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Original

Effects of bodybuilding and protein supplements in saliva, gingival crevicular fluid, and serum

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Abstract: The effects of bodybuilding and protein supplements on periodontal tissues have not yet been evaluated. The present study aimed to examine the periodontal status and interleukin (IL)-1β, apoptosis-associated speck-like protein containing C-terminal caspase-recruitment domain (ASC), and caspase 1 (CASP1) gene expression levels of body builders compared with those of controls. Twenty-five bodybuilders with gingivitis (BB-G) who used protein powder supplements were compared with 25 nonexercising males with (G) and 25 without (H) gingivitis. Saliva, gingival crevicular fluid (GCF), and serum were collected for gene expression analysis. Plaque index (PI), gingival index (GI), probing depth (PD), clinical attachment level (CAL), and bleeding on probing (BOP) were recorded. GI and BOP were higher in group BB-G and G than in group H ($P < 0.01$), but PI, PD, and CAL were similar between groups ($P > 0.05$). In GCF, CASP1, ASC, and IL-1β expression were upregulated in group G compared with groups BB-G and H ($P < 0.01$). In addition, ASC ($P < 0.05$) and IL-1β ($P < 0.01$) were downregulated in group BB-G compared with group H. CASP1, IL-1β ($P < 0.01$), and ASC in the saliva were downregulated in group BB-G compared with groups H and G ($P < 0.05$). CASP1, IL-1β, and ASC may play a role in the pathogenesis of gingivitis. Bodybuilding and supplement usage may decrease gingival inflammation by downregulating CASP1, IL-1β, and ASC.

Keywords: supplements, bodybuilding, ASC, interleukin-1beta, caspase 1, gingivitis.

Introduction

Periodontal disease, which affects 10-15% of the global population, is an inflammatory disease that destroys supporting tissues around the tooth (1). The primary etiological factor in periodontal disease is a polymicrobial biofilm, with interactions between periodontal tissues and the biofilm triggering the host response, which results in destructive inflammation in periodontal tissues (2). Gingivitis and periodontitis are common forms of periodontal disease. Gingivitis affects up to 90% of adults (3), and is associated with inflammation restricted to superficial gingival tissues and supragingival biofilms (4). It may progress to periodontitis, in which case, it is characterized by destruction of periodontal tissues (2).

In periodontal disease, inflammation in response to a biofilm is regulated by the interleukin (IL)-1 family. Within this family, IL-1β is considered an essential cytokine of the inflammatory response and is increased in gingival tissues with periodontal disease (5). The conversion of pro-IL-1β to IL-1β is regulated by inflammasomes (6). The best-defined inflammasomes are nucleotide-binding oligomerization domain-like receptors (NLRs), which are intracellular pattern recognition receptors. NLRs

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Table 1 Demographic profile of study participants

Characteristics	Group BB-G	Group G	Group H
Age (year) (mean ± SD)	23.42 ± 2.47	21.70 ± 1.70	22.26 ± 1.48
BMI (kg/m^2)	24.77 ± 1.98	22.71 ± 1.72	24.73 ± 1.81
Smoking (Y/N)	13/12	15/10	9/16
Tobacco amount per day (mean ± SD)	8.92 ± 9.46	5.00 ± 8.49	8.46 ± 8.33
Brushing times a day (1/2/3)	10/14/1	9/15/1	6/16/3
Flossing regularly (Y/N)	10/15	9/16	19/6
Alcohol consumption (Y/N)	12/13	14/11	10/15
HEI score	80.00 ± 9.60	77.00 ± 13.18	78 ± 11.56

BMI, body mass index; HEI, healthy eating index. Data was expressed as mean ± standard deviation. No statistically significant between-group differences were found in age, BMI, and the amount of tobacco smoked ($P > 0.05$). Participants from all groups brushed at least once daily, although many of the participants did not floss their teeth. The HEI score was 80.00 ± 9.60 in group BB, 76.07 ± 13.18 in group G, and 78 ± 8.76 in group H. There were no between-group differences in the HEI scores ($P > 0.05$) (Table 1).

recognize pathogen- or danger-associated molecular patterns and induce the activation of cysteine proteinase caspase 1 (CASP1), which then activates IL-1β maturation (7). Among inflammasomes, the NLR family, pyrin domain containing 3 (NLRP3) is thought to play a role in periodontal disease (8). The NLRP3 inflammasome consists of an NLRP3 scaffold, CASP1, and adaptor molecule called the apoptotic speck protein containing a C-terminal caspase recruitment domain (ASC), which mediates the interaction of NLRP3 and CASP1 (7). NLRP3 exerts its inflammatory effects through ASC (9), and co-expression of NLRP3, with activation of CASP1 by ASC resulting in IL-1β activation (10). A previous study reported that a supragingival biofilm challenge led to an increase in ASC and CASP1 gene expression but had no effect on NLRP3 (7).

