

Inflammation on the Frontlines: Linking Salivary and Blood Biomarkers to Cardiometabolic Health and Physical Performance Parameters in Firefighters

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Running Title: Inflammation and cardiometabolic health in firefighters

Objective: Firefighters experience chronic stress that contributes to elevated cardiometabolic disease (CMD) risk. This study examined associations between blood and salivary biomarkers of inflammation, stress, and oxidative stress with measures of body composition, fitness, and cardiometabolic health among firefighters (n = 140). **Methods:** Principal components analysis was used to reduce multicollinearity among body composition, physical performance, and cardiometabolic variables, followed by multivariate regression models predicting adiponectin, C-reactive protein (CRP), and interleukin-6 (IL-6) concentrations. **Results:** Body mass and composition, fitness, and atherogenic dyslipidemia were associated with multiple biomarkers. Salivary IL-6 demonstrated robust associations with body composition, physical performance, and salivary CRP. Blood adiponectin and CRP were strongly associated with atherogenic dyslipidemia, insulin resistance and oxidative stress. **Conclusions:** Salivary inflammatory biomarkers, particularly IL-6, may provide a feasible complement to blood biomarkers for identifying CMD risk among firefighters.

Keywords: firefighting, heart disease, cardiovascular disease, oxidative stress, insulin resistance

Learning Outcomes:

- Blood and salivary pro-inflammatory biomarkers are positively associated with adiposity, cardiometabolic risk, and negatively associated with physical fitness among career, structural firefighters.
- Salivary interleukin-6 demonstrated robust positive associations with metabolic risk, and negative associations with physical fitness.
- Blood markers of oxidative stress were positively associated with markers of insulin resistance and inflammation.

1. Introduction

Firefighting is one of the most physiologically and psychologically demanding occupations in contemporary society, with the National Fire Protection Association (NFPA) reporting that in 2024, approximately 60% of on-duty fatalities are attributed to overexertion and stress as the “causes” and the nature of these deaths being due to a significant cardiac event (i.e., sudden cardiac death) (1). Importantly, the prevalence of these on-duty deaths, both in cause and nature, has remained at approximately 50% over the last four decades, suggesting that cardiovascular disease (CVD) continues to represent a major concern within the fire service (1-7). These indices of cardiac fatalities among firefighters have been attributed to underlying physiological stress and cardiovascular strain (8, 9) that compound over time to increase a firefighter's exposure to oxidative stress, inflammation, and chronic stress, which can ultimately increase their risk of CVD and premature mortality (10-12). Compounding these physiological demands, firefighters are also exposed to work environments and conditions that may encourage sedentary behaviors and unhealthy dietary practices (e.g., frequent snacking), further augmenting CVD risk (13). Recent evidence also suggests that cumulative years of service may accelerate the aging process and increase the risk of poor health and physical performance, thereby compromising occupational readiness (14). Taken together, given that multiple occupation-related factors are associated with an increased risk of CVD, there is an urgent need to identify early screening parameters that can be leveraged to improve firefighter health, performance, and readiness.

Firefighters are often conceptualized as a subgroup of tactical and occupational athletes, given that their duties require performing physically demanding tasks in environmentally challenging conditions (e.g., exposure to extreme heat and smoke). Accordingly, structural firefighters routinely experience emergency responses that can result in sustained heart rates up to

approximately 84-100% of maximal heart rate (HR_{max}), peak blood lactate concentrations of 6 to 13 mmol/L, and oxygen uptake values ($\dot{V}O_{2max}$) ranging between 39 to 45 mL/kg/min or greater (15-18). Despite these occupational demands, multiple research groups (19, 20) have assessed firefighters' cardiorespiratory capacity and demonstrated that career male and female firefighters may be below the ideal $\dot{V}O_{2max}$ level recommended by the NFPA (21, 22) and researchers to meet occupational demands (13). For example, Tinsley and colleagues (20) reported that among 3,867 US firefighters (i.e., 3,651 males and 216 females), median $\dot{V}O_{2max}$ values were 32 mL/kg/min for males and 31 mL/kg/min for females. Importantly, there is also evidence suggesting that firefighters with lower fitness levels exhibit higher concentrations of key oxidative stress and inflammatory biomarkers linked to a greater CVD risk (10-12, 23). For example, McAllister et al. (12) found that firefighters with higher $\dot{V}O_{2max}$ had lower concentrations of advanced oxidation protein products (AOPP) and C-reactive protein (CRP) compared to their less-fit counterparts. Furthermore, McAllister et al. (11) and Gonzalez et al. (10) have demonstrated that blood CRP is associated with adiposity and one's aerobic capacity. However, despite this growing body of work, there remains a paucity of data examining associations between salivary inflammatory biomarkers and indices of health and physical performance among firefighters.

Several recent reports have not only demonstrated that oxidative stress and inflammatory biomarkers are associated with health and physical performance parameters among structural firefighters, but have also provided justification for the use of pragmatic proxies in place of clinical-grade laboratory-based testing procedures (10-12, 23). For instance, the findings of McAllister et al. (11) support the assessment and monitoring of simple anthropometric measures, such as waist circumference, given their association with oxidative stress and inflammatory biomarkers. This point is particularly salient, as a substantial proportion of the US fire service

(≈75%) is composed of volunteer firefighters, and some departments in predominantly rural areas may not have access to specific clinical-grade assessment tools. In this context, salivary biosampling may offer a non-invasive way to assess biomarkers, and monitoring can also be a feasible method for evaluating physiological, oxidative, and inflammatory stress. Given the established links between these types of biomarkers and CVD risk, it is reasonable that if there are associations with the salivary biomarkers, similarly to how past work has demonstrated associations with blood physiological stress, oxidative stress, and inflammation biomarkers, fire departments could leverage this non-invasive method of assessment and monitoring with partnerships between them and research teams.

