The Impacts of Ramadan Intermittent Fasting on Oxidant/Antioxidant Stress Biomarkers of Stable Chronic Obstructive Pulmonary Disease Male Patients

Sawssan Mrad, MD[1†], Hadhemi Rejeb, MD[2†], Jihene Ben Abdallah, MD[1], Hajer Graiet, PhD[3] (ID), Mouna Ben Khelifa, MD[2], Amel Abed, MD[1], Salima Ferchichi, MD[3], Khelifa Limem, MD[1], and Helmi Ben Saad, MD, PhD[4,5,6] (ID)

Abstract

No prior study has evaluated the impacts of Ramadan intermittent fasting (RIF) on oxidant/antioxidant stress (OS/AOS) biomarkers in patients with chronic obstructive pulmonary disease (COPD). The aim of this study was to assess the impacts of RIF on some OS/AOS biomarkers measured in male patients with stable COPD. Fifteen COPD patients (mean age: 71 ± 6 years) fasting Ramadan in 2017 volunteered to take part in the study. Three sessions (before Ramadan [BR], end Ramadan [ER], after Ramadan [AR]) were selected. Blood samples of OS (homocysteine [μmol/L], thiobarbituric acid reactive substances [TBARS, μmol/L]) and AOS (catalase [U/ml], ceruloplasmin [g/L], superoxide dismutase [SOD, ng/ml], zinc [μmol/L], albumin [g/L]) biomarkers were consistently taken 4.5 to 2.5 hr before the *iftar*. Findings were analyzed by applying Friedman or Kruskal–Wallis ANOVA. Comparisons of the number of patients with high OS [high homocysteine and/or TBARS] and low AOS (low catalase and/or ceruloplasmin and/or SOD and/or zinc and/or albumin) blood values between the three sessions were performed using the Cochran test. The median ± interquartile of homocysteine (BR: 21.48 [18.98–24.49], ER: 23.15 [21.77–26.45], AR: 24.87 [21.91–37.12]), ceruloplasmin (BR: 0.27 [0.24–0.30], ER: 0.28 [0.26–0.33], AR: 0.28 [0.25–0.32]), SOD (BR: 288.00 [112.00–400.00], ER: 182.00 [152.00–386.00], AR: 234.00 [190.00–420.00]) and the mean ± SD of TBARS (BR: 5.66 ± 1.26, ER: 4.59 ± 0.78, AR: 5.29 ± 1.69), catalase (BR: 120.97 ± 54.62, ER: 106.73 ± 50.92, AR: 137.39 ± 40.88), zinc (BR: 11.85 ± 2.01, ER: 12.47 ± 2.34, AR: 12.21 ± 2.58) and albumin (BR: 39.78 ± 3.19, ER: 40.74 ± 2.26, AR: 40.56 ± 2.38) were not significantly affected by RIF. The number of patients with high OS (BR [n = 13], ER [n = 15], AR [n = 14]) or low AOS (BR [n = 12], ER [n = 13], AR [n = 13]) statuses were not significantly influenced by RIF. In conclusion, RIF did not induce any significant statistical or clinical changes in OS/AOS biomarkers or statuses in COPD patients.

Chronic obstructive pulmonary disease (COPD) is the most important cause of morbidity and mortality worldwide bringing substantial and increasing social and economic problems (Global Initiative for Chronic Obstructive Lung Disease [GOLD], 2019). It is the outcome of a complex interaction of long-term cumulative exposure to noxious particles or gases, associated with a diversity of host factors (e.g., genetics, airways hyper-responsiveness, and poor lung growth during childhood; GOLD, 2019). Inhalation of such substances causes a chronic inflammatory response and oxidant stress (OS) in some patients' lungs, leading to the abnormalities characterizing COPD (Celli et al., 2015). OS is an important amplifying mechanism of COPD (Ben Moussa, Sfaxi, Tabka, Ben Saad, & Rouatbi, 2014). In the systemic circulation of COPD patients, the OS/antioxidant stress (AOS) balance is marked by an increase in OS biomarkers and a decrease in endogenous AOS (Ben Moussa, Rouatbi, & Ben Saad, 2016; Ben Moussa et al., 2014; GOLD, 2019). International scholarly societies such as

the American Thoracic Society and European Respiratory Society (Celli et al., 2015) recommend additional studies to elucidate the pathways driving OS leading to the abnormalities characterizing COPD.

