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Analysing the Impact of Resistance Training on Salivary Inflammatory Biomarkers Interleukin 6 and C - reactive protein

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ABSTRACT

Background: Chronic low-grade inflammation is a recognized precursor to cardiovascular and metabolic diseases. While systemic inflammatory markers are typically measured via serum, non-invasive salivary diagnostics offer a viable clinical alternative. This study investigated the effect of a structured 12-week resistance training (RT) program on salivary C-reactive protein (CRP) and Interleukin-6 (IL-6) levels.

Methods: A quasi-experimental pre- and post-test design was employed involving 50 healthy adults (18 males, 32 females; aged 34–45 years). Participants underwent 24 supervised resistance training sessions using progressive latex-free Theraband resistance over 12 weeks, adhering to American Heart Association (AHA) guidelines. Salivary samples were collected using the passive drool method following standardized operating procedures.

Results: Analysis of 44 paired samples revealed a highly significant reduction in salivary biomarkers post-intervention. Salivary CRP decreased from 2.99 ± 0.48 mg/L to 2.51 ± 0.48 mg/L ($t = 9.61$, $p < 0.001$), and salivary IL-6 decreased from 24.85 ± 7.30 pg/mL to 23.45 ± 7.29 pg/mL ($t = 7.90$, $p < 0.001$). /mL to 23.45 ± 7.29 pg /mL ($t = 7.90$, $p < 0.001$). Parallel serum analysis demonstrated similar directional trends, supporting the validity of saliva as a surrogate for systemic inflammatory monitoring.

Conclusion: Structured resistance training significantly attenuates salivary markers of inflammation. These findings suggest that resistance training can modulate the inflammatory milieu, potentially reducing cardiovascular and metabolic risk. The alignment between serum and salivary trends reinforces the utility of saliva as a practical, non-invasive tool for systemic biomarker assessment.

Keywords: Resistance training, salivary biomarkers, Interleukin-6, C-reactive protein, cardiovascular disease, inflammation.

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INTRODUCTION

Chronic systemic inflammation is increasingly recognized as a primary driver of various metabolic syndromes and cardiovascular diseases (CVD), characterized by the persistent elevation of pro-inflammatory mediators. Resistance training (RT)—comprising progressive weight and strength training—is traditionally prescribed to enhance muscular hypertrophy, physical strength, and metabolic efficiency by exerting force against external resistance [1]. However, emerging evidence highlights the potent immunomodulatory capacity of Resistance training, particularly its role in attenuating systemic inflammatory cascades and modulating the production of key biomarkers such as Interleukin-6 and C-reactive protein [2].

Interleukin-6 is a pleiotropic cytokine with dual functionality; while it acts as a pro-inflammatory agent in acute pathological states, it is released as a "myokine" from skeletal muscle fibers during contraction, where it exerts anti-inflammatory effects by stimulating the production of Interleukin -10 and inhibiting tumor necrosis factor-alpha [3, 4]. In the systemic circulation, Interleukin-6 serves as the primary stimulus for the hepatic synthesis of C - reactive protein, an acute-phase protein and a gold-standard clinical indicator of systemic inflammation and an independent predictor of cardiovascular risk [5]. Consequently, the ability of resistance training to downregulate the Interleukin-6 /C-reactive protein axis represents a critical mechanism for mitigating inflammation-related health risks.

Despite the established efficacy of resistance training in reducing serum concentrations of these markers, traditional monitoring relies on venous blood sampling (phlebotomy), which is invasive, costly, and impractical for high-frequency longitudinal monitoring in both clinical and athletic populations. Salivary diagnostics have emerged as a promising, non-invasive alternative for tracking physiological shifts. Recent studies suggest that salivary concentrations of Interleukin-6 and C-reactive protein correlate significantly with their serum counterparts, reflecting the systemic inflammatory status through passive diffusion and active transport across the salivary glands [6, 7].

However, there remains a paucity of data regarding the sensitivity of salivary Interleukin-6 and C -reactive protein in response to structured strength training protocols. Clarifying the relationship between resistance training and these salivary markers is essential for validating non-invasive monitoring and for the development of personalized exercise prescriptions aimed at systemic inflammation management. Therefore, the present study aims to evaluate the effect of a 12-week resistance training program on salivary Interleukin-6 and C- reactive protein levels in healthy middle-aged adults.

The study objective is to evaluate the impact of a structured 12-week resistance training program on the levels of salivary Interleukin-6 and C-reactive protein in healthy middle-aged adults.