Chronic or repeated exposure to physiological stress, oxidative stress, and inflammation is common among individuals in high-stress occupations, including firefighters, and is associated with elevated CVD risk. Although past work has demonstrated that blood-based biomarkers, such as CRP, are related to key predictors of CVD risk, such as adiposity and cardiorespiratory fitness, to the authors' knowledge, no data demonstrate similar associations with salivary biomarkers of physiological, oxidative, and inflammatory stress among firefighters. Therefore, the purpose of this study was to examine the associations between blood and salivary biomarkers of inflammation, physiological stress, and oxidative stress with various health and physical performance parameters, aiming to (1) expand current knowledge by exploring relationships among body composition, physical performance, and biomarkers of cardiometabolic health, inflammation, and oxidative stress, measured in both blood and saliva among firefighters; and (2) evaluate whether salivary inflammatory cytokines could serve as a non-invasive and practical tool for the early detection of cardiometabolic risk in firefighters.

2. Methods

2.1. Participants and Experimental Design

Data from 140 career structural firefighters employed by a South-Central U.S. fire department were analyzed. Participants gave written informed consent before completing questionnaires on health, wellness, and lifestyle history. These tools helped identify signs, symptoms, and diagnoses related to cardiometabolic and blood disorders. Eligibility included being 18–65 years old at the time of consent, currently working as a firefighter with the department, and providing consent. Data was collected in spring and summer 2024 during the department's annual clinical and fitness tests. Testing took place over two days: first, during shift change, fasting blood samples were taken; second, participants completed clinical exercise physiology assessments (see Figure 1). All procedures complied with the Declaration of Helsinki and were approved by the Institutional Review Board of Texas A&M University (IRB2023-0957D).

<<<<Insert Figure 1 about here>>>>

2.2. Testing Overview and Procedures

The testing procedures described have been previously detailed (14). Annual clinical testing over two days included bio-sample collection and lab assessments. Firefighters arrived at a central fire station at 0720 hours on the first day to provide a fasting blood sample (≥ 12 hours). Participants then visited an on-campus testing facility for clinical evaluations, including assessments of resting hemodynamics, body composition, anthropometrics, muscle strength, endurance, and flexibility, as well as a maximal cardiopulmonary exercise test (CPXT), during which $\dot{V}O_2\text{max}$ was estimated using the Foster equation based on time-to-exhaustion (24).

2.2.1. Demographics, Anthropometrics, and Body Composition Assessments

Participants initially filled out a series of health, wellness, and lifestyle questionnaires. Height and body mass were measured with a physician's scale and a height rod. Then, a laboratory research technician measured waist and hip circumferences according to World Health Organization procedures (25, 26). Finally, participants had a dual-energy X-ray absorptiometry (DXA) scan (Hologic Horizon A, Marlborough, MA) to assess fat mass, fat-free mass, body fat percentage, visceral adipose tissue, and the distribution of android and gynoid fat.

2.2.2. Physical Performance Parameters

Participants performed a sit-and-reach test following the standard procedures established by the American College of Sports Medicine (ACSM) (27), followed by a 1-minute sit-up and push-up test (27). Then, aerobic capacity was assessed via a cardiopulmonary exercise test (CPXT) performed on a treadmill equipped with a 12-lead electrocardiogram system (TM65 treadmill; Quinton Q Stress System; Cardiac Science Corporation, Bothell, WA), using the Bruce protocol.

2.2.3. Blood Collection and Analysis Procedures

Participants arrived following a minimum 12-hour overnight fast prior to biospecimen collection. Using standard venipuncture procedures, fasting blood samples were obtained from the antecubital vein by certified phlebotomists into two 8.5 mL serum separator tubes (SSTs) and one 4 mL K2-ethylenediaminetetraacetic acid (EDTA) tube (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Following collection, tubes were gently inverted to ensure adequate mixing and allowed to clot at room temperature for approximately 30 minutes before transport. Samples were subsequently transported within 1 hour of collection to a Biosafety Level 2 (BSL-2) laboratory and a certified commercial laboratory (Clinical Pathology Laboratories Inc., Austin, TX, USA) for processing and analysis.

Serum and plasma samples were analyzed for total cholesterol (TC), triglycerides (TAG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting plasma glucose, fasting plasma insulin, apolipoprotein B (ApoB), and hemoglobin A1c (HbA1c). Insulin resistance was estimated using the Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), calculated as: $\text{HOMA-IR} = (\text{fasting glucose [mmol/L]} \times \text{fasting insulin [pmol/L]}) / 405$. Stored serum aliquots were later thawed and analyzed in duplicate for adiponectin, interleukin-6 (IL-6), C-reactive protein (CRP), and advanced oxidation protein products (AOPP). Commercially available assay kits were utilized for all biomarker analyses (adiponectin: R&D Systems, Minneapolis, MN, USA; kit #SRP300; IL-6: R&D Systems, Minneapolis, MN, USA; kit #SS600C; CRP: R&D Systems, Minneapolis, MN, USA; kit #QK1707; AOPP: Cell Biolabs, San Diego, CA, USA). Absorbance values were quantified using a colorimetric microplate reader (BioTek, Winooski, VT, USA). All assays demonstrated intra-assay coefficients of variation below 10%.