Ramadan has great communal value among Muslims all over the world. During this holy month, Muslims are expected to refrain from all types of food and drink between dawn and sunset. Although there are several exemptions from Ramadan intermittent fasting (RIF), many COPD Muslim patients still choose to fast (Aydin et al., 2014). RIF involves specific dietary and lifestyle modifications, such as sudden changes in sleep cycle, physical activities, and eating schedules (i.e., eating one large meal after sunset and at least one lighter meal before dawn, food quantity and quality, nocturnal food consumption, and meal frequency; Barkia et al., 2011; Faris, Jahrami, Obaideen, & Madkour, 2019; Ramadan, 2002). These modifications may adversely affect the COPD patients' health if not addressed properly (Abolaban & Al-Moujahed, 2017). For example, the aforementioned lifestyle modifications can cause clinical circumstance to worsen due to a persistent gap between up-to-date expert information and decisive robust evidence regarding the pathophysiologic and metabolic modifications of fasting (Rejeb et al., 2018). Since personal beliefs affect patients' health behaviors and adherence to treatments, health-care providers need to learn how RIF can affect the health of their COPD Muslim patients and how to help them fast safely (Abolaban & Al-Moujahed, 2017). The possible effects of such spiritual beliefs on some biological biomarkers (e.g., inflammation and/or hematological and/or OS/AOS data) were not addressed by international scholarly societies (Celli et al., 2015; GOLD, 2019). According to a 2019 systematic review and meta-analysis including only healthy people (Faris et al., 2019), RIF provides some protection against elevated OS biomarkers, namely malondialdehyde (MDA). A 2017 systematic review including athletics ($n = 7$ studies), nonathletic healthy subjects ($n = 13$ studies) and unhealthy ones ($n = 10$ studies) concluded that RIF was less likely to enhance OS/AOS status in athletes. It has a beneficial impact on OS in nonathletic healthy subjects, and it improves OS/AOS status in patients with chronic conditions (Shephard,

2017). To the best of the authors' knowledge, no previous study has evaluated the effects of RIF on OS/AOS biomarkers in COPD patients.

The present study is the second part of a project aiming at evaluating the impacts of RIF on some biological data in COPD patients. The first part, evaluating the effects of RIF on hematological and inflammatory indices, has recently been published (Rejeb et al., 2018). It concluded that RIF had significant effects on hemoglobin, hematocrit, red and white blood cells values, but it did not induce any significant changes in inflammatory (erythrocyte sedimentation rate [ESR] and C-reactive protein [CRP]) and other hematological (platelets, mean corpuscular volume or hemoglobin) indices (Rejeb et al., 2018). Given the significant role of the balance between OS and AOS biomarkers in public health, especially in COPD fasting subjects, this study (representing the second part of the project) was carried out. Its aim was to investigate the impacts of RIF on some OS (homocysteine, thiobarbituric acid reactive substances [TBARS]) and AOS (catalase, ceruloplasmin, superoxide dismutase [SOD], zinc, albumin) biomarkers determined in male patients with stable COPD. As seen in diabetic and hypertensive patients (Al-Shafei, 2014a, 2014b), the null hypothesis was that RIF alleviates OS and increases AOS in COPD patients.