METHODS

A quasi-experimental pre- and post-test design was employed to evaluate the effect of resistance training on systemic inflammatory markers. The study protocol was reviewed and approved by the Institutional Ethics Committee (Approval No: MAHER/IEC/PhD/116/APRIL25). The trial was prospectively registered with the Clinical Trials Registry- India (CTRI: CTRI/2026/02/103151). In accordance with the Declaration of Helsinki, all participants provided written informed consent prior to enrolment. The study recruited 50 apparently healthy adults (18 males, 32 females) aged 34 to 45 years from the Meenakshi Academy of Higher Education and Research. Inclusion criteria were based on general health parameters, ensuring a baseline of health suitable for resistance training. Individuals with acute infections, chronic inflammatory diseases, recent involvement in structured exercise programs, or local oral pathology were excluded to prevent confounding inflammatory responses. Sample size was determined using G-Power software. Based on a moderate effect size (Cohen's d = 0.5), an alpha level of 0.05, and 80% statistical power, a minimum of 30–40 participants for the cohort was required to ensure statistical validity.

Saliva Collection and Processing

Salivary sampling adhered to Sal metrics standard operating procedures (SOPs) to ensure reliability and minimize contamination. To control for circadian variation, samples were collected between 7:00 AM and 9:00 AM. Participants followed a strict pre-sampling protocol: no food supplements, beverages (except water), oral hygiene products, or strenuous physical activity for at least 60 minutes prior to collection. The passive drool method was utilized to collect unstimulated saliva. Samples were immediately processed via centrifugation to remove debris and mucins and subsequently stored at -20°C until biochemical analysis.

Resistance Training Intervention

A 12-week structured Resistance training program was implemented in accordance with American Heart Association (AHA) guidelines to improve cardiometabolic and muscular strength [8]. The regimen consisted of two 30-minute supervised sessions per week (totaling 24 sessions), including a 5-minute dynamic warm-up (side-stepping, jogging, arm circles, lunges, and squats) and a 5-minute static cool-down (stretching of the buttocks, hamstrings, inner thighs, calves, and quadriceps). To ensure safety and prevent allergic reactions, latex-free Therabands were used (Figure 1). Progression was managed via a standardized overload protocol: - Weeks 1–2: Red Therabands (3.7 lbs. resistance) to establish baseline form. (Figure 2) - Weeks 3–12: Black Therabands (7 lbs. resistance) to induce progressive overload (Figure 2). The program targeted all major muscle groups with 9 specific exercises (Chest press, Push-ups, Shoulder press, Shoulder raise, Seated row, Triceps extension, Biceps curl, Squat, and Abdominal crunch), performed for 2 sets of

10 repetitions each. The standardized execution of these movements is illustrated in Figure 3 (standing upper extremity exercises) and Figure 4 (seated upper extremity exercises), demonstrating the precise posture and resistance application used during the intervention.

Figure 1: Packaging and certification of the latex-free Therabands, confirming the manufacturer's specifications and product authenticity.

Figure 2: Latex-free Therabands utilized for the intervention: Red (3 lbs. resistance) and Black (7 lbs. resistance) bands used for progressive overload.

Figure 3: In -person standing upper extremity resistance training using elastic Therabands.

Figure 4: In- person seated upper extremity resistance training using elastic bands

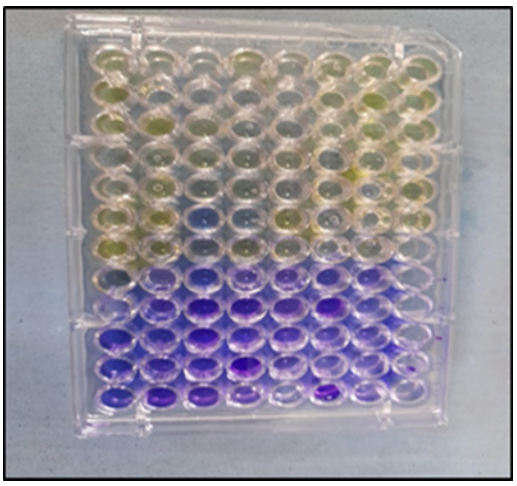
Figure 5: ELISA kit sourced from ELK Biotechnology for both quantification of C - reactive protein and Interleukin-6.

Biochemical Analysis

Concentrations of salivary C-reactive protein (CRP) and Interleukin-6 (IL-6) were quantified using high-sensitivity ELISA kits from ELK Biotechnology (Figure 5) following the manufacturer's instructions. After the final incubation and color development, the optical density of each well was measured using an automated microplate reader (Figure 6). These absorbance values were then used to calculate the exact concentrations of the biomarkers by comparing them against a standard curve generated for each assay. The resulting colorimetric intensity of the samples is illustrated in the representative ELISA microplate (Figure 7).

