Abstract

Objectives: This study aimed to determine the effect of 7-day dietary supplementation of N-acetylcysteine (NAC)/zinc/vitamin C on the time-to-exhaustion (TTE), the cardiorespiratory fitness (CRF) index, and metabolic indicators.

Methods: This study enrolled volleyball student trainees (n = 18 men) who took NAC/zinc/vitamin C (750 mg/5 mg/100 mg) for 7 days at Jouf University, Saudi Arabia. The CRF index and TTE were determined. Serum concentrations of metabolic regulators (insulin, betatrophin, and hepatocyte growth factor), biomarkers of cellular damage/hypoxia, and indicators of lipid and glycemic control were measured.

Results: Supplementation improved the TTE and CRF index, and lowered cytochrome c, C-reactive protein, hypoxia-inducible factor-1α (HIF-1α), total cholesterol, insulin, and glycated hemoglobin values. Before and after supplementation, the CRF index was negatively correlated with body mass index and positively correlated with the TTE. Before supplementation, the CRF index was positively correlated with betatrophin concentrations, and hepatocyte growth factor concentrations were positively correlated with betatrophin concentrations and negatively correlated with the homeostasis model assessment of insulin resistance index. After supplementation, the CRF index was negatively correlated with HIF-1α concentrations and metabolites. Additionally, the TTE was negatively correlated with HIF-1α, cytochrome c, and triacylglycerol concentrations.
Conclusion: Supplementation of NAC/zinc/vitamin C improves metabolic and CRF performance.

Keywords
Cytochrome c, betatrophin, hepatocyte growth factor, hypoxia-inducible factor-1α, antioxidant, cardiorespiratory fitness

Date received: 8 September 2021; accepted: 29 November 2021

Introduction
Cardiorespiratory fitness (CRF) is the capacity of the circulatory and respiratory systems to supply oxygen to mitochondria of skeletal muscles. CRF is an objective measure of health that can be tracked over time and compared across populations.1 Athletic high-intensity training increases cellular damage markers2 and oxidative stress, which induce fatigue and decreased performance.3 In contrast, enhancement of antioxidants increases the capacity to perform strenuous physical training and improves tolerance. Dietary thiol donors, such as cysteine and N-acetyl-cysteine (NAC), renew the major water-soluble antioxidant glutathione and attenuate the disadvantages of intense physical exercise.4–7

Although skeletal muscle is a major oxygen consumer, it is adaptively resilient to exercise-induced hypoxic episodes. Stabilization and activation of the oxygen-sensitive, master transcription regulator, hypoxia-inducible factor (HIF)-1α, perfects such adaptation to counteract oxidative stress, vascular endothelial dysfunction, and muscle fiber atrophy and fatigue.8,9 HIF-1α controls glucose transporters and glycolysis, metabolism, endothelial nitric acid synthase/angiogenesis, erythropoietin/erythropoiesis, mitochondrial function, and oxygen consumption, and regulates pH.8,10,11 HIF-1α deletion blunts acute skeletal muscle adaptation, which is lessened with long-term training. However, the overexpression of HIF-1α can be deleterious. Studies have shown higher concentrations of HIF-1α negative regulators in elite athletes than in control subjects.11,12

Serum concentrations of hepatocyte growth factor (HGF), a multi-functional trophic cytokine, are closely correlated with glycemic control and whole-body metabolism. HGH is important for angiogenesis and tissue regeneration.13 Betatrophin (lipasin or angiopoietin-like 8) is a pancreatic β-cell proliferation inducer and metabolic regulator. Muscle exercise-induced secretion of irisin increases betatrophin production. This production significantly improves glucose and lipid homeostasis and insulin resistance.14–18 However, blood betatrophin and HGF concentrations greatly differ among healthy adults. Additionally, an increase in blood betatrophin concentrations is positively correlated with C-reactive protein (CRP), insulin resistance, and hypertriglyceridemia.19,20 Betatrophin is a potential biomarker for physiological performance that is correlated with physical training.21–23 The trophic effect of endurance and maximal exercise is due to an increase in circulating concentrations of growth factors, including HGF, and mobilization of bone marrow-derived hemopoietic and angiogenic
progenitors. Hypoxic high-intensity training, particularly as a chronic adaptation, induces skeletal muscle satellite cells and angiogenesis, which are associated with HGF and HIF-1α.

The above-mentioned findings highlight the important role of HGF, HIF-1α, and betatrophin in physical activity. This study hypothesized that metabolic fitness combined with a healthy antioxidant armament has a positive effect on the CRF index and physical performance. This study aimed to determine the effects of a NAC/zinc/vitamin C antioxidant food supplement on the time-to-exhaustion (TTE), CRF index, and metabolic fitness. Serum indices of systemic inflammation, cellular damage and hypoxia, metabolic regulators, and glycemic and lipid control in novice volleyball players were measured.

**Subjects and methods**

Eighteen volunteer novice male volleyball trainees at Jouf University, Sakaka, Saudi Arabia were consecutively recruited for this study after signing a written informed consent form. Participants were in the middle of their training season and were apparently healthy, without a remarkable medical history. The study was conducted during the academic year of 2019 at the training rooms and play courts of the College of Dentistry under the supervision of Dr Moataz A. Kanoun, Associate Prof. of Physical Education. The study was conducted according to the guidelines of the Declaration of Helsinki, and was approved by the Bioethics Committee of Jouf University (approval #: 30-3/38-39). The age and body mass index ([BMI] weight in kg/height in m²) were prospectively measured. There were seven (39%) tobacco smokers.

The NAC/Z/C antioxidant supplement was composed of 750 mg NAC (NAC750; VitaminVersand24 DE GmbH, Stolberg, Deutschland) and 100 mg vitamin C/5 mg zinc (Limitless C-ZINC; Eva Pharma, Giza, Egypt) tablets, and was taken twice daily for 7 days. The CRF index was assessed using the following equation: CRF index = TTE/hₐ × 50, where the TTE is the time-to-exhaustion in seconds while stationary running at maximum speed, and hₐ is the summation of the recovery pulse (using the Mibest OLED Finger Pulse Oximeter; https://www.mibeststore.com/) measured at 1 to 1.5, 2 to 2.5, and 3 to 3.5 minutes post-exhaustion. An index of >96 indicates excellent fitness, 83 to 96 is good, 68 to 82 is average, 54 to 67 is low average, and <54 is poor.