Population and Methods

Study Design and Population

Since a large part of the methodology was hitherto described (Rejeb et al., 2018), only a reminder of the main points of this section and the determination way of OS/AOS biomarkers will be described. The study was performed during the summer of 2017 (Ramadan started from May 27th and ended on June 24th). Approval for the study (number 405/2017) was granted from the Ethical Committee of the Farhat HACHED Hospital. All patients signed an informed written consent prior being included. Patients were individually informed about the study purposes, and were allowed to leave it any time they desired to. Patients were not charged any charges for the accomplished exams. The elapsed time

[1]Laboratory of Biochemistry, Farhat Hached Hospital, Sousse, Tunisia
[2]Pulmonary Department, Farhat Hached Hospital, Sousse, Tunisia
[3]Research Unit of Clinical and Molecular Biology (UR17ES29), University of Monastir, Tunisia
[4]Laboratory of Physiology and Functional Explorations, Farhat Hached Hospital, Sousse, Tunisia
[5]Heart Failure (LR12SP09) Research Laboratory, Farhat Hached Hospital, Sousse, Tunisia
[6]Laboratory of Physiology, Faculty of Medicine of Sousse, University of Sousse, Tunisia

†These authors contributed equally as first authors to this study.

Corresponding Author:
Helmi Ben Saad, Laboratory of Physiology, Faculty of Medicine of Sousse, Rue Mohamed Karoui, Sousse 4002, Tunisia.
Email: helmi.bensaad@rns.tn

from dawn to sunset was ~16.5 hr. The experimental design comprised the following three sessions: BR (7–12 days before Ramadan), ER (last 4 days of end Ramadan), and AR (14–18 days after Ramadan). During the BR session, all COPD patients answered some medical questionnaires, largely described in the work of Rejeb et al. (2018). During the three sessions, anthropometric, spirometric, and blood samples data were collected. During the 60-day study period (May 15th to July 13th), the ambient temperature and humidity means ± standard deviation (SD) were 27.3 ± 3.3°C and 62.1% ± 9.0%, respectively.

A convenience sample of 15 male patients with stable COPD completed the study. Their mean ± SD of age, height, body weight, and body mass index were, respectively, 71 ± 6 years, 1.68 ± 0.05 m, 71 ± 19 kg, and 25.4 ± 7.2 kg/m^2. During the three sessions, COPD patients were questioned regarding the schedules of their medication use. In order to avoid misinterpretation of OS/AOS biomarkers, COPD patients were asked to stop, momentarily, 1 week before each session, the use of some vitamin (e.g., vitamin A, C, and E; Rossman et al., 2013). COPD diagnosis was determined from a post-bronchodilator ratio between the forced expiratory volume in 1 s and the forced vital capacity <0.70 (Ben Saad et al., 2014; GOLD, 2019).

Blood Sample: OS and AOS Biomarkers and Applied Definitions

The blood sample was collected using a 20-ml syringe at the same time of the day (approximately 455 to 275 min before sunset). It was divided into four tubes: hematological, ESR, CRP, and OS (homocysteine, TBARS) as well as AOS (catalase, ceruloplasmin, SOD, zinc, albumin) biomarkers.

Homocysteine, an enzyme biosynthesized from methionine via a multistep process, can cyclize to give homocysteine thiolactone, a five-membered heterocycle. Due to this "self-looping" reaction, homocysteine-containing peptides tend to cleave themselves by reactions generating OS (Sibrian-Vazquez, Escobedo, Lim, Samoei, & Strongin, 2010). Homocysteinemia was evaluated by an immunoturbidimetric assay (COBAS INTEGRA 400 plus analyzer). Intra- and inter-assay precision coefficient of variation (CV) of the COBAS INTEGRA homocysteine values are 1.0% and 1.4%, respectively. Adults' normal homocysteinemia is 12–15 μmol/L. TBARS, a byproduct of lipid peroxidation, are the products of the damage produced by OS (Michel, Bonnefont-Rousselot, Mas, Drai, & Thérond, 2008). The TBARS level was evaluated according to the method of Yagi (1976). The TBARS plasma normal concentrations vary from 0 to 50 μmol/L (Michel et al., 2008). Patients with high OS status were those having hyperhomocysteinemia (>15 μmol/L) and/or high TBARS (>50 μmol/ml).