Figure 6: High-sensitivity microplate reader used to measure the optical density (OD) of the ELISA samples for calculating biomarker concentrations (ELISA Reader).

Figure 7: Representative ELISA microplate with wells utilized for the quantification of salivary Interleukin-6 (IL-6) and C-reactive protein (CRP) concentrations.



Statistical Analysis

Data were analyzed using SPSS version 26. Descriptive statistics (Mean ±SD) were employed for participant demographics. To evaluate the effect of the intervention, paired-sample t-tests were used to compare pre- and post-intervention levels of salivary C-reactive protein and Interleukin-6. A p-value of < 0.05 was considered statistically significant.

RESULTS

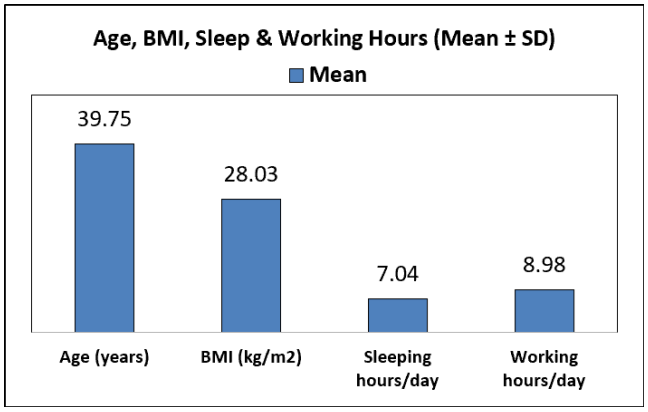
The baseline demographic and occupational characteristics of the study cohort (n=44) are summarised in the table: 1 and also represented in graph.1 and graph. 2, respectively. The sample consisted of healthy middle-aged adults with a diverse range of occupational backgrounds, ensuring that the observed effects were attributable to the intervention rather than pre-existing occupational physical activity. 50 samples were collected; 6 samples were used for standardization, and 44 yielded valid paired results for pre- and post-intervention analysis. The 12-week resistance training intervention led to a statistically significant reduction in both the inflammatory biomarkers analyzed. As shown in Table 2, there was a clear and significant decrease in the salivary C-Reactive Protein levels following the training period, falling from a baseline of 2.99 ±0.48mg/L to 2.51±0.48mg/L (t=9.61, p<0.001). A similar significant downward trend was observed for the salivary Interleukin-6, which decreased from 24.85 ± 7.30 pg. /mL at baseline to 23.45 ± 7.30 pg /mL at baseline to 23.45±7.29pg./mL in the post-intervention (t=7.90, p<0.001). These results indicate that the resistance training intervention effectively lowered systemic inflammatory markers as measured through salivary analysis. Further, to validate the use of salivary markers as a non-invasive diagnostic tool, comparative analysis between serum and salivary concentrations was performed. Representative data for selected participants (Table 3) demonstrate that while absolute concentrations remained significantly higher in the serum than in the saliva, a strong, consistent directional correlation was maintained. For instance, participants exhibiting the highest serum Interleukin-6 levels also showed the highest corresponding salivary concentrations. This consistency reinforces the validity of salivary biomarkers as a reliable proxy for monitoring systemic inflammatory changes.

Table 1: Baseline participants' demographic and occupational data for N=44

Component	Intervention group (n=44)
Gender female n (%)	40(90.9%)
Age (years)	39.75± 3.37
BMI (kg/m^2)	28.03 ±4.10
Sleeping hours per day	7.04 ±0.89
Working hours per day	8.98±1.65
Type of occupation n (%)	
Health industry based	5(11.4%)
Administration based	9(20.5%)
Finance based	12(27.3%)
Teaching based	18(40.9%)

Note: Values are expressed as Mean ± Standard Deviation (SD) or frequency (percentage). BMI: Body Mass Index.