A correlation between periodontal disease and physical activity was reported, suggesting that normal-weight individuals who consumed a high-quality diet and performed regular physical activity were 40% less likely to have periodontitis (11). In addition, a lower level of physical exercise and the consumption of a poor diet were associated with increased odds of periodontal disease (12).

Bodybuilding has become globally popular in the pursuit of an idealized muscular physique by using a regime of weight training and a tailored nutrition program (13,14). To achieve such a physique, bodybuilders undertake a high level of training. Recently, to accelerate muscle gain, the use of dietary supplements has become popular among bodybuilders. However, these supplements are associated with potential side-effects such as cardiovascular, hepatic, and psychiatric disease (15). Moreover, regular users of supplements were reported to have active caries and to be ignorant of the effect of supplements on dental health (14).

Despite the popularity of bodybuilding and the possible relationship of supplements with dental caries, to date, no study has evaluated the periodontal status of bodybuilders. Thus, the present study aimed to examine the periodontal status, gene expression levels and common biochemical markers of bodybuilders compared with those of healthy controls.

Materials and Methods

This study was approved by the Clinical Research Ethics Committee of Sifa University (#256-68) and was undertaken between December 2014 and May 2015. The study groups consisted of bodybuilders with gingivitis who used protein supplements (group BB-G) (n = 25) and nonexercising healthy males with (group G) (n = 25), and without (group H) (n = 25) gingivitis. Participants in groups G and H comprised patients who presented for periodontal treatment or maintenance to the Department of Periodontology, Faculty of Dentistry, Sifa University. Bodybuilders were invited to the same department for this study.

All participants signed an informed consent form before participating in the study. Data regarding demographic characteristics, smoking status, oral hygiene maintenance protocols, dietary habits, brand names, and types of supplements were collected using a questionnaire. A trained interviewer (EB) administered the questionnaire face-to-face to all the participants (Table 1).

Standardization of the study groups

In all the groups, patients with a history of antibiotic usage or periodontal therapy within the previous six months, any systemic disease, or who were undergoing orthodontic therapy were excluded. Participants aged 18-28 years with a normal body mass index (BMI) and >20 teeth were included. The socioeconomic and

educational statuses of all participants were standardized based on the information supplied in the questionnaire. Only individuals with a high socioeconomic status and education level (i.e., college/university or higher) were included.

Bodybuilders who remained in training and had used protein powder supplements regularly for 6 months to 1 year were included. Bodybuilders who used other types of supplements such as steroids, drugs, or substances were excluded. The supplement types and brands were also standardized. Only three of the most commonly used brands were included. Individuals who used nonbranded products were excluded. The amount of supplement that each bodybuilder consumed daily was the same. Before commencement of the study, 57 bodybuilders were invited to the department for periodontal examinations. Of these, 32 were excluded during standardization: seven because of steroid usage, nine because of the use of more than five different supplements, nine because of exercising 3 or fewer times a week, and seven because of the use of antibiotics within the previous 3 months. Finally, 25 bodybuilders who exercised regularly 5 times a week using the same sort of program and who used similar types (and amounts) of protein powder supplements, were included in this study. Nine of the 25 bodybuilders used a combination of whey, glutamine, branched-chain amino acids (BCAAs), and creatine (CREA); eight used a combination of whey, glutamine, and BCAAs; and eight used a combination of whey, BCAAs, and CREA.

Dietary adherence

Meal consumption of all participants was standardized by including participants who consumed a balanced diet. At the first appointment, food records were analyzed using the Healthy Eating Index (HEI) (16). The HEI has 10 dietary components, with each scored from 0 to 10. HEI scores > 80 indicated a good diet, scores of 51-80 indicated a need for improvement, and scores < 51 indicated a poor diet. To assess potential differences between groups, all participants provided a three-day dietary recall log.