Morning fasting peripheral venous blood samples (2.5 mL) were aseptically collected for recovering serum after clotting at room temperature on the first day before supplementation and on the last day of the study. Specific enzyme-linked immunosorbent assay-based quantitative kits were used to determine concentrations of glycated hemoglobin (HbA1c), insulin, CRP, HIF-1α, and cytochrome c (catalog numbers: CEA190Hu, CEA448Hu, SEA821Hu, SEA798Hu, and SEA594Mi, respectively; Cloud-Clone Corp., Wuhan, Hubei, China), as well as betatrophin and HGF (catalog numbers: SK00528-08/SK00331-02; Aviscera Bioscience, Inc., Santa Clara, CA, USA). Enzymatic colorimetric assays were used to measure serum glucose, triacylglycerol (TAG), and total cholesterol concentrations (Human, Gesellschaft fur Biochemica und Diagnostica mbH, Wiesbaden, Germany). The homeostatic model was used to calculate insulin resistance (https://www.thebloodcode.com/homa-ir-calculator/).

Except for smoking, continuous normally distributed data are presented as the mean ± standard deviation. The changes in TTE, the CRF index, and the other investigated parameters before and after NAC/Z/C supplementation were compared using
the Wilcoxon matched-pairs signed rank test. Within group correlations (i.e., before and after supplementation) were tested by non-parametric Spearman’s analysis, and r and P values are presented. Smoking was represented by 1 and nonsmoking was represented by 0. Prism 7.0 software (GraphPad Software, Inc, San Diego, CA, USA) was used for analysis. Significance was set at P ≤ 0.05.

Results

Changes in the investigated parameters after supplementation

Table 1 shows the characteristics of the male participants and changes in the investigated parameters before and after providing NAC/Z/C supplementation. The TTE (P = 0.003) and the CRF index (P < 0.001) were significantly higher after supplementation than before supplementation. Concentrations of cytochrome c (P < 0.001), CRP (P = 0.004), HIF-1α (P < 0.001), insulin (P < 0.001), HbA1c (P < 0.001), total cholesterol (P = 0.023), and the homeostasis model assessment of insulin resistance (HOMA-IR) index (P < 0.001) were significantly lower after supplementation than before supplementation. Betatrophin, HGF, glucose, and TAG concentrations were not significantly different after supplementation compared with before supplementation.

Table 1. Parameters measured before and after 1 week of N-acetyl-cysteine/zinc/vitamin C antioxidant food supplementation to novice volleyball players (total n = 18).

| Parameter                          | BS                        | AS                        | P     |
|-----------------------------------|----------------------------|----------------------------|-------|
| Age, years                        | 21.78 ± 2.157 (19.0–26.0) | –                          | –     |
| BMI, kg/m²                        | 25.44 ± 5.02 (17.0–35.0)  | –                          | –     |
| Time-to-exhaustion, s             | 168.2 ± 81.01 (76–300)    | 194.9 ± 93.79 (90–320)    | 0.003 |
| Cardiorespiratory fitness index   | 54.44 ± 25.43 (21.35–98.04) | 65.23 ± 27.45 (27.22–99.34) | <0.001|
| Cytochrome c, ng/mL               | 17.29 ± 3.868 (11.83–29.30) | 15.14 ± 4.465 (7.932–29.020) | <0.001|
| C-reactive protein, pg/mL         | 428 ± 226.5 (179.7–1055.0) | 317.6 ± 158.3 (154.9–650.3) | 0.004 |
| Hypoxia-inducible factor-1α, pg/mL| 46.12 ± 19.43 (17.16–72.22) | 34.29 ± 16.65 (13.79–68.85) | <0.001|
| Betatrophin, ng/mL                | 1.113 ± 2.008 (0.052–5.678) | 1.403 ± 2.728 (0.104–8.541) | 0.262 |
| Hepatocyte growth factor, pg/mL   | 785.2 ± 501.2 (147.3–1955) | 958.2 ± 331.5 (242.1–1512) | 0.137 |
| Total cholesterol, mmol/L         | 4.302 ± 0.716 (2.994–5.385) | 4.056 ± 0.709 (2.994–5.053) | 0.023 |
| Triacylglycerols, mmol/L          | 1.420 ± 0.312 (1.007–2.076) | 1.275 ± 0.253 (0.838–1.696) | 0.077 |
| Insulin, μIU/mL                   | 8.147 ± 0.802 (6.613–9.643) | 7.365 ± 0.605 (6.081–8.284) | <0.001|
| Glucose, mmol/L                   | 4.381 ± 0.545 (3.398–5.043) | 4.126 ± 0.631 (3.368–5.441) | 0.147 |
| HOMA-IR index                     | 1.583 ± 0.266 (1.1–2.1)    | 1.35 ± 0.273 (1.0–2.0)     | <0.001|
| HbA1c, %                          | 5.437 ± 0.743 (4.14–6.946) | 4.348 ± 0.885 (3.262–5.779) | <0.001|

Data are mean ± standard deviation (range). Values were compared before (BS) and after (AS) supplementation. BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; HbA1c, glycated hemoglobin.

Correlations between indices of systemic inflammation, cellular damage and hypoxia, metabolic regulators, and glycemic and lipid control

Correlation analysis among parameters in the participants before supplementation is shown in Table 2. Age was significantly positively correlated with the TTE (r = 0.456, P = 0.029) and the CRF index (r = 0.440, P = 0.039), but negatively correlated with TAG concentrations (r = –0.457, P = 0.028). BMI was negatively correlated with the CRF index (r = –0.410, P = 0.05).
Table 2. Correlations of demographic and anthropometric characteristics with other parameters before antioxidant supplementation in the participants.