Catalase, an enzyme found in cells exposed to oxygen, catalyzes the decomposition of hydrogen peroxide (H_2O_2) to H_2O and oxygen (Góth, 1991). It protects the cell from OS by reactive oxygen species (ROS). The catalase activity was evaluated by decreasing H_2O_2 quantity at a wavelength of 240 nm using the spectrophotometry method (Góth, 1991). The normal values of catalasemia activity in healthy subjects is approximately 20.4 U/ml (Góth, 1991). Ceruloplasmin, an enzyme synthesized in the liver, is associated with oxidation of ferrous iron into ferric iron, thus assisting in its transport in the plasma in association with transferrin, which can carry iron only in the ferric state (Song & Dunaief, 2013). Ceruloplasminemia was evaluated by an immunoturbidimetric assay (COBAS INTEGRA 400 plus analyzer). Intra- and inter-assay precision CV of the COBAS INTEGRA ceruloplasmin values are 3.6% and 3.9%, respectively, at 0.2 g/L. Normal ceruloplasminemia is 0.2–0.6 g/L. SOD, an enzyme that alternately catalyzes the dismutation of the superoxide radical into either oxygen or H_2O_2, is an antioxidant defense in the cells exposed to oxygen (Hayyan, Hashim, & AlNashef, 2016). SOD level was determined by a BioVendor ELISA kit (Cu/Zn SOD Human ELISA). According to the manufacturers' guide, the blood SOD upper limit of normal value was 35.2 ng/ml. Intra- and inter-assay precision CV are 5.1% and 5.8%, respectively. Zinc is an AOS, an anti-inflammatory agent, an inhibitor of nicotinamide adenine dinucleotide phosphate oxidase, and a co-factor of SOD (Prasad, 2014). It induces the generation of metallothionein, which is very rich in cysteine and is an excellent scavenger of hydroxide. Zincemia level was determined by a manual kit RANDOX (colorimetric method) and normal values are 9.18–18.4 μmol/L (Prasad, 2014). Intra- and inter-assay precision CV are 3.8% and 6.1%, respectively, at 24 μmol/L. Serum albumin, the most abundant blood plasma protein, represents an important circulating AOS. Albuminemia was determined by the immunoturbidimetric assay (COBAS INTEGRA 400 plus analyzer) and its normal range is 35.6–46.1 g/L. Intra- and inter-assay precision CV are 1.83% and 1.36%, respectively, at 25 g/L. The patients with low AOS status, were those having hypocatalasemia (<20.4 U/ml) and/or hypoceruloplasminemia (<0.2 g/L), and/or low SOD (<35.2 ng/ml), and/or hypozincemia (<9.18 μmol/L), and/or hypoalbuminemia (<35.6 g/L).

Statistical Analysis

The Kolmogorov–Smirnov test was used to analyze the distribution of variables. When the distribution was normal and the variances were equal, the results were expressed by their mean ± *SD* and 95% confidence

Table 1. Impacts of Ramadan Intermittent Fasting on Oxidant/Antioxidant Stress Biomarkers of 15 Stable Chronic Obstructive Pulmonary Disease Male Patients.

	Before Ramadan	End Ramadan	After Ramadan	ANOVA
Oxidant stress biomarkers				
Homocysteine	21.48 (18.98–24.49)	23.15 (21.77–26.45)	24.87 (21.91–37.12)	0.315
(μmol/L)[a]	95% CI [18.11, 29.02]	95% CI [20.56, 32.21]	95% CI [22.03, 34.58]	
Thiobarbituric acid reactive substances	5.66 ± 1.26	4.59 ± 0.78	5.29 ± 1.69	0.083
(μmol/L)[b]	95% CI [4.96, 6.36]	95% CI [4.16, 5.02]	95% CI [4.35, 6.22]	
Antioxidant stress biomarkers				
Catalase	120.97 ± 54.62	106.73 ± 50.92	137.39 ± 40.88	0.243
(U/ml)[b]	95% CI [90.72, 151.22]	95% CI [78.54, 134.93]	95% CI [114.75, 160.03]	
Ceruloplasmin	0.27 (0.24–0.30)	0.28 (0.26–0.33)	0.28 (0.25–0.32)	0.914
(g/L)[a]	95% CI [0.23, 0.35]	95% CI [0.25, 0.31]	95% CI [0.25, 0.30]	
Superoxide dismutase	288.00 (112.00–400.00)	182.00 (152.00–386.00)	234.00 (190.00–420.00)	0.701
(ng/ml)[a]	95% CI [177.31, 472.42]	95% CI [165.47, 379.46]	95% CI [201.54, 441.66]	
Zinc	11.85 ± 2.01	12.47 ± 2.34	12.21 ± 2.58	0.768
(μmol/L)[b]	95% CI [10.74, 12.96]	95% CI [11.17, 13.76]	95% CI [10.78, 13.64]	
Albumin	39.78 ± 3.19	40.74 ± 2.26	40.56 ± 2.38	0.752
(g/L)[b]	95% CI [38.01, 41.54]	95% CI [39.49, 42.00]	95% CI [39.25, 41.88]	