Training protocols

All bodybuilders performed the same weekly exercise program. The protocol consisted of three exercises per session. On the 1st and 4th days, they performed chest, biceps, and abdominal exercises. On the 2nd and 5th days, they performed back, triceps, and abdominal exercises. On the 3rd day, they performed shoulder exercises and leg workouts. The 6th and 7th days were rest periods. The training protocols were prepared by their personal trainers.

Periodontal examination

Each participant received a full-mouth periodontal charting by a trained and experienced periodontologist (KA). Periodontal measurements were recorded at six sites per tooth (mesiobuccal, midbuccal, distobuccal, distolingual, midlingual, and mesiolingual). The measurements recorded included plaque index (PI) (17), gingival index (GI) (18), probing depth (PD) (19), bleeding on probing (BOP) (20), and clinical attachment level (CAL) (21). The BOP percentage was calculated by dividing the number of bleeding sites by the total number of sites examined in each participant. In all cases, a periodontal probe (Hu-Friedy, Chicago, IL, USA) was used for the periodontal measurements. In addition, all participants underwent a radiological examination to confirm the presence/absence of periodontal disease.

Gingival crevicular fluid (GCF), saliva, and serum sampling

Serum, saliva, and GCF were obtained immediately before periodontal examination from all participants 4 h after they had consumed the same lunch. The participants were instructed to completely rinse their mouths with water. Subsequently, unstimulated whole saliva was collected from each participant by spitting into a disposable collection tube. After collection, 1 mL of saliva was centrifuged at 2,600 g for 15 min at 4°C (22). Cell-free supernatants containing an RNA stabilization reagent (RNAprotect Saliva Reagent, Qiagen, Valencia, CA, USA) were stored at −80°C until analysis (23).

After sample collection, the participants rinsed their mouths with water. GCF samples were collected at six Ramfjord teeth of each individual. The sampling areas were dried and then isolated using cotton rolls to prevent contamination. Supragingival plaque was removed using a curette. Paper strips (Periopaper, Oraflow, NY, USA) were gently placed into the sulcus and left for 30 s. Any strip contaminated by blood or saliva was discarded. All the strips belonging to each participant were pooled in a tube containing 0.5 mL of an RNA stabilization reagent (RNAlater, Ambion, Austin, TX, USA) and stored at −80°C until laboratory analysis (24).

Venous blood was collected from the antecubital fossa, allowed to stand at room temperature for 30 min and centrifuged at 3,500 rpm for 5 min. Serum biochemistry analyses were performed on the same day. Subsequently, the remaining serum supernatants were transferred into tubes for serum biochemistry analysis and stored at −80°C until analysis. For gene expression analysis,

Table 2 Demographic profile of study participants

Serum marker	Group BB-G	Group G	Group H	Normal range
ALT	15.23 ± 6.42	18.50 ± 20.88	14.83 ± 8.02	10-45 U/L
ALP	61.00 ± 20.08	69.30 ± 16.51	55.73 ± 14.76	30-120 U/L
AST	26.10 ± 14.50*$^{\varepsilon}$	15.73 ± 8.91$^{\varepsilon}$	15.14 ± 2.78*	0-31 U/L
Creatinine	0.92 ± 0.17$^{\gamma}$	0.78 ± 0.08$^{\gamma}$	0.82 ± 0.12	0.7-1.3 mg/dl
CK-MB	29.02 ± 45.35	8.65 ± 5.85	15.04 ± 24.96	0-25 U/L
GGT	15.24 ± 10.45	30.00 ± 32.04	14.60 ± 7.88	0-55 U/L
BUN	36.50 ± 11.37*$^{\varepsilon}$	22.62 ± 4.15$^{\varepsilon}$	24.98 ± 5.35*	7.9-21 mg/dl
Hs-TNT	6.10 ± 4.62	4.52 ± 0.93	5.63 ± 2.89	3-14 ng/L

ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; CK-MB, creatine kinase MB; GGT, gamma-glutamyl transpeptidase; BUN, blood urea nitrogen; hs-TnT, high-sensitivity troponin T. Data are expressed as mean ± standard deviation. Creatinine levels were higher in group BB-G than in group G ($P < 0.05$), and AST and BUN levels were higher in group BB-G than in groups H and group G ($P < 0.01$). *, $^{\varepsilon}$ $P < 0.01$, $^{\gamma}$ $P < 0.05$

different tubes containing an RNA stabilization reagent (RNAlater, Ambion, Austin, TX, USA) were used. Gene expression of the samples was immediately analyzed.