2.2.4. Saliva Collection and Analysis Procedures

Saliva biosamples were collected using a passive drool technique (Salimetrics, PA) as previously described (28) and analyzed in duplicate for IL-6 and CRP concentrations. Prior to sample collection, participants were instructed to rinse their mouths with water approximately 10 minutes beforehand. Following collection, samples were transported to a laboratory and stored at -80°C . All saliva samples were shipped via FedEx to a laboratory for analysis (Salimetrics, SalivaLab, Carlsbad, CA). Commercially available assay kits were used for determining concentrations of IL-6 and CRP (Salimetrics, Carlsbad, CA). Due to the low IL-6 concentration in the saliva samples, a modified 1:4 dilution was used in addition to the incorporation of an additional low standard at

0.78pg/mL. All assays demonstrated intra-assay and inter-assay coefficients of variation below 10%.

2.3. Statistical Analysis

All statistical analyses were conducted using R statistical software (v4.5.1; R Core Team 2025). To address potential multicollinearity among predictors, dimension reduction was performed using principal components analysis (PCA). For body composition, physical performance, and cardiometabolic health variables, PCA was conducted using varimax rotation, and principal components (PCs) with eigenvalues greater than 1.0 were retained for subsequent analyses.

The retained PCs were used as predictors in multivariate linear regression models predicting concentrations of key biomarkers, including adiponectin, CRP, and IL-6. Separate models were estimated for each dependent variable and each set of PCs, and results are presented by the variable group from which the PCs originated. Each model was estimated both with and without age adjustment. To account for multiple comparisons across dependent variables, p-values were corrected using the false-discovery rate (FDR) procedure (29). Family-wise error rate was defined based on the dependent variables and age adjustment. Finally, associations among key biomarkers were examined using zero-order Pearson correlation coefficients.

3. Results

3.1. Descriptive results

Descriptive statistics for the 140 career firefighters included in the analysis are presented in **Table I**. As shown, some saliva and blood-chemistry analytes exceeded or fell below the detection limits of their respective assays, resulting in differential missingness across variables.

<<<Table I about here>>>

3.2. Dimension Reduction

Body composition variables included android fat (%), gynoid fat (%), fat mass (kg and %), lean mass (kg), waist circumference (cm), waist-to-hip ratio, and body weight (kg). Principal components analysis (PCA) of these variables yielded three factors with eigenvalues >1.0 , collectively accounting for approximately 94% of the variance (**Table II**). The first PC (62.1% variance explained) was interpreted as capturing aspects of “body mass & composition”, with all variables contributing positively, though the highest loadings (>0.4) were for android/gynoid fat distribution and fat mass (kg and %). The second PC (18.4% variance explained) was interpreted as capturing “lean mass and fat distribution,” characterized by a strong positive loading for lean mass (0.6) and moderate loadings for some variables related to fat distribution (e.g., gynoid fat = -0.42). The third PC (13.4% variance explained) was interpreted as capturing “body roundness,” with higher values corresponding to lower waist-hip ratio (loading = -0.74) and higher lean mass (loading = 0.44).

Physical performance variables included time to exhaustion (TTE), push-ups, sit-ups, and sit-and-reach performance. PCA of these variables produced a single PC with an eigenvalue >1.0 representing a “general fitness” dimension and account for 55.5% of the variance. Cardiometabolic health variables included HDL, LDL, and total cholesterol, triglycerides, ApoB, glucose, insulin, and HbA1c. PCA of these variables yielded three PCs with eigenvalues >1.0 , collectively accounting for 80.8% of the variance. The first PC (44.9% variance explained) was interpreted as capturing “atherogenic dyslipidemia,” with higher values conveying greater cardiometabolic health risk (e.g., loading for HDL and LDL cholesterol were -0.19 and 0.48, respectively). The second PC (22.7% variance explained) was interpreted as capturing “glycemic control”, with high scores being associated with lower concentrations of glucose and insulin (loadings were -0.44 and

-0.47, respectively). Although a third component exceeded the eigenvalue threshold, it was not retained due to its marginal eigenvalue (1.03) and limited interpretability, suggesting that this component primarily captured residual rather than substantively meaningful variation in the data.

<<<Table II about here>>>

3.3. *Multivariate results*

3.3.1. Body composition

Associations between body composition principal components and key biomarkers are presented in Table III. Body mass & composition (PC1) was associated with all key dependent variables. With the exception of adiponectin, which demonstrated a negative association ($\beta = -0.24$, 95% CI = [-0.42, -0.06], $p = 0.011$), all observed associations were positive. The association with adiponectin and CRP (blood) ($\beta = 0.41$, 95% CI = [0.23, 0.6], $p < 0.001$) was the only association to remain statistically significant after multiple-testing (FDR) adjustment. Adjustment for age did not materially attenuate these associations; rather, effect estimates generally increased in size, suggesting the presence of age-related patterns among many of the biomarkers under investigation. After accounting for the linear relationship between age and the other predictors, adiponectin and IL-6 (saliva) were also found to be associated with body mass & composition at a level that was robust to FDR adjustment. Lean mass/fat distribution (PC2) was associated with IL-6 (blood) ($\beta = 0.19$, 95% CI = [0.01, 0.36], $p = 0.045$); however, this association was not robust to FDR adjustment, before or after including age in the model. Body roundness (PC3) was associated with IL-6 (saliva) ($\beta = 0.32$, 95% CI = [0.07, 0.56], $p = 0.014$); however, as with lean mass/fat distribution, this association was not robust to FDR adjustment before or after accounting for age.

<<<Table III about here>>>

3.3.2. Physical Fitness Metrics

General fitness (PC1; **Table IV**) was negatively associated with blood CRP ($\beta = -0.3$, 95% CI = [-0.49, -0.1], $p = 0.004$). When age was included as a covariate, additional associations emerged with adiponectin ($\beta = 0.26$, 95% CI = [0.03, 0.48], $p = 0.026$) and salivary IL-6 ($\beta = -0.5$, 95% CI = [-0.83, -0.17], $p = 0.004$), though only the association with IL-6 was robust to FDR adjustment.