Note. Data were [a]median (interquartile) (95% confidence interval) or [b]mean ± SD (95% confidence interval). ANOVA = Friedman or Kruskal–Wallis analysis of variance between the three sessions.

interval (95% CI). If the distribution was not normal, the results were expressed by their medians (interquartile; 95% CI). Qualitative data (high OS or low AOS statuses) were expressed by numbers. Comparisons of the OS/AOS biomarkers data were made between the three sessions. Results were obtained by applying repeated measures analysis of variance (Friedman or Kruskal–Wallis ANOVA). Comparisons of the number of COPD patients with high OS or low AOS statuses between the three sessions (BR, ER, and AR) were performed via the Cochran test (Q). Analyses were carried out using the Statistica software (Statistica Kernel version 6; StatSoft, Paris, France). Alpha was set at $p < .05$.

Results

Table 1 presents the OS/AOS biomarkers data of the 15 COPD patients. Its main conclusion was that RIF had no effect on the seven biomarkers.

The number of patients with high OS status (BR [$n = 13$], ER [$n = 15$], AR [$n = 14$]) or low AOS status (BR [$n = 12$), ER [$n = 13$], AR [$n = 13$]) were not significantly influenced by RIF ($Q = 3.00$; $df = 2$, $p < .223$; $Q = 0.33$, $df = 2$, $p < .846$, respectively).

Discussion

This present study, involving 15 males with stable COPD, identified that RIF did not bring about any significant statistical variations in their OS/AOS blood biomarkers or any significant clinical variations in their OS/AOS statuses.

The survival of aerobic tissues is provided by a delicate balance between the AOS defense mechanisms and the cellular systems generating OS (Sülü, Özturk, Güven, & Kiliç, 2010). OS is the set of intra- or extra-cellular situations leading to the metabolic or chemical generation of ROS or reactive nitrogen species (RNS; e.g., superoxide radicals, H_2O_2, hydroxyl radicals, lipid hydroperoxides; Ibrahim, Habib, Jarrar, & Al Baz, 2008). ROS and RNS can cause OS to vital cellular elements (e.g., membrane lipids, proteins, DNA) which means a vital menace to cell liveliness (Ibrahim et al., 2008; Squadrito & Pryor, 1998). The aerobic tissues are secured from OS by exogenous AOS available via nutrition (e.g., vitamin A, E, and C) and endogenous AOS (e.g., glutathione [GSH], glutathione peroxidase [GPx], catalase and SOD; Ibrahim et al., 2008). OS intermediated by ROS/RNS is involved as an important influencing factor in the process of aging as well as in several chronic conditions (e.g., cancer, COPD, diabetes, cardiovascular and degenerative diseases; Al-Shafei, 2014a, 2014b; Ibrahim et al., 2008; Packer & Cadenas, 2007; Shephard, 2017). Although the effects of RIF on OS/AOS biomarkers in healthy humans and/or in patients with chronic conditions have been reported (Adawi et al., 2017; Al-Shafei, 2014b; Asgary et al., 2000; BaHammam, Pandi-Perumal, & Alzoghaibi, 2016; Ibrahim et al., 2008; Shephard, 2017; Sülü et al., 2010), studies on its effects on OS/AOS biomarkers in patients with chronic respiratory diseases (e.g., COPD and/or asthma) are lacking. To the best of the authors' knowledge, this study is the first to include COPD patients. The main results of the studies including

athletic, nonathletic healthy and unhealthy subjects were summarized in two recent systematic reviews (Faris et al., 2019; Shephard, 2017).