IL-1β, CASP1, and ASC gene expression analysis by real time-polymerase chain reaction (RT-PCR)

CASP1, IL-1β, and ASC gene expression in GCF, saliva, and serum were analyzed using RT-PCR. All gene mRNA expression quantitation was performed with total RNA prepared from samples by extraction kits (RNeasy Plus Micro and Mini kits, Qiagen, Hilden, Germany), according to the manufacturer's instructions. After quantification of the total RNA obtained, 20 μL were denatured and reverse transcribed to cDNA molecules by using cDNA synthesis kits (SuperScript III RT, Invitrogen, CA, USA). Relative quantification of all the genes was performed using RT-PCR kits (Quantitect, Qiagen). Specific primers and probes were used for IL-1β, CASP1, and ASC, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) for control. The primer sequences were as follows: ASC, forward primer (5′-CTTATCGCGAGGGTCACAAA-3′), and reverse primer (5′-AGCTTCCGCATCTTGCTT-3′); CASP1, forward primer (5′-ACACAAGAAGGGAGGAGAGA-3′), and reverse primer (5′-CTTCACCCATGGAACGGATAA-3′); IL-1β, forward primer (5′-CAAAGGCGGCCAGGATATAA-3′), and reverse primer (5′-CTAGGGATTGAGTCCACA TTCAG-3′); GAPDH, forward primer (5′-GGTGTG AACCATGAGAAGTATGA-3′), and reverse primer (5′-GAGTCCTTCCACGATACCAAAG-3′). The expression analyses were determined using real-time systems (ABI PRISM 7000, Applied Biosystems, Foster City, USA). C_T values were obtained for ASC, CASP1, IL-1β, and GAPDH. C_T values and the log of RNA concentrations of the samples showed a reverse linear correlation ($R^2 = 0.99$). To assess changes, the fold change in gene expression of target mRNA was normalized to that of internal control gene mRNA using the$^{-\Delta\Delta CT}$ method.

Serum biochemistry analysis

Serum samples were examined for gamma-glutamyl transpeptidase (GGT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatine kinase MB (CK-MB), high-sensitivity troponin T (hs-TnT), blood urea nitrogen (BUN), and creatinine levels (Table 2). The age- and gender-specific normal reference ranges for these biomarkers are enlisted in Table 2.

Statistical analysis

To estimate the sample size, a power analysis was performed using a program‡‡ (G*Power version 3.1.7, Franz Faul, Christian-Albrechts-University, Kiel, Germany). According to the results, a total sample size of 75 individuals would provide a power of 86% (actual power, 0.8688; critical F, 3.1239; noncentrality parameter, 12.00) to detect a significant difference with an effect size of 0.40 at a significance level of $\alpha = 0.05$. The normal distribution of data was determined using the Kolmogorov-Smirnov test. Comparisons of normally distributed data were analyzed using an ANOVA statistical test. Post-hoc comparisons were performed using the Tukey-Kramer statistical test. A Kruskal-Wallis test was used to determine the differences between skewed variables across independent groups. Statistical analysis was conducted using a statistical package program (SPSS 20.0, IBM, Chicago, IL, USA). The data were expressed as mean ± standard deviation. A P value < 0.05 was considered statistically significant.