<<<Table IV about here>>>

3.3.3. Cardiometabolic Health Risk

Atherogenic dyslipidemia (PC1) is associated with lower concentrations of adiponectin ($\beta = -0.31$, 95% CI = [-0.48, -0.14], $p < 0.001$) and elevated concentrations of blood CRP ($\beta = 0.33$, 95% CI = [0.14, 0.52], $p = 0.001$). Both associations were robust to the inclusion of age in the model and multiple testing (with/without age included). Glycemic control (PC2) was negatively associated with adiponectin ($\beta = -0.19$, 95% CI = [-0.36, -0.01], $p = 0.038$) and positively associated with IL-6 in blood ($\beta = 0.21$, 95% CI = [0.03, 0.39], $p = 0.024$). Neither association remained statistically significant following FDR adjustment. As seen in earlier models, including age in the model actually strengthened the observed associations. In the present case, the age-adjusted associations with glycemic control increased to a level that was robust to FDR adjustment, suggesting age-related variation in the independent variables in the model. These data are shown in **Table V**.

<<<Table V about here>>>

3.7. Correlational analysis of key biomarkers

Zero-order (pairwise) Pearson correlations were estimated among all key biomarkers and between AOPP and HOMA-IR. **Table VI** presents these correlations in the lower triangle and corresponding pairwise sample sizes in the upper triangle, reflecting differential missingness across biomarkers. Adiponectin demonstrated negative associations with nearly all other

biomarkers, though only three reached statistical significance: CRP in blood ($r = -0.22$), AOPP ($r = -0.28$), and HOMA-IR ($r = -0.29$). In contrast, all other biomarkers produced a positive manifold, with the two strongest associations emerging between CRP and IL-6 ($r = 0.43$; both measured in saliva) and AOPP and HOMA-IR ($r = 0.48$).

<<<Table VI about here>>>

4. Discussion

The primary findings of this study indicate that both blood and salivary inflammatory biomarkers are associated with markers of cardiometabolic health among career firefighters. Specifically, salivary IL-6 demonstrated significant associations with multiple variables, and these associations remained statistically significant following adjustment for age and correction for multiple comparisons, particularly in relation to body mass & composition, general fitness, and salivary CRP concentrations. In addition, salivary CRP and IL-6 were statistically significantly associated with blood-based markers of oxidative stress and insulin resistance, including AOPP and HOMA-IR. Consistent with prior work, blood adiponectin and CRP were significantly related with atherogenic dyslipidemia, body mass & composition, and adiponectin was also inversely associated with biomarkers of cardiometabolic disease risk: CRP, AOPP, and HOMA-IR. Altogether, these findings suggest that both blood and salivary biomarkers provide complementary information regarding cardiometabolic disease risk among career firefighters.

Previous research has demonstrated that salivary inflammatory biomarkers, such as CRP, are indicative of cardiometabolic disease risk, including chronic heart disease (30) and acute myocardial infarction (31). While saliva CRP concentrations have been shown to demonstrate statistically significant associations with blood CRP concentrations (32), this finding has been inconsistent (33) since salivary biomarkers of inflammation, such as CRP and IL-6, can be largely

impacted by oral inflammation (34). Regardless, the present findings are consistent with prior work demonstrating that body composition is a significant predictor of salivary CRP (35) and salivary IL-6 concentrations (36), supporting their potential utility as non-invasive markers for cardiometabolic disease (37). With respect to general fitness, past work has also shown that salivary CRP is significantly related to low aerobic fitness (35); however, the present study involved career firefighters and the findings from Naidoo et al. (2012), included a cross-sectional analysis of South African overweight children (35). Thus, the varying populations may explain the conflicting results.

With respect to blood-based markers of inflammation, blood CRP, IL-6, and adiponectin have been consistently linked to markers of oxidative stress (38, 39) and are recognized predictors of cardiometabolic health status (40-42). Regarding career firefighters, much of the existing literature has focused on acute elevations in inflammatory biomarkers in response to structural and wildland firefighting activities (43, 44). In this context, the present data are unique in demonstrating a significant relationship between blood adiponectin and CRP with atherogenic dyslipidemia among career firefighters. Moreover, blood adiponectin was also related to body mass & composition and biomarkers of cardiometabolic disease risk: CRP, AOPP, and HOMA-IR.

Adiponectin is an adipokine that is released by adipose tissue in an *inverse* relationship with body fat percentage (45). This physiological relationship was supported by the present data, as adiponectin was inversely related to markers of cardiometabolic disease risk. Adiponectin concentrations have also been shown to increase in response to dietary interventions such as caloric restriction, Mediterranean diets, DASH, and plant-based diets (46). Given its anti-inflammatory properties and favorable effects on insulin sensitivity, lipid metabolism, and glucose homeostasis

(47), the observed associations underscore the potential relevance of adiponectin as a meaningful biomarker of cardiometabolic health among career firefighters.

It is important to note that the present study is not without limitations. First, our sample is a non-probabilistic convenience sample, which limits the ability to generalize findings beyond the study population. Accordingly, our results should not be interpreted as representative of firefighters more broadly. Second, omitted-variable bias may have influenced the observed associations. Although age was statistically controlled and sex was addressed by design, biomarker levels and health more generally are known to differ across key axes of variation not measured in the current study (e.g., race/ethnicity, socioeconomic status). Third, the cross-sectional nature of the data precludes inference regarding temporal order or causality among the observed associations. Given these limitations, we advise readers that all results reported herein are specific to the present sample and should be interpreted as descriptive, pending future studies employing longitudinal designs and broader sampling strategies to support statistical inference.