|                  | BMI  | Smoking | TTE | CRF  | CytC | CRP  | BT  | HGF  | HIF-1α | Ins  | Glucose | HOMA-IR index | HbA1c | TC  | TAGs |
|------------------|------|---------|-----|------|------|------|-----|------|--------|------|---------|---------------|-------|-----|------|
| Age              | ns   | ns      | 0.456/0.029 | 0.440/0.039 | ns   | ns   | ns  | ns   | ns     | ns   | ns      | ns            | ns    | ns  | ns   |
| BMI              |      |         | 0.454/0.029 | 0.410/0.05   | ns   | ns   | ns  | ns   | 0.544/0.010 | ns   | 0.399/0.05 | 0.439/0.034   | ns    | 0.390/0.05 |
| Smoking          |      |         | ns   | 0.703/0.001 | ns   | ns   | ns  | ns   | ns     | ns   | ns      | ns            | ns    | ns  | ns   |
| TTE              |      | 0.934/0.001 |      | 0.405/0.05  | ns   | ns   | ns  | ns   | ns     | ns   | ns      | ns            | ns    | ns  | ns   |
| CRF              |      |         | ns   | 0.399/0.05  | ns   | ns   | ns  | ns   | ns     | ns   | ns      | ns            | ns    | ns  | ns   |
| CytC             |      |         | ns   | ns         | ns   | 0.634/0.002 | ns  | 0.395/0.05 | ns     | ns   | ns      | ns            | ns    | ns  | ns   |
| CRP              |      |         | ns   | ns         | ns   | ns   | ns  | ns   | ns     | ns   | ns      | ns            | ns    | ns  | ns   |
| BT               |      |          |      | 0.577/0.006 | ns   | ns   | ns  | ns   | ns     | ns   | ns      | ns            | ns    | ns  | ns   |
| HGF              |      |          |      |            | ns   |         | ns  | 0.399/0.05 | ns     | ns   | ns      | ns            | ns    | ns  | ns   |
| HIF-1α           |      |          |      |            | ns   |          | ns  | 0.399/0.05 | ns     | ns   | ns      | ns            | ns    | ns  | ns   |
| Ins              |      |          |      |            | ns   |          | ns  | 0.690/0.001 | 0.474/0.024 | ns   | ns      | ns            | ns    | ns  | ns   |
| Glucose          |      |          |      |            | ns   |          | ns  | 0.765/0.001 | 0.516/0.014 | ns   | ns      | ns            | ns    | ns  | ns   |
| HOMA-IR index    |      |          |      |            | ns   |          | ns  | 0.765/0.001 | 0.516/0.014 | ns   | ns      | ns            | ns    | ns  | ns   |
| HbA1c            |      |          |      |            | ns   |          | ns  | 0.516/0.014 | 0.525/0.013 | ns   | ns      | ns            | ns    | ns  | ns   |
| TC               |      |          |      |            | ns   |          |     |            | ns     | ns   | ns      | ns            | ns    | ns  | ns   |
| TAGs             |      |          |      |            | ns   |          |     |            | ns     | ns   | ns      | ns            | ns    | ns  | ns   |

Data are r/P values.

BMI, body mass index; TTE, time-to-exhaustion; CRF, cardiorespiratory fitness; CytC, cytochrome c; CRP, C-reactive protein; BT, betatrophin; HGF, hepatocyte growth factor; HIF-1α, hypoxia-inducible factor-1α; Ins, insulin; HOMA-IR, homeostasis model assessment of insulin resistance; HbA1c, glycated hemoglobin; TC, total cholesterol; TAGs, triacylglycerols, ns, nonsignificant.
and positively correlated with insulin concentrations \( (r = 0.544, P = 0.010) \) and HbA1c values \( (r = 0.439, P = 0.034) \). Smoking was negatively correlated with the TTE \( (r = -0.473, P = 0.024) \) and betatrophin concentrations \( (r = -0.703, P = 0.001) \). The TTE was positively correlated with the CRF \( (r = 0.934, P < 0.001) \) and betatrophin concentrations \( (r = 0.405, P = 0.05) \). Cytochrome c concentrations were positively correlated with HIF-1z concentrations \( (r = 0.634, P = 0.002) \). Betatrophin concentrations were positively correlated with HGF concentrations \( (r = 0.577, P = 0.006) \). Insulin concentrations were positively correlated with the HOMA-IR index \( (r = 0.690, P = 0.001) \) and HbA1c values \( (r = 0.474, P = 0.024) \). Glucose concentrations were positively correlated with the HOMA-IR index \( (r = 0.765, P = 0.001) \) and total cholesterol concentrations \( (r = 0.516, P = 0.014) \). The HOMA-IR index was positively correlated with total cholesterol concentrations \( (r = 0.525, P = 0.013) \). HGF concentrations were negatively correlated with the HOMA-IR index \( (r = -0.499, P = 0.018) \), insulin concentrations \( (r = -0.399, P = 0.05) \), and TAG concentrations \( (r = -0.413, P = 0.05) \).

Correlation analysis among parameters in the participants after supplementation is shown in Table 3. Age was significantly positively correlated with the TTE \( (r = 0.399, P = 0.050) \) and the CRF index \( (r = 0.453, P = 0.029) \). BMI was negatively correlated with the CRF index \( (r = -0.399, P = 0.05) \), and positively correlated with CRP \( (r = 0.425, P = 0.039) \), glucose \( (r = 0.488, P = 0.020) \), HbA1c \( (r = 0.436, P = 0.035) \) and TAG \( (r = 0.430, P = 0.037) \) values. Smoking was negatively correlated with the TTE \( (r = -0.478, P = 0.023) \) and betatrophin concentrations \( (r = -0.561, P = 0.008) \). The TTE was negatively correlated with cytochrome c \( (r = -0.409, P = 0.05) \), HIF-1z \( (r = -0.401, P = 0.05) \) and TAG \( (r = -0.436, P = 0.035) \) concentrations, and positively correlated with the CRF \( (r = 0.890, P < 0.001) \). The CRF was negatively correlated with HbA1c \( (r = -0.439, P = 0.034) \), TAG \( (r = -0.491, P = 0.019) \), glucose \( (r = -0.410, P = 0.05) \), and HIF-1z \( (r = -0.403, P = 0.05) \) concentrations. Cytochrome c concentrations were positively correlated with HIF-1z \( (r = 0.566, P = 0.007) \) and total cholesterol \( (r = 0.454, P = 0.029) \) concentrations. Betatrophin concentrations were negatively correlated with HIF-1z concentrations \( (r = -0.440, P = 0.034) \). HGF concentrations were positively correlated with HIF-1z concentrations \( (r = 0.399, P = 0.05) \) and negatively correlated with TAG concentrations \( (r = -0.447, P = 0.032) \). HIF-1z concentrations were positively correlated with glucose concentrations \( (r = 0.408, P = 0.050) \). The HOMA-IR index was positively correlated with HbA1c \( (r = 0.436, P = 0.035) \), glucose \( (r = 0.813, P < 0.001) \), and insulin \( (r = 0.619, P = 0.003) \) values. Glucose concentrations were positively correlated with HbA1c concentrations \( (r = 0.409, P = 0.05) \) and total cholesterol values \( (r = 0.421, P = 0.041) \).