Results

There were no differences in the ages of participants in

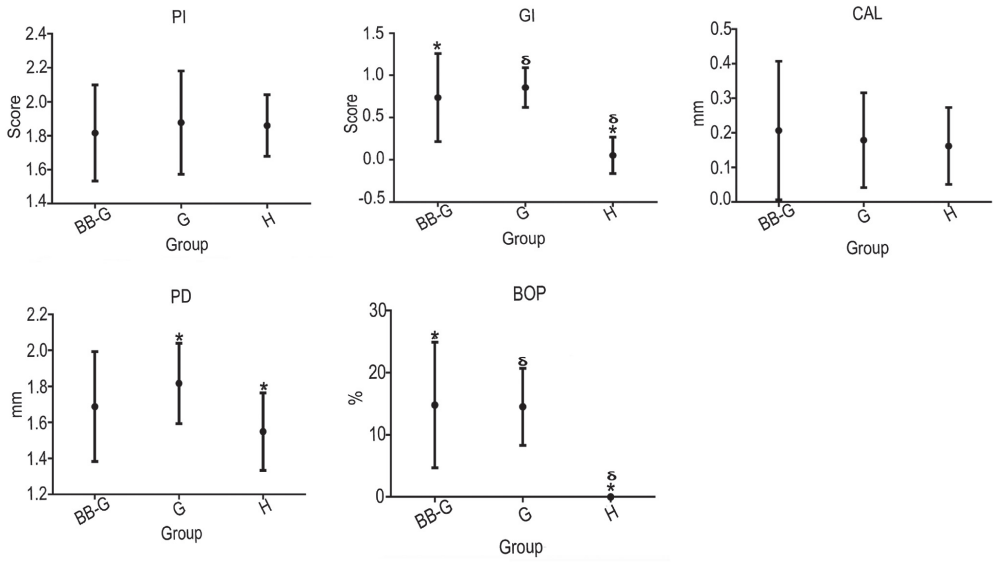


Fig. 1 Clinical periodontal parameters of the study groups. PI, plaque index; GI, gingival index; PD, probing depth; CAL, clinical attachment level; BOP, bleeding on probing. The clinical periodontal examination revealed no statistically significant differences in PI, CAL, and PD levels among the groups ($P > 0.05$). However, BOP and GI values were significantly higher in groups BB-G and G than in group H. $^{*,\delta}$ $P < 0.01$

the BB-G (23.42 ± 2.47 years), G (21.70 ± 1.70 years), and H (22.26 ± 1.48 years) groups ($P > 0.05$). In addition, no significant differences were observed in the BMI values (kg/m^2) of the BB-G (24.77 ± 19.98), G (22.71 ± 1.72), and H (24.73 ± 1.81) groups ($P > 0.05$). There were 13 smokers in group BB-G, 15 in group G, and nine in group H. There were no between-group differences in the amount of tobacco consumed per day ($P > 0.05$). Participants who regularly consumed alcohol were as follows: 13 in group BB-G, 14 in group G, and 10 in group H. All participants brushed at least once daily. As shown in Table 1, HEI scores were 80.00 ± 9.60, 77.00 ± 13.18, and 78 ± 11.56 in groups BB-G, G, and H, respectively, with no significant differences between the groups ($P > 0.05$).

Periodontal examination

Clinical periodontal examinations revealed no differences in PI, PD, and CAL levels among the groups ($P > 0.05$). However, GI and BOP values in groups BB-G and G were higher than those in group H ($P < 0.01$), and no significant differences were detected in GI and BOP values in groups G and BB-G ($P > 0.05$) (Fig. 1).

Serum biochemistry analysis results

Serum biomarkers were examined in serum samples obtained from all groups. No differences were observed in the ALT, ALP, CK-MB, GGT, and hs-TNT levels among all groups ($P > 0.05$). The creatinine levels in group BB-G were higher than those in group G ($P < 0.05$). However, the mean creatinine levels in group BB-G were within the normal range. Moreover, BUN and AST levels were higher in group BB-G than in groups G and H ($P < 0.01$). Furthermore, the mean levels of BUN but not AST were higher than normal in group BB-G.

IL-1β, CASP1, and ASC gene expression in saliva, GCF, and serum

In GCF, CASP1, IL-1β, and ASC gene expressions were significantly upregulated in group G compared with groups BB-G and H ($P < 0.01$). In addition, CASP1 ($P < 0.01$), IL-1β ($P < 0.01$), and ASC ($P < 0.05$) were significantly downregulated in group BB-G compared with group H (Fig. 2). In saliva, no significant differences were observed in the gene expression of ASC, IL-1β, and ASC in groups G and H ($P > 0.05$). However, as shown in Fig. 3, IL-1β, CASP1, and ASC (compared with G; $P < 0.05$) were significantly downregulated in group BB-G compared with group H ($P < 0.01$). IL-1β, ASC, and CASP1 were detectable in the serum of only a few participants. Thus, no differences were detected among the groups.