5. Conclusion

The findings of the present study demonstrate that both blood and salivary biomarkers are associated with markers of cardiometabolic disease among career firefighters, as evidenced by their relationships with body composition, physical fitness, and traditional cardiovascular disease risk factors, including lipids and glucose indices. Although prior work has reported similar associations, the present findings contribute to the existing literature by extending evidence for the relevance of inflammatory and oxidative stress biomarkers in this occupational group for potentially scientific and practical relevance. Importantly, these results suggest that both blood- and saliva-based biomarkers may have utility not only in acute monitoring contexts but also in the

design and evaluation of chronic health and performance interventions aimed at reducing cardiometabolic disease risk among firefighters.

ACCEPTED

References

1. Campbell RP, J. Fatal Firefighter Injuries in the United States. National Fire Protection Association; 2025 10 Jun 2025.
2. Fahy RF. US fire service fatalities in structure fires, 1977–2009. National Fire Protection Research Foundation, Quincy. 2010.
3. Fahy RF, LeBlanc PR, Molis JL. Firefighter fatalities in the United States-2011: NFPA Emmitsburg, MD; 2012.
4. Fahy RF, LeBlanc PR, Molis JL. Firefighter fatalities in the United States-2005: National Fire Protection Association. Fire Analysis and Research Division; 2006.
5. Fahy RF, Petrillo JT. Firefighter fatalities in the US in 2020. National Fire Protection Association. 2021.
6. Kales SN, Smith DL. Firefighting and the Heart. *Circulation*. 2017;135(14):1296–9.
7. Soteriades ES, Smith DL, Tsismenakis AJ, Baur DM, Kales SN. Cardiovascular disease in US firefighters: a systematic review. *Cardiol Rev*. 2011;19(4):202–15.
8. Huang CJ, Webb HE, Evans RK, McCleod KA, Tangsilsat SE, Kamimori GH, et al. Psychological stress during exercise: immunoendocrine and oxidative responses. *Exp Biol Med* (Maywood). 2010;235(12):1498–504.
9. Huang CJ, Webb HE, Zourdos MC, Acevedo EO. Cardiovascular reactivity, stress, and physical activity. *Front Physiol*. 2013;4:314.
10. Gonzalez DE, Coles ME, Tanksley PT, M MH, Martin SE, McAllister MJ. Relationships Between Physiological Stress Biomarkers and Cardiovascular Disease Risk Factors Among Career Firefighters. *J Occup Environ Med*. 2025;67(7):535–41.

11. McAllister MJ, Gonzalez DE, Leonard M, Martaindale MH, Bloomer RJ, Pence J, et al. Risk Factors for Cardiometabolic Disease in Professional Firefighters. *J Occup Environ Med.* 2023;65(2):119–24.
12. McAllister MJ, Gonzalez DE, Leonard M, Martaindale MH, Bloomer RJ, Pence J, et al. Firefighters With Higher Cardiorespiratory Fitness Demonstrate Lower Markers of Cardiovascular Disease Risk. *J Occup Environ Med.* 2022;64(12):1036–40.
13. Gonzalez DE, McAllister MJ, Waldman HS, Ferrando AA, Joyce J, Barringer ND, et al. International society of sports nutrition position stand: tactical athlete nutrition. *J Int Soc Sports Nutr.* 2022;19(1):267–315.
14. Chun J, Conner MJ, Mota JA, Newman B, Dawes JJ, Martin SE, et al. Impact of Age and Years in the Fire Service on Firefighter Health and Physical Performance Outcomes. *Healthcare.* 2025;13(16):1946.
15. Sothmann MS, Saupe K, Jasenof D, Blaney J. Heart rate response of firefighters to actual emergencies: Implications for cardiorespiratory fitness. *Journal of Occupational and Environmental Medicine.* 1992;34(8):797–800.
16. McAllister MJ, Gonzalez AE, Waldman HS. Time restricted feeding reduces inflammation and cortisol response to a firegrounds test in professional firefighters. *Journal of occupational and environmental medicine.* 2021;63(5):441–7.
17. Lemon P, Hermiston RT. The human energy cost of fire fighting. *Journal of Occupational Medicine.* 1977:558–62.
18. Gledhill N, Jamnik V. Characterization of the physical demands of firefighting. *Canadian journal of sport sciences= Journal canadien des sciences du sport.* 1992;17(3):207–13.

19. Miller P, Conner M, Burnham R, Wohlgemuth K, Mota JA. One job, one standard: how the revised NFPA standard 1580 alters firefighter fit for duty status across age. *Appl Physiol Nutr Metab.* 2025;50:1–9.
20. Tinsley GM, Mota JA, Conner MJ, Jesko A, Wohlgemuth KJ, Rodriguez C. Reference Values for Body Composition, Graded Exercise Testing, Hemodynamics, and Pulmonary Function in Male and Female Firefighters. *J Occup Environ Med.* 2025;67(9):723–31.
21. Association NFP. NFPA 1582: Standard for Emergency Responder Occupational Health and Wellne. Quincy, MA; 2022.
22. Association NFP. NFPA 1580: Standard for Emergency Responder Occupational Health and Wellness. Quincy, MA; 2025.
23. Guerra BC, Martin SE, Colvin LC, Dawes JJ, McAllister MJ, Gonzalez DE. Firefighters Versus Law Enforcement Officers: A Comparison of Cardiovascular Disease Risk. *Int J Exerc Sci.* 2025;18(6):659–71.
24. Foster C, Jackson AS, Pollock ML, Taylor MM, Hare J, Sennett SM, et al. Generalized equations for predicting functional capacity from treadmill performance. *American heart journal.* 1984;107(6):1229–34.
25. Organization WH. WHO guidelines on drawing blood: best practices in phlebotomy: World Health Organization; 2010.
26. Organization WH. Waist circumference and waist-hip ratio: report of a WHO expert consultation, Geneva, 8-11 December 2008. 2011.
27. Liguori G, Medicine ACoS. ACSM's guidelines for exercise testing and prescription: Lippincott Williams & Wilkins; 2020.