Discussion concerning several points related to the methodology applied and/or results were addressed in some previous local papers (Ben Saad, 2018; Fenneni et al., 2014, 2015, 2017; Latiri et al., 2017; Rejeb et al., 2018; Zouari et al., 2018). Only the choice of OS/AOS biomarkers will be discussed in this study. In the systematic review and meta-analysis aiming to evaluate the impacts of RIF on OS biomarkers in healthy subjects, only MDA was tested. MDA, a biological marker commonly used to assess OS in COPD patients (Ben Moussa et al., 2014), is a highly toxic product associated with lipid peroxidation and react with protein and deoxyribonucleic-acid (Kanabrocki et al., 2006). Since MDA is the prototype of the so-called TBARS (Tsikas, 2017), in this study, the OS status was evaluated via TBARS values in addition to homocysteine. The latter had some merits during COPD. On the one hand, TBARS are sensitive OS biomarkers in early COPD (Zinellu et al., 2016) and were negatively associated with COPD spirometric data (Ochs-Balcom et al., 2006). On the other hand, homocysteinemia is significantly elevated in COPD patients and is related to severity (Seemungal et al., 2007). The AOS status was evaluated via the catalase, ceruloplasmin, SOD, zinc, and albumin data, which also had some advantages during COPD. First, catalasemia is proportionate to the COPD severity (Singh et al., 2017). Second, ceruloplasminemia increased in severe COPD and it may play a part in the pathogenesis of pulmonary emphysema (Pedersen & Franck, 1987). Third, SOD is a protein that protects the lungs from free radical damage and chronic inflammation (Oberley-Deegan, Regan, Kinnula, & Crapo, 2009). Fourth, zincemia of COPD patients is lower than that of healthy nonsmokers (Karul et al., 2003). Finally, in COPD patients, albumin levels decrease during the acute phase response due to the increase in the catabolism of albumin (Banh, 2006).

RIF represents an exceptional model to evaluate the impacts of consistent food restriction for a fixed time duration (8–18 hr) and for 29 or 30 days on different biochemical data of the human body (Faris et al., 2019). According to this study findings, RIF did not bring about any significant statistical changes to OS/AOS biomarkers in COPD patients or any significant clinical changes to their OS/AOS statuses. Given the pioneering nature of the present study, only the data from the studies aiming at evaluating the impact of RIF on OS/AOS biomarkers in healthy subjects and in patients with other frequent chronic conditions will be reported.