Discussion

Periodontal diseases are reported to be the most common chronic inflammatory diseases among men (2). Gingivitis, a common form of periodontal disease, is associated with inflammation of gingival tissues; clinically evident as redness, swelling, and BOP. Although gingivitis is initiated by dental plaque accumulation,

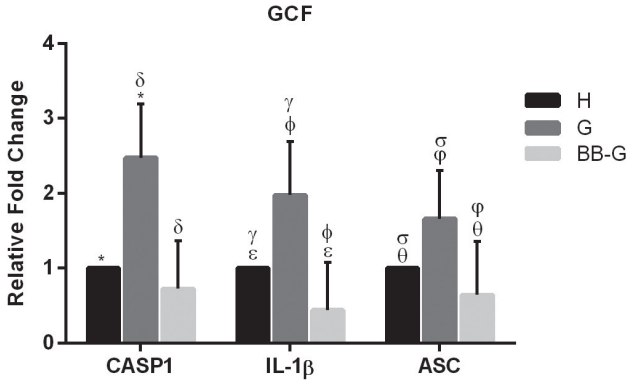


Fig. 2 Relative fold change of GCF levels of CASP1, IL-1β, and ASC. CASP1, IL-1β, and ASC were significantly upregulated in group G compared with groups H and BB-G ($P < 0.01$). CASP1, IL-1β ($P < 0.01$), and ASC ($P < 0.05$) were significantly downregulated in group BB-G compared with group H. $^{*, \varepsilon, \gamma, \delta, \sigma, \varphi, \phi}$ $P < 0.0$, $^{\theta}$ $P < 0.05$

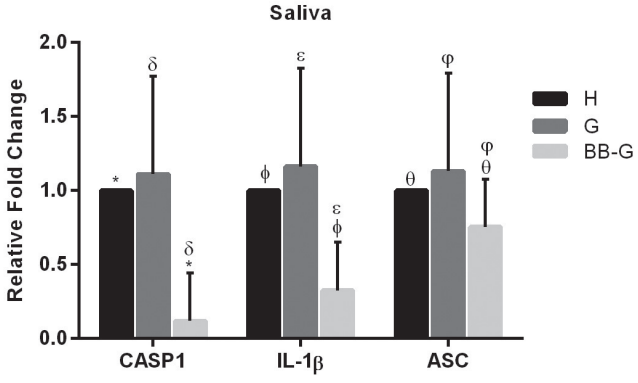


Fig. 3 Relative fold change in CASP1, IL-1β, and ASC levels in the saliva. No mean differences in ASC, IL-1β, and CASP1 were found in group G versus group H ($P > 0.05$). ASC, IL-1β, and CASP1 were significantly downregulated in group BB-G compared with groups G and H. $^{*, \varepsilon, \delta, \varphi, \phi}$ $P < 0.01$, $^{\theta}$ $P < 0.05$

the inflammatory response of the host to bacteria is also important (4). In the present study, PI and PD levels of the three groups were similar ($P > 0.05$). However, BOP and GI values were higher in groups BB-G and G than in group H ($P < 0.01$). GI and BOP values of the bodybuilders and gingivitis groups were similar ($P > 0.05$). The healthy periodontal condition in group H may be the result of relatively better oral hygiene practices such as flossing, and relatively lower number of smokers in group H compared with other groups (25). A previous study of 302 athletes who participated in 25 sport events in the 2012 London Olympic Games reported that 76% had gingivitis and that 55% had evidence of cavities (26). Moreover, a systematic review reported that the oral health of athletes was poor (27). Consistent with the literature, all bodybuilders in the present study were diagnosed with gingivitis. Thus, the control groups in this study were planned to consist of participants with and without gingivitis.