28. Gonzalez DE, Dillard CC, Johnson SE, Martin SE, McAllister MJ. Physiological Stress Responses to a Live-Fire Training Evolution in Career Structural Firefighters. *J Occup Environ Med.* 2024;66(6):475–80.
29. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal statistical society: series B (Methodological).* 1995;57(1):289–300.
30. Al-Rawi NH, Shahid AM. Oxidative stress, antioxidants, and lipid profile in the serum and saliva of individuals with coronary heart disease: is there a link with periodontal health? *Minerva Stomatol.* 2017;66(5):212–25.
31. Miller CS, Foley JD, 3rd, Floriano PN, Christodoulides N, Ebersole JL, Campbell CL, et al. Utility of salivary biomarkers for demonstrating acute myocardial infarction. *J Dent Res.* 2014;93(7 Suppl):72s–9s.
32. Foley JD, 3rd, Sneed JD, Steinhubl SR, Kolasa JR, Ebersole JL, Lin Y, et al. Salivary biomarkers associated with myocardial necrosis: results from an alcohol septal ablation model. *Oral Surg Oral Med Oral Pathol Oral Radiol.* 2012;114(5):616–23.
33. Pay JB, Shaw AM. Towards salivary C-reactive protein as a viable biomarker of systemic inflammation. *Clin Biochem.* 2019;68:1–8.
34. Bahbah EI, Noehammer C, Pulverer W, Jung M, Weinhaeusel A. Salivary biomarkers in cardiovascular disease: An insight into the current evidence. *Febs j.* 2021;288(22):6392–405.
35. Naidoo T, Konkol K, Biccard B, Dudose K, McKune AJ. Elevated salivary C-reactive protein predicted by low cardio-respiratory fitness and being overweight in African children. *Cardiovasc J Afr.* 2012;23(9):501–6.

36. Ostrowska L, Smarkusz-Zarzecka J, Gornowicz A, Lenzion K, Zyśk B, Pogodziński D. Analysis of Selected Salivary Adipokines and Cytokines in Patients with Obesity-A Pilot Study. *Int J Mol Sci.* 2023;24(4).
37. Selvaraju V, Babu JR, Geetha T. Association of salivary C-reactive protein with the obesity measures and markers in children. *Diabetes Metab Syndr Obes.* 2019;12:1239–47.
38. Ramos-González EJ, Bitzer-Quintero OK, Ortiz G, Hernández-Cruz JJ, Ramírez-Jirano LJ. Relationship between inflammation and oxidative stress and its effect on multiple sclerosis. *Neurologia (Engl Ed).* 2024;39(3):292–301.
39. Mahmoud AM, Wilkinson, F.L., Sandhu, M.A., Lightfoot, A.P. The Interplay of Oxidative Stress and Inflammation: Mechanistic Insights and Therapeutic Potential of Antioxidants. *Oxidative Medicine and Cellular Longevity.* 2021;2021(1).
40. Mainous A, Jo, A., Sharma, P. Systemic Inflammation Among Adults with Undiagnosed Cardiometabolic Conditions: Is This A Neglected Focus for Prevention? *The Annals of Family Medicine.* 2023;21.
41. Killeen SL, Byrne DF, Geraghty AA, Kilbane MT, Twomey PJ, McKenna MJ, et al. Higher Inflammation Is Associated with Cardiometabolic Phenotype and Biochemical Health in Women with Obesity. *Ann Nutr Metab.* 2022;78(3):177–82.
42. Tate AR, Rao GHR. Inflammation: Is It a Healer, Confounder, or a Promoter of Cardiometabolic Risks? *Biomolecules.* 2024;14(8).
43. Allsopp GL, Main LC. Acute and chronic inflammation in firefighters: A narrative review. *Mutat Res Rev Mutat Res.* 2025;796:108569.

44. Esteves F, Madureira J, Barros B, Alves S, Pires J, Martins S, et al. Impact of occupational exposure to wildfire events on systemic inflammatory biomarkers in Portuguese wildland firefighters. *Environ Res.* 2025;277:121608.
45. Kern PA, Di Gregorio GB, Lu T, Rassouli N, Ranganathan G. Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor- α expression. *Diabetes.* 2003;52(7):1779–85.
46. Janiszewska J, Ostrowska J, Szostak-Węgierek D. The Influence of Nutrition on Adiponectin-A Narrative Review. *Nutrients.* 2021;13(5).
47. Lei X, Qiu S, Yang G, Wu Q. Adiponectin and metabolic cardiovascular diseases: Therapeutic opportunities and challenges. *Genes Dis.* 2023;10(4):1525–36.

Figure Legends

Figure 1: overview of experimental procedures.

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Table 1. Descriptive statistics of analytic variables.

Characteristic	N	Mean	SD	Range
Demographics				
Age (years)	140	34	9	19, 58
Body Composition				
Waist cir. (cm)	140	95	13	56, 183
Waist-hip ratio	140	0.91	0.10	0.56, 1.78
Weight (kg)	140	94	16	34, 159
Fat mass (%)	140	23.9	5.2	13.8, 39.2
Fat mass (kg)	140	24	9	9, 65
Lean mass (kg)	140	70	10	28, 100
Android fat (%)	140	28	7	13, 48
Gynoid fat (%)	140	25.3	4.9	15.3, 40.1
Physical Fitness				
Time to exhaustion	140	11.09	1.69	6.52, 15.62
Sit ups	139	42	9	14, 70
Push ups	136	51	18	0, 95
Sit and reach	140	15.7	3.4	6.5, 27.0
Cardiometabolic				
HDL cholesterol	139	51	11	28, 83
LDL cholesterol	139	117	32	57, 193
Total cholesterol	139	188	37	125, 273
Triglycerides (blood)	139	102	61	35, 310
ApoB	138	95	24	56, 155
Glucose (blood)	139	90	10	72, 121

