain biomarker responses to decompression stress.

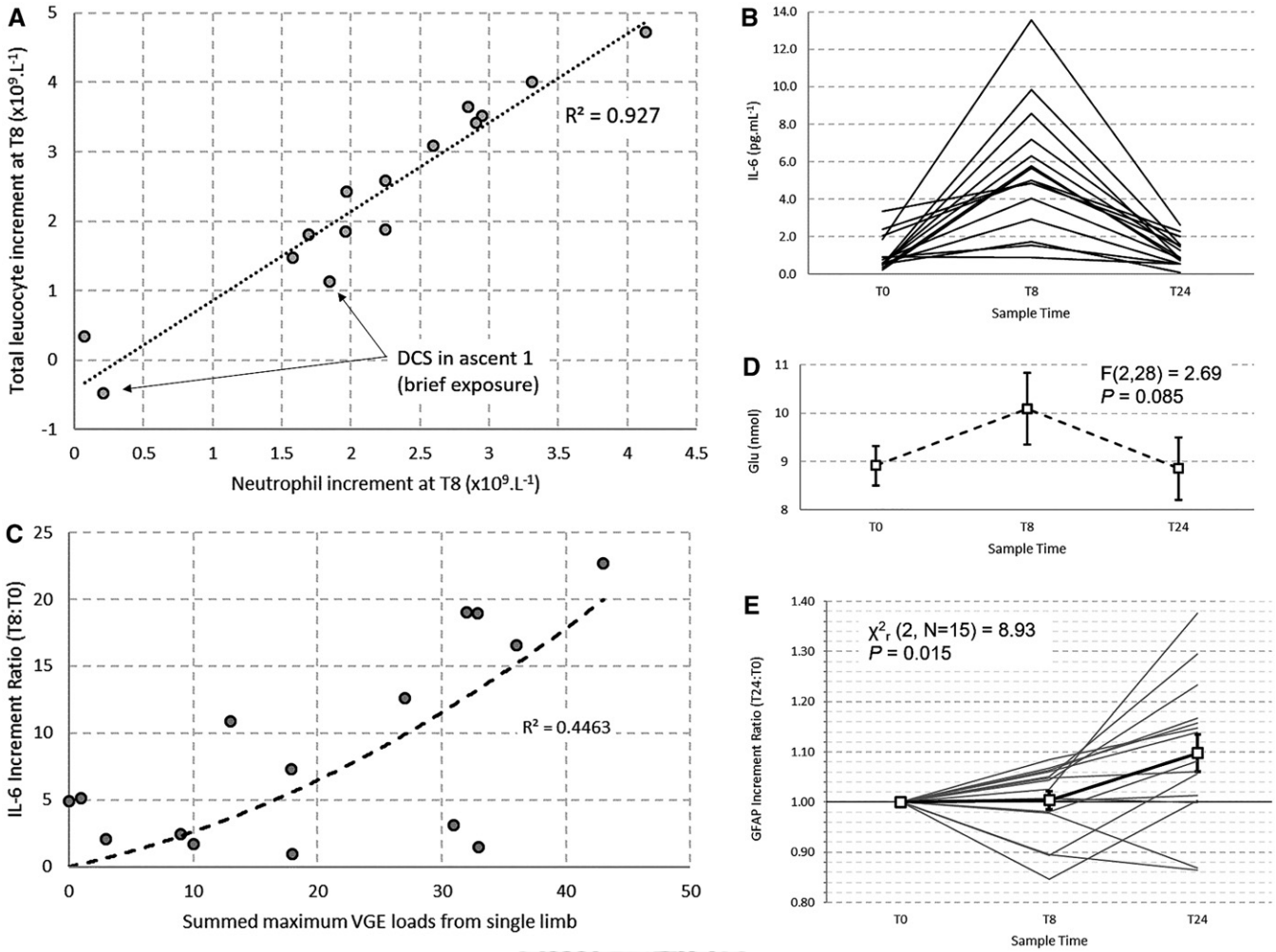

Fig. 5. Detailed blood, cytokine, and neurometabolite responses. A) Relationship between total leukocyte increment and neutrophil increment at T8 relative to baseline (T0); B) individual IL-6 responses ($N = 15$); C) relationship of IL-6 response at T8 to cumulative maximum load of venous gas emboli (VGE) from a single limb, summed over all test epochs;[6] D) cohort Glu data ($N = 15$); and E) individual and cohort GFAP responses relative to baseline at T0 ($N = 15$). For abbreviations, see caption under Table I.

Other results reported here may be summarized briefly as suggestive of endothelial dysfunction and oxidative stress. Overall, the data have implications for recovery time following hypobaric stress that imposes risk of DCS. Although hematological and cytokine responses normalized by T24, numerous markers remained elevated, including C5a, CRP, NGAL, TF, total EMPs, and GFAP, indicating ongoing inflammatory response, endothelial dysfunction, and incomplete recovery. Additional decompression under these conditions might be associated with exaggerated responses, possibly increasing risk of DCS, and warrants further investigation. Evaluation of responses to hypobaric decompression on consecutive days would be illuminating and should encompass additional indicators of neurological insult.

The main limitation of this study is the inability to disambiguate the relative influences of the three stressors that comprise the composite environmental stress, specifically hyperoxia, decompression (microbubble generation), and moderate physical activity at altitude. Six sets of control exposures would be required to isolate fully the individual and pairwise responses

Table IV. Hematological Inflammatory Indices.

INDEX	BASELINE (T0) (MEAN ± SD)	POST-EXPOSURE (T8) (MEAN ± SD)	RECOVERY (T24) (MEAN ± SD)	rmANOVA
NLR	2.13 ± 0.87	3.16 ± 0.75	2.13 ± 0.72	$F(2, 28) = 42.3$, $P < 0.00001$
PLR	152 ± 55	148 ± 35	153 ± 35	$F(1.22, 17.04) = 0.29$, $P = 0.644$
SII	513 ± 247	804 ± 260	511 ± 202	$F(2, 28) = 44.01$, $P < 0.00001$

$N = 15$. The SII is the product of the neutrophil and platelet counts divided by the lymphocyte count. The normality assumption was met for all repeated measures analysis of variance (rmANOVA). Sphericity correction was applied for PLR analysis.

to these three stressors, plus a seventh "zero exposure" control for diurnal influences, far beyond the original scope of the work.[6] Nonetheless, the composite stress is representative of occupational exposure profiles, with direct relevance to military parachutist dispatchers working at high altitude. Additional limitations relate to analytical power and range. Subject numbers, biomarker targets, and sample times were all constrained by available resources. A study with greater power may have identified additional statistically significant effects, particularly for SOD and Glu. Additionally, three exposures were unavoidably inconsistent, curtailed prematurely due to limb bend DCS, and followed by 1 h of hyperoxia at ground level prior to taking T8 venous samples. While no age-related effects were apparent in the biomarker data, two-thirds of our test cohort were over 40 yr of age and all were men, almost exclusively Caucasian. A cohort with a different age–sex distribution and/or ethnic composition might behave quite differently.

In summary, the early pathophysiological response to exertional, hyperoxic, hypobaric decompression stress, in a cohort of predominantly middle-aged men, is proinflammatory, generating an acute phase response whereby mean CRP level remains elevated by 100% above baseline the day after exposure. Further work is required to validate and extend these observations, and to explore the disconcerting possibility of unexplained brain insult suggested by GFAP elevation the day after decompression.

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