The novel aspect of this study is that it is the first evaluation of gene expression levels of IL-1β, ASC, and CASP1 in the GCF, saliva, and serum of patients, with and without gingivitis compared with those of bodybuilders with gingivitis. CASP1 and ASC and NLRP3 scaffolds are part of the NLRP3 inflammasome (7), which is thought to play a role in periodontal disease (8). The co-expression of NLRP3 and ASC activates CASP1, which then activates IL-1β (10). A previous study reported that a supragingival biofilm challenge led to an increase in the gene expression of CASP1, ASC, and IL-1β but that it had no effect on the expression of NLRP3 (7). In the present study, ASC, CASP1, and IL-1β gene expression were significantly upregulated in the GCF of group G compared with the GCF of group H (Fig. 2). In fact, among all the genes, IL-1β was the only one that was examined and found to be involved in periodontal tissue destruction in GCF in a previous study (28). In addition, the expression levels of ASC and CASP1 were significantly upregulated in the GCF of patients with gingivitis compared with healthy controls in the present study (Fig. 2). In contrast, no significant differences were observed in the expression of any of the evaluated genes in the saliva of both gingivitis and control groups (Fig. 3). Furthermore, no significant differences were observed in the expression of any of the evaluated genes in the serum samples of all groups. This finding may be explained by the limited severity of plaque-induced gingival inflammation (29). A previous study detected IL-1β at extremely low concentrations in the serum of periodontally healthy patients and gingivitis patients (29). In addition, salivary IL-1β levels of gingivitis patients and healthy controls were reported to be similar (30). In accordance with the findings in the literature, in the present study, salivary levels of the evaluated genes were similar in groups G and H (Fig. 3).

A previous study reported that regular exercise induced positive biological changes and reduced inflammatory markers and that IL-1β in saliva had anti-inflammatory effects on periodontal tissues (31). In the bodybuilders, expression levels of all the genes were significantly downregulated in both GCF and saliva compared with those in the gingivitis and healthy control groups (Figs. 2, 3). However, no differences were found in the serum because of the detection of these genes in extremely few patients. We can speculate that bodybuilding and supplement usage result in destruction of periodontal tissues by

downregulating IL-1β, ASC, and CASP1. However, as this study did not include a bodybuilder group without supplement usage, it is not possible to determine the effect of exercise alone versus the effects of exercise and supplements. In the present study, almost all bodybuilders who presented at the department used supplements in the belief that they increased muscle gain. It would likely be extremely difficult to find a cohort of bodybuilders who do not use supplements.

According to previous research, more than half of the US population consumes dietary and herbal supplements, with increasing consumption (32). Male bodybuilders were reported to most commonly consume minerals, vitamins, protein powder/liquids, and amino acids (33), and bodybuilders who participated in competitions were reported to generally consume three types of supplements (34). Although the present study included only noncompetitive bodybuilders who used protein powder supplements alone, the combination of supplements (CREA, BCAAs, glutamine, and whey) was similar to that reported in the literature (34). The amount of these supplements consumed daily by each bodybuilder was equivalent to each other. However, given the number of supplement brands available in the market, it was impossible to standardize the specific type of supplement consumed.

Bodybuilders commonly consume CREA to increase their strength. CREA monohydrate is legally available and has been reported to be a safe supplement, without any adverse effects on the kidney and liver (35). However, serum creatinine levels were reported to be increased in certain individuals in the absence of a clinical cause of renal failure (36). In the present study, BUN and creatinine markers of kidney function were also analyzed. The BUN levels were higher in group BB-G than in groups G and H ($P < 0.01$), and the mean BUN levels in group BB (36.50 ± 11.37) were higher than the normal values (7.9-21 mg/dL). The creatinine levels in group BB were higher than those in group G ($P < 0.05$), but were similar to those in group H ($P > 0.05$). As only eight bodybuilders used CREA in the present study, the effects of CREA alone could not be determined.

In the present study, the mean serum creatinine levels in each study group were within normal limits; however, the mean serum BUN levels in groups BB-G and H were higher than the normal values. BUN and creatinine are important indicators of renal health. However, serum creatinine is a much more reliable indicator of renal function than serum BUN because BUN is far more likely to be affected by dietary conditions (such as high protein consumption) not related to renal function (37). Thus, considering that serum creatinine levels were within the normal range in all groups, increased BUN levels in groups BB-G and H may not be significant in the present study.

Glutamine is a widely consumed nutritional supplement, with supplementation below 14 g/day reported to be safe in adults (38). In the present study, 17 of the 25 bodybuilders used glutamine at a dose of 10 g/day, in combination with other supplements. Thus, the effects of glutamine alone could not be clearly identified. However, according to a consensus report in 2011, L-glutamine supplements should not be used as an aid to sports and exercise (39).