Characteristic	N	Mean	SD	Range
Insulin	139	1.80	1.23	0.37, 6.83
HbA1c (%)	138	5.41	0.24	4.84, 6.00
HOMA-IR	139	0.40	0.29	0.07, 1.47
Adiponectin	124	5,987	2,994	1,372, 14,275
Inflammation & Oxidative Stress Markers				
AOPPs	124	100	46	58, 333
CRP (blood)	106	1,278	1,224	120, 5,165
CRP (saliva)	113	737	1,115	33, 4,557
IL-6 (blood)	124	1.63	1.28	0.43, 7.13
IL-6 (saliva)	65	15	20	0, 81

Table 2. Principal components and Pearson's *r* of body composition, fitness, and cardiometabolic variables.

	Principal Components			Pearson's <i>r</i>						
	PC1	PC2	PC3	1	2	3	4	5	6	7
Body Composition										
1 Android fat (%)	0.40	-0.24	-0.07							
2 Gynoid fat (%)	0.36	-0.42	0.03	0.79						
3 Fat mass (kg)*	0.43	-0.10	0.12	0.88	0.80					
4 Fat mass (%)	0.41	-0.30	-0.01	0.94	0.90	0.92				
5 Lean mass (kg)	0.21	0.60	0.44	0.19	0.03	0.39	0.16			
6 Waist cir. (cm)*	0.38	0.32	-0.29	0.66	0.46	0.73	0.63	0.52		
7 Waist-hip ratio*	0.20	0.37	-0.74	0.31	0.12	0.26	0.25	0.17	0.75	
8 Weight (kg)	0.36	0.27	0.39	0.58	0.48	0.77	0.61	0.73	0.68	0.20
<i>% Variance Explained</i>	62.1	18.4	13.4							
Fitness										
1 Time to exhaustion	0.56									
2 Push ups	0.57			0.61						
3 Sit ups	0.50			0.48	0.50					
4 Sit and reach	0.34			0.30	0.29	0.17				
<i>% Variance Explained</i>	55.5									
Cardiometabolic Health										
1 HDL cholesterol	-0.19	0.46	0.52							
2 LDL cholesterol	0.48	0.26	0.10	-0.11						
3 Total cholesterol	0.45	0.35	0.17	0.10	0.96					

	Principal Components			Pearson's <i>r</i>						
	PC1	PC2	PC3	1	2	3	4	5	6	7
4 Triglycerides (blood)*	0.42	-0.13	-0.36	-0.50	0.56	0.55				
5 ApoB	0.49	0.19	0.04	-0.20	0.94	0.91	0.61			
6 Glucose (blood)	0.15	-0.44	0.48	-0.19	0.09	0.03	0.13	0.13		
7 Insulin*	0.27	-0.47	-0.09	-0.45	0.20	0.14	0.49	0.30	0.45	
8 HbA1c (%)	0.10	-0.36	0.58	-0.17	0.06	0.02	0.08	0.08	0.33	0.23
<i>% Variance Explained</i>	44.9	22.7	13.3							

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Table 3. Body composition principal components predicting adiponectin, CRP, and IL-6 in firefighters.

	Adiponectin			CRP (blood)			CRP (saliva)			IL-6 (blood)			IL-6 (saliva)		
	Beta ¹	SE	P	Beta ¹	SE	P	Beta ¹	SE	P	Beta ¹	SE	P	Beta ¹	SE	P
Unadjusted															
General adiposity (PC1)	-0.24*	0.092	0.011	0.41**	0.093	<0.001	0.23*	0.096	0.021 ²	0.20*	0.091	0.027 ²	0.29*	0.122	0.019 ²
Lean mass/fat dist. (PC2)	-0.11	0.092	0.2	-0.06	0.094	0.5	-0.07	0.096	0.5	0.19*	0.091	0.045 ²	0.09	0.124	0.5
Body roundness (PC3)	0.04	0.092	0.6	0.10	0.094	0.3	0.06	0.096	0.6	0.06	0.091	0.5	0.32*	0.125	0.014 ²
R ²	0.070			0.189			0.062			0.079			0.163		
Age Adjusted															
General adiposity (PC1)	-0.29*	0.101	0.005	0.38**	0.103	<0.001	0.27*	0.105	0.013 ²	0.22*	0.101	0.029 ²	0.38*	0.135	0.007
Lean mass/fat dist. (PC2)	-0.11	0.092	0.3	-0.05	0.095	0.6	-0.08	0.097	0.4	0.18*	0.092	0.049 ²	0.05	0.126	0.7
Body roundness (PC3)	0.10	0.105	0.3	0.13	0.107	0.2	0.01	0.110	>0.9	0.04	0.105	0.7	0.23	0.138	0.11
R ²	0.082			0.193			0.070			0.080			0.194		
No. Obs.	115			97			106			115			61		

¹p<0.05; **p<0.01; ***p<0.001

²p>.05 after multiple-testing adjustment.

Adiponectin			CRP (blood)			CRP (saliva)			IL-6 (blood)			IL-6 (saliva)		
Beta ¹	SE	P	Beta ¹	SE	P	Beta ¹	SE	P	Beta ¹	SE	P	Beta ¹	SE	P

Abbreviations: CI = Confidence Interval, SE = Standard Error

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Table 4. Fitness principal component predicting adiponectin, CRP, and IL-6 in firefighters.