Discussion

Oxidative homeostasis balances the endogenously generated and exogenously acquired prooxidant free radicals and oxidants versus the enzymatic, and endogenous and exogenously acquired nonenzymatic antioxidants (e.g., vitamins C and E, lipoate, CoQ, glutathione, carotenoids, and zinc). Antioxidant supplementation has beneficial outcomes with physical activity, particularly in high-intensity, short-duration, vigorous, and exhaustive exercise, and for physical inactivity compared with low-to-moderate intensity and long-term exercises. NAC is a non-essential precursor of cysteine and glutathione; the major water-soluble antioxidant in muscle.
Table 3. Correlations of demographic and anthropometric characteristics with other parameters after antioxidant supplementation in the participants.

|          | BMI  | Smoking | TTE   | CRF   | CytC  | CRP   | BT    | HGF   | HIF-1\(\alpha\) | Ins   | Glucose | HOMA-IR index | HbA1c | TC   | TAGs |
|----------|------|---------|-------|-------|-------|-------|-------|-------|-----------------|-------|---------|----------------|-------|------|------|
| Age      |      | ns      |       |       |       |       |       |       |                 | ns    | ns      |                 |        |      |      |
| BMI      |      |         | ns    |       |       |       |       |       |                 | ns    | ns      |                 |        |      |      |
| Smoking  |      |         |       |       |       |       |       |       |                 | ns    | ns      |                 |        |      |      |
| TTE      |      |         |       |       |       |       |       |       |                 | ns    | ns      |                 |        |      |      |
| CRF      |      |         |       |       |       |       |       |       |                 | ns    | ns      |                 |        |      |      |
| CytC     |      |         |       |       |       |       |       |       |                 | ns    | ns      |                 |        |      |      |
| CRP      |      |         |       |       |       |       |       |       |                 | ns    | ns      |                 |        |      |      |
| BT       |      |         |       |       |       |       |       |       |                 | ns    | ns      |                 |        |      |      |
| HGF      |      |         |       |       |       |       |       |       |                 | ns    | ns      |                 |        |      |      |
| HIF-1\(\alpha\) |      |         |       |       |       |       |       |       |                 | ns    | ns      |                 |        |      |      |
| Ins      |      |         |       |       |       |       |       |       |                 | ns    | ns      |                 |        |      |      |
| Glucose  |      |         |       |       |       |       |       |       |                 | ns    | ns      |                 |        |      |      |
| HOMA-IR index |      |         |       |       |       |       |       |       |                 | ns    | ns      |                 |        |      |      |
| HbA1c    |      |         |       |       |       |       |       |       |                 | ns    | ns      |                 |        |      |      |
| TC       |      |         |       |       |       |       |       |       |                 | ns    | ns      |                 |        |      |      |

Data are r/P values.

BMI, body mass index; TTE, time-to-exhaustion; CRF, cardiorespiratory fitness; CytC, cytochrome c; CRP, C-reactive protein; BT, betatrophin; HGF, hepatocyte growth factor; HIF-1\(\alpha\), hypoxia-inducible factor-1\(\alpha\); Ins, insulin; HOMA-IR, homeostasis model assessment of insulin resistance; HbA1c, glycated hemoglobin; TC, total cholesterol; TAGs, triacylglycerols, ns, nonsignificant.
NAC also enhances potassium homeostasis and potentiates sarcoplasmic Na⁺/K⁺- and Ca²⁺-ATPases. However, counteracting the reactive oxygen species deprives cells from their physiological roles. This is the reason for the outcomes of antioxidant supplementation with substantial physical activity being controversial. High doses of antioxidant supplementation lead to prooxidant effects and systemic and muscle harmful effects by inhibiting the anabolic signaling pathways (JNK, p65phox, and Akt/mTOR/p70s6K/p38MAPK) that blunt the expression of adaptation proteins. These effects apply to vitamins C and E and NAC among others.

CRF declines in untrained individuals, and fitness is inversely correlated with age, BMI, metabolic syndrome, diabetes, and dyslipidemia as risk factors for cardiovascular disease. In this study, NAC/Z/C antioxidant supplementation deceased cellular damage and hypoxia, as well as inflammatory markers (cytochrome c, HIF-1α, and CRP), and improved metabolic efficiency (decreased total cholesterol, HbA1c, insulin, and HOMA-IR values). These metabolic effects were paralleled by a nonsignificant increase in serum concentrations of betatrophin and HGF and a decrease in glucose and TAG concentrations. The large interindividual variation might have nullified the significance of differences among these variables. However, strengthened metabolic fitness was found as shown by a significant improvement in the TTE and CRF index. An improved metabolic capacity and oxygen supply enable skeletal muscle to adapt to long-term aerobic performance at higher intensities. Vitamin C suppresses endothelial cell apoptosis by increasing the bioavailability of intracellular nitric oxide, which prevents cytochrome c release and blocks apoptosis signaling.

A meta-analysis of previous studies that measured changes in circulating HIF-1α concentrations after endurance exercise showed various profiles of a reduction, increase, and no change. Expression of muscle HGF is mostly increased after endurance exercise. Serum betatrophin concentrations are mostly decreased after endurance exercise. Most previous studies on endurance exercise showed increased gene expression for cytochrome c in muscles. Endurance exercise mostly acutely reduces serum insulin concentrations. HIF-1α, which is induced by acute exercise, unfavorably inhibits oxygen consumption and aerobic mitochondrial metabolism. Such HIF-1α activation is blunted by its negative regulators that are enhanced by long-term aerobic endurance exercise training.

HbA1c values indirectly indicate a dominant inflammatory/oxidative stress status and correlate with CRP concentrations. Therefore, a reduction in HbA1c, CRP, and cytochrome c values in the present study reflects the metabolic efficacy of the antioxidant cocktail used.

In the present study, age was significantly positively correlated with the TTE and CRF index before and after supplementation. However, age was only significantly negatively correlated with serum TAG concentrations before supplementation. BMI was negatively correlated with the CRF index before and after supplementation. BMI was also positively correlated with serum insulin and HbA1c values before supplementation, and positively correlated with serum CRP, glucose, HbA1c and TAG values after supplementation. Smoking was negatively correlated with the TTE and serum betatrophin concentrations before and after supplementation. Previous studies have shown negative correlations of CRF and physical activity with BMI and smoking. However, although smoking and physical activity are largely incongruent behaviors, their relationship is often attenuated or reversed in male adolescents and for moderate (vs. vigorous) exercise.
This finding is consistent with our findings in young adult participants.

Aerobic and muscle fitness are independently, inversely associated with serum CRP concentrations. In contrast, independently of lifestyle behavior and BMI, an improvement in and/or high CRF reduces CRP concentrations. CRF in adults is inversely correlated with BMI, CRP concentrations, smoking, and resting heart rate. CRP concentrations are significantly associated with BMI, fasting glucose concentrations, and HbA1c values, while they are negatively correlated with a high fitness level, prolonged exercise training, and antioxidants. Antioxidants, including vitamin C and NAC, significantly reduce CRP concentrations and other inflammatory markers and cytokines, which are key adaptive changes to training, by inhibiting nuclear factor kappa B. Zinc counteracts insulin resistance, reduces CRP concentrations, and protects against cardiovascular disease. Zinc produces these effects because it is an essential trace element in many enzymes, particularly the antioxidant superoxide dismutase, and is involved in insulin homeostasis. The present study also showed a similar reduction in biomarkers of inflammation, cellular damage, and hypoxia, which indicated the effectiveness of the antioxidant cocktail used.