Whey protein is popular among bodybuilders for its effects on muscle size, and it is generally used in combination with amino acids and CREA (40). A previous study reported a daily intake of 40-80 g among bodybuilders (41). In the present study, all bodybuilders used 50 g of whey protein in combination with BCAA and/or glutamine and CREA. Thus, its effects alone could not be determined.

BCAAs account for 14-18% of amino acids in skeletal muscle proteins, and they are popular supplements among bodybuilders (42). Among BCAAs, leucine is a popular supplement, with a safe upper limit of 550 mg/kg bodyweight/day in adult men (43). In the present study, the bodybuilders used a mixture of BCAAs at 625 mg/kg bodyweight/day. Manuel and Heckman (44) reported the possible role of BCAAs in amyotrophic lateral sclerosis (ALS), one of the most common neuromuscular diseases worldwide, when used by athletes as a dietary supplement to increase muscle gain. Furthermore, BCAAs were reported to be a possible cause of the high incidence of ALS among American football players (45). In the sporadic form of ALS, inflammation is driven by stimulation of macrophages through CASP1, IL-1, IL-6, and chemokine signaling in the spinal cord and peripheral blood (45). In the present study, serum levels of IL-1β, CASP1, and ASC in group BB-G were similar to those in the other groups ($P > 0.05$). In the present study, the bodybuilders had used BCAA supplements for only six months to one year. Future research in bodybuilders who had used these supplements for several years would be needed to shed light on their potential adverse health effects.

Previous research has reported a significant correlation between a low-quality diet, as determined by HEI, and risk of periodontal disease (calculus formation) (46). In the present study, the dietary quality, as determined by the HEI score, was not different among the groups ($P > 0.05$). Thus, the potential effect of dietary quality could

not be evaluated.

In addition to supplements, strenuous exercise can also affect serum biochemical markers (47,48). Elevation of CK-MB levels, which is related to myocardial infarction, was detected, mimicking that seen in myocardial infarction in female athletes (47). In the present study, the levels of CK-MB and those of another cardiac marker, hs-TNT, were similar and within normal limits in all groups ($P > 0.05$). In addition, weightlifting was reported to increase liver-function parameters (AST and ALT) after exercise in healthy males (48). In the present study, no differences were observed in ALT levels among all groups ($P > 0.05$). AST levels were higher in group BB-G than in groups G and H but within normal values. Although the mean AST levels in groups G and H were within the normal range, they were higher in group BB-G ($P < 0.01$). The discordance in the results compared with those in the literature may be because of the different sampling times used in the studies. As a result, serum markers may be different in healthy controls compared with those in bodybuilders. Thus, medical professionals should be aware of these effects.

Numerous studies have evaluated the effects of periodontal status on physical exercise (26,27,49). Muscle damage was also shown to reduce physical fitness and induce serum levels of proinflammatory cytokines such as IL-1β and IL-6 (50). As the same proinflammatory biomarkers are involved in both periodontal disease and muscle metabolism (50), the systemic challenge created by periodontal disease also affects physical fitness. Oliveira et al. suggested that periodontal disease may be a risk indicator for poor physical fitness (49). A systematic review reported that oral health of athletes was poor and that this had a negative effect on athletes' training and performance across a wide range of sports (27). The possible relationship between poor oral health in sports may be explained by the frequent intake of carbohydrates, decrease in salivary flow and drying of the mouth during exercise (51) and exercise-induced immune suppression (52).

Bodybuilders who used anabolic steroids for long periods were reported to have an increased risk of severe arteriosclerosis; moreover, such drugs were associated with fatalities. Furthermore, dietary supplements may pose less of a threat to a bodybuilder's health than anabolic steroids (33). Thus, the possible effects of steroids on periodontal status must be clarified in future studies.

In conclusion, the outcomes of the present study indicated that CASP1, IL-1β, and ASC may play vital roles in the pathogenesis of gingivitis. Bodybuilding and protein powder-supplement usage may decrease gingival inflammation by downregulating CASP1, IL-1β, and ASC.

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Conflict of interest

The authors report no conflict of interest related to this study.

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