Graph 1: Demographic characteristics of the study population (n = 44)



Graph 2: Distribution of baseline occupational categories among participants (n = 44)

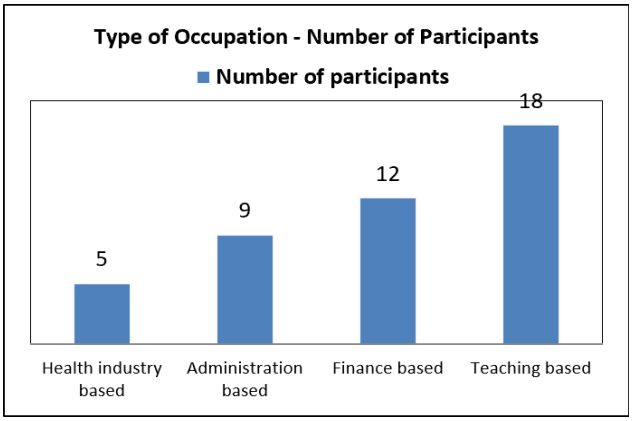
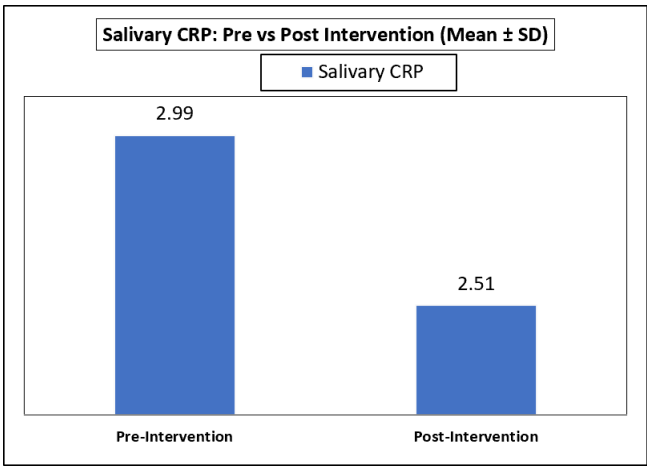


Table 2: Salivary levels of C - reactive protein (mg/L) and Interleukin-6 (pg. /ml) pre- and post-intervention of a 12-week resistance training exercise program. Values are expressed as mean ± SD

Salivary bio-marker	Pre-Inter-vention	Post-Inter-vention	t- statistics	P-value
Salivary CRP	2.99±0.48	2.51± 0.48	9.61	0.001***
Salivary IL-6	24.85 ±7.30	23.45 ±7.29	7.90	0.001***

Note: n=44; indicates standard deviation. *** Significant at p<0.001.

Graph 3: Represents the salivary C-reactive protein pre- vs. post-intervention (mean ±SD)



Graph 4: Represents the salivary interleukin-6 pre- vs. post-intervention (mean ±SD)

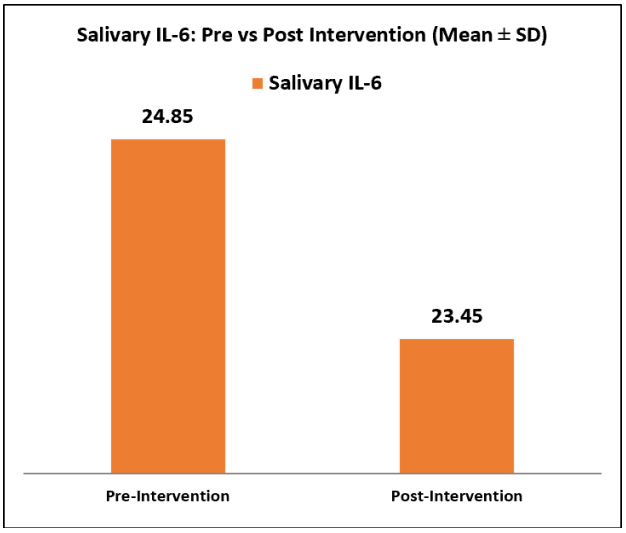


Table 3: Representative comparison of Serum and Salivary inflammatory biomarkers in selected participants

Serum IL-6 (pg./mL)	Saliva IL-6 (pg./mL)	Serum CRP (mg/L)	Saliva CRP (mg/L)
45	18	27	3.3
39	15	14.3	1.9
73	32	20.3	2.6
67	24	17.9	2.2
58	19	28.2	3.4

Note: Data demonstrate a consistent directional correlation between serum and salivary concentrations, validating the use of saliva as a non-invasive proxy for systemic inflammatory status.