In the present study, correlation analysis before supplementation showed the following significant positive correlations: 1) TTE with the CRF index and serum betatrophin concentrations, 2) serum cytochrome c with HIF-1α concentrations, 3) serum betatrophin with HGF concentrations, 4) serum insulin concentrations with HOMA-IR and HbA1c values, 5) serum glucose concentrations with the HOMA-IR index and total cholesterol concentrations, and 6) the HOMA-IR index with serum total cholesterol concentrations. HGF concentrations were negatively correlated with the HOMA-IR index, and insulin and TAG concentrations. Mitochondrial dysfunction, which improves with physical activity and fitness, has been implicated in the development of metabolic disorders, such as insulin resistance and type 2 diabetes mellitus. Mitochondrial dysfunction and free cytoplasmic cytochrome c, which is involved in oxidative stress and O2/energy shortage, activate caspase 9 as the primary event in apoptosis. This finding was the rationale for using cytochrome c and HIF-1α as biomarkers in the present study.

Circulating HGF concentrations are high in healthy athletes, irrespective of the type of exercise. This finding may explain the present finding of no significant increase in HGF concentrations, which showed large inter-individual variation, because basal concentrations were already high. Large individual variations in serum HGF concentrations have been previously reported. HGF induces bel-2 and inhibits translocation of bax from the cytosol to the mitochondria to prevent endothelial cell apoptosis, which is induced by high glucose concentrations. HGF attenuates oxidative stress and cytochrome c release in retinal pigment epithelium cells treated with buthionine-(S,R)-sulfoximine, which depletes glutathione. In contrast, HGF concentrations are negatively correlated with aerobic and muscle fitness, and positively correlated with CRP, interleukin-6, and tumor necrosis factor-α concentrations.

The present study showed no significant increase in serum betatrophin concentrations after supplementation, with large inter-individual variation. Serum betatrophin concentrations were positively correlated with the TTE and HGF concentrations before supplementation, and negatively correlated with HIF-1α and smoking after supplementation. Betatrophin concentrations show large inter-individual differences and are positively correlated with age, glucose, TAG, total cholesterol, and HbA1c values in healthy adults and
patients.\textsuperscript{20,66,67} CRP and betatrophin concentrations are higher in patients with obesity and in those with metabolic syndrome compared with healthy lean controls, and they are positively correlated with each other. A 3-month physical activity program greatly reduced circulating betatrophin concentrations.\textsuperscript{19} Additionally, a study showed that betatrophin concentrations were increased following aerobic and resistance exercise, and were positively correlated with body weight and HOMA-IR and negatively correlated with total cholesterol and TAG concentrations.\textsuperscript{18} However, 6-month physical exercise did not change betatrophin concentrations in healthy subjects.\textsuperscript{68} In contrast, a study showed that betatrophin concentrations were significantly decreased after three types of exercise training in participants with obesity, and were positively associated with fasting blood glucose concentrations before and after exercise.\textsuperscript{69}

The present study showed better metabolic cytoprotective action, anti-inflammatory effects, and resilience to hypoxia after supplementation, as reflected by a significant improvement in the TTE and CRF index in the participants. This finding was observed despite the fact that glycemic and lipid profile parameters were within the normal physiological range. Ten years previously, our research group was one of the first groups worldwide to characterize the changes in circulating concentrations of HIF-1\(\alpha\) as a hypoxic cellular stress and damage biomarker, which is consistent with the present results.\textsuperscript{70–73} In the present study, the distinct improvement in the performance of the participants regarding the TTE, the CRF index, and metabolic fitness compared with previous studies on well-trained athletes,\textsuperscript{28–30} could be attributed to the following. 1) This study used a strong antioxidant cocktail, 2) the participants’ athletic performance was novice in nature, and 3) the cardiorespiratory fitness (\(n=9;\) 50% with a poor index) and the metabolic fitness (\(n=11;\) >61% with a BMI of \(\geq 25\) kg/m\(^2\)) of the participants were poor, which suggested physical inactivity and/or unhealthy nutrition. However, validation of the utility of the biomarkers used in this study requires larger studies in the future.

**Conclusions**

The antioxidant NAC/Z/C food supplement applied in this study led to a significant improvement in the TTE and CRF index because it supported cellular viability (cytochrome c and HIF-1\(\alpha\)), reduced inflammation (CRP) and insulin resistance (HbA1c and HOMA-IR), and improved the lipid profile (total cholesterol) and glycemic control indices (insulin and HbA1c). These findings were correlated with a nonsignificant increase in metabolic regulators of cytoprotection (betatrophin and HGF), and a decrease in glucose and TAG concentrations. BMI and smoking negatively affected these benefits. HIF-1\(\alpha\) concentrations were positively correlated with cytochrome c and glucose concentrations, and negatively correlated with the CRF index and TTE, which suggest that HIF-1\(\alpha\) is a marker of damage. The marked improvements observed in this study are reasonable in consideration of the initial low physical fitness and CRF of our participants, and this suggests unhealthy nutrition. Larger and longer term studies are required in the future to confirm the generalization of our results and the utility of the biomarkers used, particularly for trained athletes.

**Acknowledgements**

The author is highly indebted to Prof. Tarek H. El-Metwally (Biochemistry Division, Pathology Department, College of Medicine, Jouf University, Sakaka, Saudi Arabia) for help during the planning and execution of lab work of this study and to Prof. Hossam M. Omar (Assiut University, Assiut, Egypt) for assistance.
of final drafting of the manuscript. The training and field data collection were sponsored by Dr. Moataz A. Kanoun (Physical Education Department, Jouf University, Sakaka, Saudi Arabia) and Dr. Mohammad Salahuddin (Department of Physiology, College of Medicine, Jouf University, Sakaka, Saudi Arabia).

Declaration of conflicting interest
The authors declare that there is no conflict of interest.

Funding
The authors disclosed receipt of the following financial support for the research, authorship, and publication of this article: The authors were supported by the Deanship of Scientific Research, the Vice Presidency for Postgraduate Studies and Scientific Research, Jouf University, Sakaka, Saudi Arabia for funding of this work (project fund number: 39/564).