In the 2019 systematic review and meta-analysis (Faris et al., 2019), only four studies including healthy subjects were retained and only MDA values were reported. These four studies (Asgary et al., 2000; BaHammam et al., 2016; Ibrahim et al., 2008; Sülü et al., 2010) reported mixed results concerning MDA values (μmol/L): significant reduction was observed at ER compared with BR (1.73 ± 0.38 vs. 1.9 ± 0.51 in 50 Iranian males aged 30–60 years [Asgary et al., 2000]; 9.3 ± 3.2 vs. 5.8 ± 2.2 in 22 Turkish females aged 27 ± 4 years [Sülü et al., 2010]); no significant change was observed at ER compared with BR (1.31 ± 0.15 vs. 1.41 ± 0.13 in 14 Emiratis [9 males] aged 25–58 years [Ibrahim et al., 2008]; 8.9 ± 6.8 vs. 7.0 ± 3.2 in 23 Turkish males aged 30 ± 7 years [Sülü et al., 2010]), no significant change was observed after 2 weeks of fasting compared with BR (70.1 ± 32.9 vs. 75.5 ± 28.3 in eight Saudis aged 27 ± 5 years [BaHammam et al., 2016]). The authors of the systematic review and meta-analysis calculated the MDA "Hedge's g" to be 0.219 with a low heterogeneity assessment ($I^2 = 0.0\%$), and they concluded that the impacts of RIF in terms of a decrease in OS biomarkers was small, suggesting that healthy Muslims fasters may develop some short-term protection against low-grade systemic OS (Faris et al., 2019). The aforementioned four studies also reported conflicting results concerning some additional OS/AOS biomarkers. First, while albumin mean values (g/L) increased between BR and ER sessions (44 ± 2 vs. 46 ± 2 for males; 42 ± 3 vs. 44 ± 2 for females, respectively) in Turkish subjects (Sülü et al., 2010), similar mean values (41.8 ± 5.4 vs. 39.8 ± 5.0, respectively) were noted in Emiratis subjects (Ibrahim et al., 2008). Second, while some AOS biomarkers (i.e., α- and ϒ-tocopherol, retinol, vitamin C, α- and β-carotene, lycopene, lutein, and zeaxanthin) remained unchanged between BR and ER sessions, a significant reduction of some others (i.e., total plasma carotenoids and β-cryptoxanthin) was noted in the Emiratis study (Ibrahim et al., 2008). Third, in the Turkish study (Sülü et al., 2010), GSH values (mmol/L) decreased in males (BR: 1.1 ± 0.7; ER: 0.9 ± 0.6) and increased in females (BR: 0.8 ± 0.1; ER: 1.0 ± 0.6). Fourth, no significant change was observed concerning blood values of conjugated dienes (an OS biomarker; BR: 0.43 ± 0.15, ER: 0.45 ± 0.15 μmol/L [Asgary et al., 2000]). Lastly, the authors of an Iranian study (23 healthy subjects [16 males] aged 25–65 years) observed an insignificant impact of RIF on H_2O_2 mean ± standard error data measured at ER when compared with BR (80 ± 4 vs. 81 ± 5%, respectively; Delpazir et al., 2015). Several hypotheses were advanced to explain the divergence of results (BaHammam et al., 2016; Sülü et al., 2010): different socioeconomic conditions between countries, different nutrition styles, immune system and metabolic structure of people, hormonal difference between males and females, differences in climate, variations in the season in which Ramadan falls, health status of the subjects, lack of control over

potential confounders that may affect the OS/AOS balance (e.g., meal composition, physical activity, sleep duration, fasting duration, smoking status, medication use).

In patients with chronic conditions, and contrarily to the present study findings, accumulative investigations support the health benefits of RIF on several organs/systems (Adawi et al., 2017; Al-Shafei, 2014b; Shephard, 2017). Two examples are highlighted. First, it seems that RIF alleviates OS in diabetic patients: compared to BR, the ER blood MDA level decreased by 46.6%, however, the ER GSH level increased by 59.4%. Moreover, AR MDA, and GSH biomarkers were maintained, respectively, lower by 22.7% and higher by 31.3% (Al-Shafei, 2014b). Second, it appears that RIF also ameliorates OS in hypertensive patients: compared to BR, the ER blood MDA and GSH levels were, respectively, lowered by 45.6% and raised by 56.8%. Furthermore, AR, MDA, and GSH biomarkers were maintained, respectively, lower by 24.3% higher by 30.2% (Al-Shafei, 2014a). The 2017 systematic review concluded that RIF improves OS/AOS status of patients with clinical conditions (e.g., hypertension, diabetes, polycystic ovary syndrome, cardiac or cerebrovascular diseases, metabolic syndrome, obesity; Shephard, 2017): among the 10 studies including patients with chronic diseases, 8 studies identified an improvement of OS status during RIF, one study ($n = 56$ patients with stable cardiac disease) identified a nonsignificant trend to a decrease of CRP during RIF; and the other study ($n = 82$ patients with coronary or cerebrovascular disease or metabolic syndrome) registered no change in either CRP or homocysteine concentrations.

Why Did OS/AOS Biomarkers and Statuses in COPD Remain Unchanged During RIF?