DISCUSSION

The present study demonstrates that a structured 12-week resistance training (RT) program significantly attenuates salivary concentrations of C-reactive protein (CRP) and Interleukin-6 (IL-6) in healthy middle-aged adults. The observed approximately 16% decrease in salivary C-reactive protein (CRP), coupled with a significant reduction in Interleukin-6, indicates a systemic transition toward a more favorable anti-inflammatory profile. These findings suggest that progressive resistance exercise may serve as a potent non-pharmacological intervention to mitigate chronic low-grade inflammation, a condition closely linked to the pathogenesis of metabolic syndrome and atherosclerosis [11, 12]. The biochemical foundation of these results lies in the endocrine capacity of skeletal muscle. During mechanical loading and muscle contraction, skeletal muscle fibers act as endocrine organs, secreting a variety of peptides known as myokines [13]. Central to this process is the release of muscle-derived Interleukin-6. Unlike the pro-inflammatory Interleukin-6 produced by macrophages during infection, exercise-induced Interleukin-6 functions as an anti-inflammatory modulator [14]. Specifically, muscle-derived Interleukin-6 stimulates the systemic release of Interleukin-10 (IL-10) and Interleukin-1 receptor antagonist (IL-1ra), while simultaneously inhibiting the production of Tumor Necrosis Factor-alpha (TNF-alpha) [15]. Because hepatic synthesis of C-reactive protein (CRP) is primarily driven by Interleukin-6 in a pro-inflammatory context, the shift toward an anti-inflammatory myokine environment likely suppresses the liver's C-reactive protein production, explaining the significant decrease in C-reactive protein observed in this cohort [16]. Beyond the immediate cytokine shift, these results contribute to the understanding of "inflammation"—the chronic, sterile, low-grade inflammation that characterizes the aging process [17]. By reducing the baseline levels of creatine protein and interleukin-6, resistance training may decelerate the biological aging of the vascular system and skeletal muscle, potentially delaying the onset of sarcopenia and frailty in middle-aged populations [18]. This suggests that the 12-week protocol used in this study does not merely produce a temporary effect but may reprogram the systemic inflammatory set-point. Furthermore, the reduction in systemic inflammation likely has profound implications for glucose metabolism and insulin sensitivity. Elevated C - reactive protein and interleukin-6 are known to interfere with insulin signaling by promoting the phosphorylation of Insulin Receptor Substrate-1 (IRS-1), thereby inducing insulin resistance [19]. The down-regulation of these markers observed in our study suggests a synergistic benefit: resistance training improves muscle glucose uptake via GLUT4 translocation while simultaneously removing the inflammatory "brake" on insulin signaling [20]. This dual mechanism explains why resistance training is a cornerstone in the prevention and management of Type 2 Diabetes Mellitus. This systemic improvement is further enhanced by the muscle-liver-adipose crosstalk. Regular Resistance Training promotes the "browning" of white adipose tissue and reduces the secretion of adipokines from visceral fat, which are primary sources of systemic Interleukin-6 [21]. By reducing the adipose-derived inflammatory load and increasing the muscle-derived anti-inflammatory output, resistance training creates a systemic environment that favors metabolic homeostasis over inflammatory dysfunction [22]. Our findings are consistent with recent meta-analyses which have reported that resistance training effectively lowers systemic Creatine protein levels in middle-aged and older populations [23].

While some studies have reported inconsistent trends in Interleukin-6 reduction [24], this can be attributed to the transient nature of Interleukin-6. As a pleiotropic cytokine, Interleukin-6 exhibits high volatility and acute spikes immediately post-exercise, whereas creative protein represents a more stable, integrated marker of systemic inflammation [25].

Finally, this study provides critical validation for the use of salivary diagnostics. As demonstrated by the representative correlation observed between our salivary and serum data, salivary creatine protein and Interleukin-6 are reliable proxies for systemic inflammatory status. This supports the transition toward point-of-care (POC) monitoring, offering a non-invasive alternative to venous phlebotomy, which is particularly valuable for longitudinal athletic monitoring and clinical screenings. These physiological optimizations complement the findings of Moses et al. (2021) [26], who demonstrated that structured resistance training significantly enhances the power and strength of lower limbs. By improving the physiological capacity of the skeletal muscle, resistance training not only enhances physical performance but also optimizes the muscle's endocrine function, thereby facilitating the release of anti-inflammatory myokines and the subsequent downregulation of systemic C-reactive protein and Interleukin-6.

CONCLUSION

This study demonstrates that a 12-week structured resistance training intervention leads to statistically and clinically meaningful reductions in salivary CRP and IL-6. The strong alignment between salivary and serum trends validates the use of saliva as a non-invasive indicator of systemic inflammation. These results reinforce the role of resistance training as a potent non-pharmacological intervention for modulating low-grade inflammation and improving long-term cardio metabolic health. Future research should employ larger randomized controlled trials (RCTs) with deeper mechanistic measures to further elucidate these inflammatory pathways.

Conflicts of interest

No conflicts of interest.

Declaration of Generative AI

The authors stated that the only purpose of using generative artificial intelligence techniques was to improve the manuscript's clarity and revise its wording. The authors are solely responsible for the work's scientific content, data analysis, results interpretation, and conclusions.

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