ORCID iD
Abdulrahman A. Alduraywish https://orcid.org/0000-0002-2710-3187

References
1. Tomkinson GR, Lang JJ and Tremblay MS. Temporal trends in the cardiorespiratory fitness of children and adolescents representing 19 high-income and upper middle-income countries between 1981 and 2014. Br J Sports Med 2019; 53: 478–486. DOI: 10.1136/bjsports-2017-097982.
2. Freitas VH, Nakamura FY, Miloski B, et al. Sensitivity of physiological and psychological markers to training load intensification in volleyball players. J Sports Sci Med 2014; 13: 571–579.
3. Souglis A, Bogdanis GC, Giannopoulou I, et al. Comparison of inflammatory responses and muscle damage indices following a soccer, basketball, volleyball and handball game at an elite competitive level. Res Sports Med 2015; 23: 59–72. DOI: 10.1080/15438627.2014.975814.
4. Slattery KM, Dascombe B, Wallace LK, et al. Effect of N-acetylcysteine on cycling performance after intensified training. Med Sci Sports Exerc 2014; 46: 1114–1123. DOI: 10.1249/MSS.0000000000002222.
5. McLeay Y, Stannard S, Houldham S, et al. Dietary thiols in exercise: oxidative stress defence, exercise performance, and adaptation. J Int Soc Sports Nutr 2017; 14: 12. DOI: 10.1186/s12970-017-0168-9.
6. Leelarungrayub D, Khansuwan R, Pothongsunun P, et al. N-acetylcysteine supplementation controls total antioxidant capacity, creatine kinase, lactate, and tumor necrotic factor-alpha against oxidative stress induced by graded exercise in sedentary men. Oxid Med Cell Longev 2011; 2011: 329643. DOI: 10.1155/2011/329643.
7. de Jesus Pires de Moraes A, Andreato LV, Branco B, et al. Effects of N-acetylcysteine supplementation on cellular damage and oxidative stress indicators in volleyball athletes. J Exerc Rehabil 2018; 14: 802–809. DOI: 10.12965/jer.1836152.076.
8. Chee NT, Lohse I and Brothers SP. mRNA-to-protein translation in hypoxia. Mol Cancer 2019; 18: 49. DOI: 10.1186/s12943-019-0968-4.
9. Valle-Tenney R, Rebolledo D, Acuña MJ, et al. HIF-hypoxia signaling in skeletal muscle physiology and fibrosis. J Cell Commun Signal 2020; 14: 147–158. DOI: 10.1007/s12079-018-00553-8.
10. Favier FB, Britto FA, Freyssenet DG, et al. HIF-1-driven skeletal muscle adaptations to chronic hypoxia: molecular insights into muscle physiology. Cell Mol Life Sci 2015; 72: 4681–4696. DOI: 10.1007/s00018-015-2025-9.
11. Lindholm ME and Rundqvist H. Skeletal muscle hypoxia-inducible factor-1 and exercise. Exp Physiol 2016; 101: 28–32. DOI: 10.1113/EP085318.
12. Sato S, Basse AL, Schönke M, et al. Time of Exercise Specifies the Impact on Muscle Metabolic Pathways and Systemic Energy Homeostasis. Cell Metab 2019; 30: 92–110. e4. DOI: 10.1016/j.cmet.2019.03.013.
13. Konya H, Miuchi M, Satani K, et al. Hepatocyte growth factor, a biomarker of macroangiopathy in diabetes mellitus. World J Diabetes 2014; 5: 678–688. DOI: 10.4239/wjd.v5.i5.678.
14. Xie X, Gao T, Yang M, et al. Associations of beta-trophin levels with irisin in Chinese women with normal glucose tolerance. *Diabetol Metab Syndr* 2015; 7: 26. DOI: 10.1186/s13098-015-0019-2.

15. Amri J, Parastesh M, Sadegh M, et al. High-intensity interval training improved fasting blood glucose and lipid profiles in type 2 diabetic rats more than endurance training; possible involvement of irisin and beta-trophin. *Physiol Int* 2019; 106: 213–224. DOI: 10.1556/2060.106.2019.24.

16. Sadeghipour HR, Yeganeh G, Zar A, et al. The effect of 4-week endurance training on serum levels of irisin and beta-trophin in streptozotocin-induced diabetic rats. *Arch Physiol Biochem* 2020; 2020: 1–7. DOI: 10.1080/13813455.2020.1849310.

17. Akbarzadeh S and Bernardi M. The effect of 4-week endurance training on serum levels of irisin and beta-trophin in streptozotocin-induced diabetic rats. *Arch Physiol Biochem* 2020: 1–7. DOI: 10.1080/13813455.2020.1849310.

18. Tavassoli H and Heidarianpour A. Associations between Betatrophin with Irisin and Metabolic Factors: Effects of Exercise Training in Diabetic Rats. *Am J Med Sci* 2021; 362: 496–505. DOI: 10.1016/j.amjms.2021.05.023.

19. Abu-Farha M, Sriraman D, Cherian P, et al. Circulating ANGPTL8/Betatrophin Is Increased in Obesity and Reduced after Exercise Training. *PLoS One* 2016; 11: e0147367. DOI: 10.1371/journal.pone.0147367.

20. Alduraywish A. Circulating betatrophin and hepatocyte growth factor in type 2 diabetic patients: Their relationship with disease prognosis. *J Endocrinology and Metabolism* 2017; 7: 31–43. DOI: 10.14740/jem405w.

21. Gao T, Jin K, Chen P, et al. Circulating Betatrophin Correlates with Triglycerides and Postprandial Glucose among Different Glucose Tolerance Statuses - A Case-Control Study. *PLoS One* 2015; 10: e0133640. DOI: 10.1371/journal.pone.0133640.

22. Fu Z, Berhanef F, Fite A, et al. Elevated circulating lipasin/betatrophin in human type 2 diabetes and obesity. *Sci Rep* 2015; 4: 5013. DOI: 10.1038/srep05013.

23. Susanto H, Taufiq A, Sugiharto, et al. Moderate-Intensity Exercise and Musical Co-Treatment Decreased the Circulating Level of Betatrophin. *International J Endocrinology* 2020; 2020: 3098261. DOI: 10.1155/2020/3098261.

24. Bonsignore MR, Morici G, Riccioni R, et al. Hemopoietic and angiogenetic progenitors in healthy athletes: different responses to endurance and maximal exercise. *J Appl Physiol (1985)* 2010; 109: 60–67. DOI: 10.1152/japplphysiol.01344.2009.