Two hypotheses could be advanced to explain the stability of OS/AOS biomarkers and statuses during the three sessions. The first one is connected to the relationship between body weight and blood OS level. In fact, increased body weight is associated with increased levels of OS in the human body (Fernández-Sánchez et al., 2011). For example, when adipose tissue increases, the activity of AOS enzymes (e.g., SOD, catalase, GPx) significantly diminishes (Fernández-Sánchez et al., 2011). In this study, there was no significant impact of RIF on the body weight of the 15 COPD patients (BR: 71 ± 19; ER: 71 ± 19, AR: 72 ± 19 kg, $p = .679$; Rejeb et al., 2018). The second hypothesis is related to the relationship between COPD systemic inflammation and OS biomarkers (Bailey, Goraya, & Rennard, 2012; Mosrane et al., 2017; Tkacova, Kluchova, Joppa, Petrasova, & Molcanyiova, 2007). Among the different mediators of

the systemic inflammation (circulating inflammatory cells, inflammatory mediators, growth factors, etc.), OS keeps a crucial place (Bailey et al., 2012; Tkacova et al., 2007). For example, erythrocytic GPx activity was inversely related to neutrophil count and high-sensitivity CRP (Tkacova et al., 2007). In this study, RIF did not induce any significant changes in the median (lower-upper quartiles) inflammatory data of ESR (BR: 3 [2–9], ER: 7 [0–13], AR: 9 [5–15] mm/hr, $p = .884$) or CRP (BR: 20 [11–38], ER: 15 [9–34], AR: 20 [12–46] mg/L, $p = .349$; Rejeb et al., 2018).

Study Limitations

This study presents four limitations. First, the convenience sampling was a confounding factor (Sousa, Zauszniewski, & Musil, 2004). It can lead to the under/over representation of particular groups inside the population (e.g., 12/15 patients belonged to GOLD D (i.e. high symptom burden and high risk of exacerbation) [Rejeb et al., 2018]). Second, the noninclusion of a control group of nonfasting COPD patients is a limitation since the validity of this study results cannot be completely linked to RIF (Latiri et al., 2017; Rejeb et al., 2018). In this regard, it has to be highlighted that reaching nonfasting groups in Muslim states is challenging, due to religious ideologies (Rejeb et al., 2018). Third, it was desirable to assess the COPD patients' nourishment. In fact, dietary aberrations could influence the OS/AOS biomarkers (Lu et al., 2018; Matin, Nemati, Ghobadi, Alipanah-Moghadam, & Rezagholizadeh, 2018). For example, it was demonstrated that oligomeric proanthocyanidins supplementation was effective in increasing the AOS capacity in patients with COPD (Lu et al., 2018). Fourthly, it was desirable to estimate the physical activity status of the patients since it impacts the OS/AOS balance (Ryrsø et al., 2018). For example, exercise training was proved to induce an upregulation of muscle AOS capacity (Ryrsø et al., 2018).

This study including 15 stable COPD patients fasting the 2017 holy month of Ramadan, concluded that RIF did not induce any significant statistical or clinical changes in OS/AOS biomarkers or statuses.

List of Abbreviations

ANOVA: Analysis of variance
AOS: Antioxidant stress
AR: After-Ramadan
BR: Before-Ramadan
CI: Confidence interval
COPD: Chronic obstructive pulmonary disease
CRP: C-reactive protein
CV: Coefficient of variation
ER: End-Ramadan
ESR: Erythrocyte sedimentation rate

GPx: Glutathione peroxidase
GSH: Glutathione
H$_2$O$_2$: Hydrogen peroxide
MDA: Malondialdehyde
OS: Oxidant stress
RIF: Ramadan intermittent fasting
ROS: Reactive oxygen species
SD: Standard deviation
SOD: Superoxide dismutase
TBARS: Thiobarbituric acid reactive substances
RNS: Reactive nitrogen species

Authors' Contributions

SM, HR, and HBS: Literature search, data collection, study design, data analysis, manuscript preparation, and review of manuscript.
JBA, MBK, HG, AA, SF, and KL: Study design, data analysis, manuscript preparation, and review of manuscript.
All authors read and approved the final manuscript.

ORCID iDs

Hajer Graiet [iD] https://orcid.org/0000-0002-5274-5325

Helmi Ben Saad [iD] https://orcid.org/0000-0002-7477-2965

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