	Adiponectin			CRP (blood)			CRP (saliva)			IL-6 (blood)			IL-6 (saliva)		
	Beta ¹	SE	P	Beta ¹	SE	P	Beta ¹	SE	P	Beta ¹	SE	P	Beta ¹	SE	P
Unadjusted															
General fitness (PC1)	0.17	0.094	0.068	-0.30*	0.100	0.004	-0.11	0.099	0.3	-0.08	0.095	0.4	-0.18	0.130	0.2
R ²	0.030			0.087			0.013			0.006			0.033		
Age Adjusted															
General fitness (PC1)	0.26*	0.114	0.026 ²	-0.26*	0.123	0.034 ²	-0.19	0.120	0.12	-0.12	0.116	0.3	-0.50*	0.169	0.004
R ²	0.045			0.089			0.024			0.010			0.150		
No. Obs.	111			94			103			111			59		
¹ p<0.05; **p<0.01; ***p<0.001															
² p>.05 after multiple-testing adjustment.															
Abbreviations: CI = Confidence Interval, SE = Standard Error															

Table 5. Cardiometabolic principal components predicting adiponectin, CRP, and IL-6 in firefighters.

	Adiponectin			CRP (blood)			CRP (saliva)			IL-6 (blood)			IL-6 (saliva)		
	Beta ¹	SE	P	Beta ¹	SE	P	Beta ₁	SE	P	Beta ₁	SE	P	Beta ₁	SE	P
Unadjusted															
Atherogenic dyslipidemia (PC1)	-0.31**	0.089	<0.001	0.33*	0.097	0.001	0.05	0.098	0.066	0.06	0.093	0.5	0.10	0.130	0.4
Glycemic control (PC2)	-0.19*	0.089	0.038 ²	0.15	0.097	0.13	0.14	0.098	0.02	0.21*	0.093	0.024 ²	0.08	0.130	0.5
R ²	0.129			0.128			0.020			0.048			0.017		
Age Adjusted															
Atherogenic dyslipidemia (PC1)	-0.47**	0.105	<0.001	0.34*	0.120	0.006	0.08	0.119	0.05	0.12	0.112	0.3	0.29	0.154	0.062
Glycemic control (PC2)	-0.22*	0.087	0.014	0.15	0.098	0.13	0.14	0.099	0.02	0.22*	0.094	0.019 ²	0.14	0.129	0.3
R ²	0.182			0.128			0.022			0.056			0.091		
No. Obs.	113			95			105			113			61		
¹ p<0.05; **p<0.01; ***p<0.001															
² p>.05 after multiple-testing adjustment.															
Abbreviations: CI = Confidence Interval, SE = Standard Error															

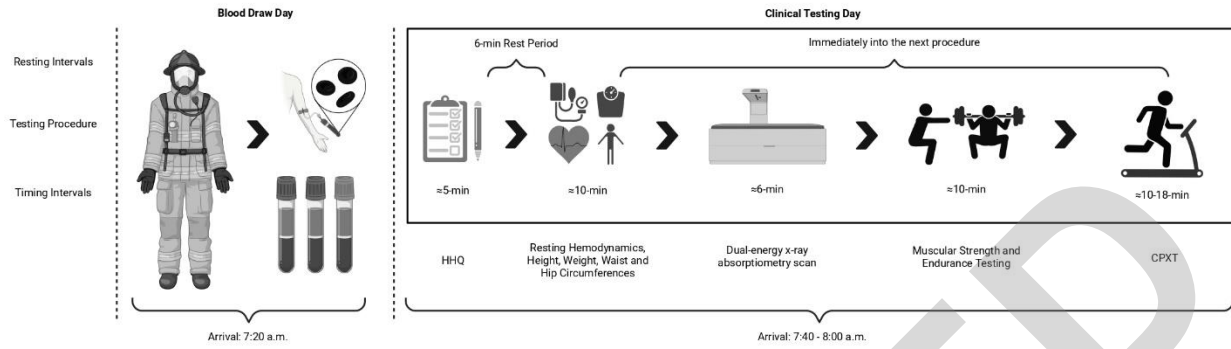
Table 6. Pairwise correlations and sample sizes between focal dependent variables.

	Adiponectin	CRP (blood)	CRP (saliva)	IL-6 (blood)	IL-6 (saliva)	AOPP	HOMA-IR
Adiponectin		106	113	124	65	124	115
CRP (blood) ¹	-0.22*		98	106	54	106	97
CRP (saliva) ¹	-0.17	0.30**		113	64	113	106
IL-6 (blood) ¹	-0.10	0.30**	0.22*		65	124	115
IL-6 (saliva) ¹	0.05	0.29*	0.43***	0.29*		65	61
AOPP ¹	-0.28**	0.28**	0.02	0.12	0.13		115
HOMA-IR ¹	-0.29**	0.30**	0.17	0.23*	0.09	0.48***	

Abbreviations: CRP=C-reactive protein; IL-6=interleukin-6; AOPP=advanced oxidative protein products; HOMA-IR=homeostatic model assessment of insulin resistance.

¹Log-transformed.

Figure 1



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Are stress biomarkers associated with cardiometabolic health and physical performance in firefighters?

Firefighters experience elevated cardiometabolic disease risk due to chronic physiological and occupational stress



Blood and saliva biomarkers were used to capture inflammatory, oxidative, and physiological stress alongside fitness and health markers

Biomarkers were meaningfully associated with cardiometabolic health and physical performance outcomes in firefighters



Inflammation on the Frontlines: Linking Salivary and Blood Biomarkers to Cardiometabolic Health and Physical Parameters in Firefighters
Matthew J. McAllister, PhD; Peter T. Tanksley, PhD; M. Hunter Martaindale, PhD; Ashlyn DeArman, BS; Milena Samora, PhD; Steven E. Martin, PhD; Drew E. Gonzalez, PhD



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