25. Schmid M, Kröpf JM and Spengler CM. Changes in Circulating Stem and Progenitor Cell Numbers Following Acute Exercise in Healthy Human Subjects: a Systematic Review and Meta-analysis. *Stem Cell Rev Rep* 2021; 17: 1091–1120. DOI: 10.1007/s12015-020-10105-7.

26. Nagahisa H, Mukai K, Ohmura H, et al. Effect of High-Intensity Training in Normobaric Hypoxia on Thoroughbred Skeletal Muscle. *Oxid Med Cell Longev* 2016; 2016: 1535367. DOI: 10.1155/2016/1535367.

27. Ghai CL (ed). *A Textbook of Practical Physiology*. 8th ed. New Delhi, India: Jaypee Brothers Medical Publishers (P) LTD, 2013, p.208.

28. Antonioni A, Fantini C, Dimauro I, et al. Redox homeostasis in sport: do athletes really need antioxidant support? *Res Sports Med* 2019; 27: 147–165. DOI: 10.1080/15438627.2018.1563899.

29. Sharifi-Rad M, Anil Kumar NV, Zucca P, et al. Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases. *Front Physiol* 2020; 11: 694. DOI: 10.3389/fphys.2020.00694.

30. Taherkhani S, Valaei K, Arazi H, et al. An Overview of Physical Exercise and Antioxidant Supplementation Influences on Skeletal Muscle Oxidative Stress. *Antioxidants (Basel)* 2021; 10: 1528. DOI: 10.3390/antiox10101528.

31. Ferreira LF, Campbell KS and Reid MB. N-acetylcysteine in handgrip exercise: plasma thiol and adverse reactions. *Int J Sport Nutr Exerc Metab* 2011; 21: 146–154. DOI: 10.1123/ijsnem.21.2.146.
32. Paschalis V, Theodorou AA, Margaritelis NV, et al. N-acetylcysteine supplementation increases exercise performance and reduces oxidative stress only in individuals with low levels of glutathione. *Free Radic Biol Med* 2018; 115: 288–297. DOI: 10.1016/j.freeradbiomed.2017.12.007.

33. Gomez-Cabrera MC, Salvador-Pascual A, Cabo H, et al. Redox modulation of mitochondrial biogenesis in exercise. Does antioxidant supplementation blunt the benefits of exercise training? *Free Radic Biol Med* 2015; 86: 37–46. DOI: 10.1016/j.freeradbiomed.2015.04.006.

34. Trewin AJ, Lundell LS, Perry BD, et al. Effect of N-acetylcysteine infusion on exercise-induced modulation of insulin sensitivity and signaling pathways in human skeletal muscle. *Am J Physiol Endocrinol Metab* 2015; 309: E388–E397. DOI: 10.1152/ajpendo.00605.2014.

35. Higgins MR, Izadi A and Kaviani M. Antioxidants and Exercise Performance: With a Focus on Vitamin E and C Supplementation. *Int J Environ Res Public Health* 2020; 17: 8452. DOI: 10.3390/ijerph17228452.

36. Myers J, Kokkinos P and Nyelin E. Physical Activity, Cardiorespiratory Fitness, and the Metabolic Syndrome. *Nutrients* 2019; 11: 1652. DOI: 10.3390/nu11071652.

37. Rössig L, Hoffmann J, Hugel B, et al. Vitamin C inhibits endothelial cell apoptosis in congestive heart failure. *Circulation* 2001; 104: 2182–2187. DOI: 10.1161/hc4301.098284.

38. Exercise Transcriptome Meta-analysis. https://extrameta.org/

39. Li J, Li Y, Atakan MM, et al. The Molecular Adaptive Responses of Skeletal Muscle to High-Intensity Exercise/Training and Hypoxia. *Antioxidants (Basel)* 2020; 9: 656. DOI: 10.3390/antiox9080656.

40. Sarinnapakorn V and Wanicagool W. Association between hs-CRP and Hba1c in over-weight type 2 diabetic female patients. *J Med Assoc Thai* 2013; 96: S54–S58.

41. Bray JJH, Foster-Davies H, Salem A, et al. Glucagon-like peptide-1 receptor agonists improve biomarkers of inflammation and oxidative stress: A systematic review and meta-analysis of randomised controlled trials. *Diabetes Obes Metab* 2021; 23: 1806–1822. DOI: 10.1111/dom.

42. Großchädl F, Titze S, Burkert N, et al. Moderate- and vigorous-intensity exercise behaviour according to the Transtheoretical Model: associations with smoking and BMI among Austrian adults. *Wien Klin Wochenschr* 2013; 125: 270–278. DOI: 10.1007/s00508-013-0361-z.

43. Mondal H and Mishra SP. Effect of BMI, Body Fat Percentage and Fat Free Mass on Maximal Oxygen Consumption in Healthy Young Adults. *J Clin Diagn Res* 2017; 11: CC17–CC20. DOI: 10.7860/JCDR/2017/25465.10039.

44. Salin K, Kankaanpää A, Hirvensalo M, et al. Smoking and Physical Activity Trajectories from Childhood to Midlife. *Int J Environ Res Public Health* 2019; 16: 974. DOI: 10.3390/ijerph16060974.

45. Haapala EA, Lee E and Laukkanen JA. Associations of cardiorespiratory fitness, physical activity, and BMI with arterial health in middle-aged men and women. *Physiol Rep* 2020; 8: e14438. DOI: 10.14814/phy2.14438.

46. Hemmingsson E, Väisänen D, Andersson G, et al. Combinations of BMI and cardiorespiratory fitness categories: trends between 1995 and 2020 and associations with CVD incidence and mortality and all-cause mortality in 471 216 adults. *Eur J Prev Cardiol* 2021: zwab169. DOI: 10.1093/eurjpc/zwab169.

47. Kaczynski AT, Manske SR, Mannell RC, et al. Smoking and physical activity: a systematic review. *Am J Health Behav* 2008; 32: 93–110. DOI: 10.5555/ajhb.2008.32.1.93.

48. Vaara JP, Santtila M, Vasankari T, et al. Cardiorespiratory and muscular fitness in young adult Finnish men between 2003 and 2015. *Scand J Med Sci Sports* 2020; 30: 716–724. DOI: 10.1111/sms.13619.

49. Steene-Johannessen J, Kolle E, Andersen LB, et al. Adiposity, aerobic fitness, muscle fitness, and markers of inflammation in children. *Med Sci Sports Exerc* 2013; 45: 714–721. DOI: 1249/MSS.0b013e3182797070a.

50. Agostinis-Sobrinho C, Ramirez-Vélez R, García-Hermoso A, et al. The combined association of adherence to Mediterranean
diet, muscular and cardiorespiratory fitness on low-grade inflammation in adolescents: a pooled analysis. *Eur J Nutr* 2019; 58: 2649–2656. DOI: 10.1007/s00394-018-1812-0.

51. González-Gil EM, Santaliestra-Pasías AM, Buck C, et al. Improving cardiorespiratory fitness protects against inflammation in children: the IDEFICS study. *Pediatr Res* 2021 (ahead of print). DOI: 10.1038/s41390-021-01471-0.

52. Zeier J, Ombrellaro KJ, Perumal N, et al. Correlates and Determinants of Cardiorespiratory Fitness in Adults: a Systematic Review. *Sports Med Open* 2019; 5: 39. DOI: 10.1186/s40798-019-0211-2.

53. Silva LA, Silveira PC, Pinho CA, et al. N-acetylcysteine supplementation and oxidative damage and inflammatory response after eccentric exercise. *Int J Sport Nutr Exerc Metab* 2008; 18: 379–388. DOI: 10.1123/ijsnem.18.4.379.

54. Hong S, Lee J, Park J, et al. Association between cardiorespiratory fitness and the prevalence of metabolic syndrome among Korean adults: a cross sectional study. *BMC Public Health* 2014; 14: 481. DOI: 10.1186/1471-2458-14-481.

55. Rahimlou M, Asadi M, Banaei Jahromi N, et al. Alpha-lipoic acid (ALA) supplementation effect on glycemic and inflammatory biomarkers: A Systematic Review and meta-analysis. *Clin Nutr ESPEN* 2019; 32: 16–28. DOI: 10.1016/j.clnesp.2019.03.015.

56. Żychowska M, Grzybkowska A, Zasada M, et al. Effect of six weeks 1000 mg/day vitamin C supplementation and healthy training in elderly women on genes expression associated with the immune response - a randomized controlled trial. *J Int Soc Sports Nutr* 2021; 18: 19. DOI: 10.1186/s12970-021-00416-6.

57. Kerksick C and Willoughby D. The antioxidant role of glutathione and N-acetyl-cysteine supplements and exercise-induced oxidative stress. *J Int Soc Sports Nutr* 2005; 2: 38–44. DOI: 10.1186/1550-2783-2-2-38.

58. Seo JA, Song SW, Han K, et al. The Associations between Serum Zinc Levels and Metabolic Syndrome in the Korean Population: Findings from the 2010 Korean National Health and Nutrition Examination Survey. *PLoS One* 2014; 9: e105990. DOI: 10.1371/journal.pone.0105990.

59. Prasad AS. Zinc is an antioxidant and anti-inflammatory agent: Its role in human health. *Front Nutr.* 2014; 1: 14. DOI: 10.3389/fnut.2014.00014.

60. El Dib R, Gameiro OL, Ogata MS, et al. Zinc supplementation for the prevention of type 2 diabetes mellitus in adults with insulin resistance. *Cochrane Database Syst Rev* 2015; 5: CD005525. DOI: 10.1002/14651858.CD005525.pub3.

61. Abdul-Ghani MA and DeFronzo RA. Mitochondrial dysfunction, insulin resistance, and type 2 diabetes mellitus. *Curr Diab Rep* 2008; 8: 173–178. DOI: 10.1007/s11892-008-0030-1.

62. Chen Q, Chai YC, Mazumder S, et al. The late increase in intracellular free radical oxygen species during apoptosis is associated with cytochrome c release, caspase activation, and mitochondrial dysfunction. *Cell Death Differ* 2003; 10: 323–334. DOI: 10.1038/sj.sedd.4401148.

63. Papandreou I, Cairns RA, Fontana L, et al. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metab* 2006; 3: 187–197. DOI: 10.1016/j.cmet.2006.01.012.

64. Nakagami H, Morishita R, Yamamoto K, et al. Hepatocyte growth factor prevents endothelial cell death through inhibition of bax translocation from cytosol to mitochondrial membrane. *Diabetes* 2002; 51: 2604–2611. DOI: 10.2337/diabetes.51.8.2604.

65. Jin M, Yang J, Kannan R, He S, et al. Hepatocyte Growth Factor Protects RPE Cells from Apoptosis Induced by Glutathione Depletion. *Invest Ophthalmol Vis Sci* 2005; 46: 4311–4319. https://doi.org/10.1167/iovs.05-0353.

66. Ghasemi H, Tavilani H, Khodadadi I, et al. Circulating Betatrophin Levels Are Associated with the Lipid Profile in Type 2 Diabetes. *Chonnam Med J* 2015; 51: 115–119. DOI: 10.4068/cmj.2015.51.3.115.

67. Chen CC, Susanto H, Chuang WH, et al. Higher serum betatrophin level in type 2 diabetes subjects is associated with urinary albumin excretion and renal function.
68. Hu H, Yuan G, Wang X, et al. Effects of a diet with or without physical activity on angiopoietin-like protein 8 concentrations in overweight/obese patients with newly diagnosed type 2 diabetes: A randomized controlled trial. *Endocr J* 2019; 66: 89–105. DOI: 10.1507/endocrj.EJ18-0191.

69. Abu-Farha M, Abubaker J, Al-Khairi I, et al. Circulating angiopoietin-like protein 8 (betatrophin) association with HsCRP and metabolic syndrome. *Cardiovasc Diabetol* 2016; 15: 25. DOI: 10.1186/s12933-016-0346-0.

70. Hamed EA, El-Abaseri TB, Mohamed AO, et al. Hypoxia and oxidative stress markers in pediatric patients undergoing hemodialysis: Cross section study. *BMC Nephrol* 2012; 13: 136–142. DOI: 10.1186/1471-2369-13-136.

71. Elsayh KI, Zahran AM, El-Abasery TB, et al. Hypoxia biomarkers, oxidative stress, and circulating microparticles in pediatric patients with thalassemia in Upper Egypt. *Clin Appl Thromb Hemost* 2014; 20: 536–545. DOI: 10.1177/1076029612472552.

72. Alduraywish AA, Almaeen AH, El-Moukhedem NS, et al. Changes in Hypoxia, Glycemic Control and Antioxidants Biomarkers after 3,000 meter Steeplechase Running: Effect of Antioxidant Liver-Supportive Supplementation. *International Medical Journal* 2020a; 27: 284–288. https://seronjihou.files.wordpress.com/2021/06/273284-2.pdf

73. Alduraywish AA, Almaeen AH, Mobasher MA, et al. Systemic hypoxia biomarkers in asymptomatic simple non-alcoholic hepatosteatosis. *African Journal of Biomedical Research* 2020b; 23: 385–390. https://www.ajol.info/index.php/ajbr/article/view/202341