

Effects of 16:8 intermittent fasting on inflammatory markers and oxidative stress in obese women

Effets du jeûne intermittent 16:8 sur les marqueurs inflammatoires et le stress oxydatif chez les femmes obèses

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ABSTRACT

Background: The efficiency of intermittent fasting (IF) in promoting weight loss and improving metabolic parameters in obese individuals has been demonstrated. This study aims to evaluate the impact of intermittent fasting on inflammatory and oxidative stress parameters compared to continuous caloric restriction (CCR), three months after intervention.

Methods: Over three months, we enrolled obese women practicing intermittent fasting (16/8) and compared them to a group following continuous caloric restriction. Anthropometric characteristics were measured using a bioelectrical impedance meter, and biological parameters, including inflammatory markers (hs-CRP and interleukin-6) and oxidative stress markers (8-hydroxyguanosine and 8-isoprostaglandin F_{2α}), were evaluated before and after the intervention.

Results: The mean age and initial body mass index (BMI) were 35.5±9.13 years and 38.3±4.8 kg/m² in the IF group, and 36.3±9.27 years and 36.8±4.3 kg/m² in the CCR group, respectively. Weight loss was statistically significant in both groups. However, no effect was observed on inflammatory markers (hs-CRP and interleukin-6) in either group. Similarly, regarding oxidative stress markers, the changes in plasma concentrations of 8-OHdG (from 78.3±50.2 pg/ml to 85.7±39.3 pg/ml in the CCR group and from 81.7±26.8 pg/ml to 92.3±36.3 pg/ml in the IF group) and 8-iso-PGF_{2α} (from 240±176 pg/ml to 194±146 pg/ml in the CCR group and from 194±153 pg/ml to 184±176 pg/ml in the IF group) were not statistically significant.

Conclusion: Despite its positive effects on weight and metabolic parameters, intermittent fasting has not demonstrated efficacy in reducing inflammation and oxidative stress in obese women.

Keywords: Obesity ; Intermittent fasting ; Low-calorie diet ; Inflammation, Oxidative stress

RÉSUMÉ

Contexte : L'efficacité du jeûne intermittent (JI) pour favoriser la perte de poids et améliorer les paramètres métaboliques chez les personnes obèses a été démontrée. Cette étude vise à évaluer l'impact du jeûne intermittent sur les paramètres de stress inflammatoire et oxydatif, par rapport à la restriction calorique continue (CCR), trois mois après l'intervention.

Méthodes : Pendant trois mois, nous avons recruté des femmes obèses pratiquant le jeûne intermittent (16/8) et les avons comparées à un groupe suivant une restriction calorique continue. Les caractéristiques anthropométriques ont été mesurées à l'aide d'un indicateur d'impédance bioélectrique, et des paramètres biologiques, y compris les marqueurs inflammatoires (hs-CRP et interleukine-6) et les marqueurs de stress oxydatif (8-hydroxyguanosine et 8-isoprostaglandine F_{2α}), ont été évalués avant et après l'intervention.

Résultats : L'âge moyen et l'indice initial de masse corporelle (IMC) étaient respectivement de 35,5±9,13 ans et 38,3±4,8 kg/m² dans le groupe FI, et respectivement 36,3±9,27 ans et 36,8±4,3 kg/m² dans le groupe CCR. La perte de poids était statistiquement significative dans les deux groupes. Cependant, aucun effet n'a été observé sur les marqueurs inflammatoires (hs-CRP et interleukine-6) dans l'un ou l'autre groupe. De même, concernant les marqueurs de stress oxydatif, les variations des concentrations plasmatiques de 8-OHdG (de 78,3±50,2 pg/ml à 85,7±39,3 pg/ml dans le groupe CCR et de 81,7±26,8 pg/ml à 92,3±36,3 pg/ml dans le groupe FI) et de 8-iso-PGF_{2α} (de 240±176 pg/ml à 194±146 pg/ml dans le groupe CCR et de 194±153 pg/ml à 184±176 pg/ml dans le groupe FI) n'étaient pas statistiquement significatives.

Conclusion : Malgré ses effets positifs sur le poids et les paramètres métaboliques, le jeûne intermittent n'a pas démontré d'efficacité pour réduire l'inflammation et le stress oxydatif chez les femmes obèses.

Mots clés: Obésité, Jeûne intermittent, Inflammation, Oxydatif de contrainte

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INTRODUCTION

Obesity has become one of the leading global public health concerns. According to the World Health Organization (WHO), its prevalence has tripled since 1975, affecting 16% of the global adult population in 2022 (1). In Tunisia, the prevalence of obesity has nearly doubled over the past two decades, increasing from 14% in 1997 to 26% in 2016 (2). This chronic condition, associated with low-grade inflammation, has serious health implications, including an increased risk of cardiovascular diseases, type 2 diabetes, and other metabolic disorders. Chronic inflammation and oxidative stress, resulting from an imbalance between the production of free radicals and antioxidant defense mechanisms, are key contributors to obesity-related complications (3). The study of oxidative stress in obese individuals often involves assessing various markers, such as reactive oxygen species (ROS), enzymatic antioxidants (e.g., superoxide dismutase and catalase), DNA damage markers (e.g., 8-hydroxydeoxyguanosine), and lipid peroxidation levels measured by biomarkers like malondialdehyde (MDA) (4). These markers provide valuable insights into the altered redox balance associated with obesity and can serve as targets to evaluate the effectiveness of nutritional interventions and bariatric surgery (5, 6).

Among dietary approaches for weight management and metabolic health, 16:8 intermittent fasting has gained attention for its ability to promote weight loss and improve insulin sensitivity (7, 8). However, its potential to modulate inflammatory status remains controversial. While some studies have reported reductions in pro-inflammatory markers such as C-reactive protein (CRP) and interleukin-6 (IL-6) (9), others have found no significant changes (10). Moreover, its impact on oxidative stress markers in obese individuals remains an underexplored area.

To our knowledge, no study in Tunisia has evaluated the impact of 16:8 intermittent fasting on inflammatory and oxidative stress markers in obese individuals. In this context, we conducted this study with the primary objective of comparing the effects of 16:8 intermittent fasting and continuous calorie restriction on inflammatory and oxidative stress markers in obese women after a 3-month follow-up.

METHODS

Study design

This study was a non-randomized, controlled, interventional clinical trial involving two parallel groups over a three-month intervention period. The two groups were matched for age and body mass index (BMI). It was conducted at the Obesity Research Unit of the National Institute of Nutrition and Food Technology in Tunis between October 2022 and March 2023.

Participants

The inclusion criteria included women aged 18 to 50 years

with a BMI ≥ 30 kg/m² who provided informed consent to participate in the study and adhere to its protocol. Women were excluded if they were pregnant, breastfeeding, or menopausal; had a diagnosis of diabetes, hypertension, or thyroid disorders (hyperthyroidism or hypothyroidism); or were taking medications (e.g., corticosteroids) or specific supplements (e.g., probiotics) that could influence weight changes. Additionally, those with a history of bariatric surgery, recent dietary modifications within the 12 weeks preceding the study, severe eating disorders, or psychiatric illnesses were not eligible.

Participants were withdrawn from the study if they became pregnant, discontinued the diet for more than one week, became unreachable, developed an acute illness (e.g., viral or bacterial infection) or any intercurrent condition during follow-up, or were newly diagnosed with diabetes or hypertension requiring treatment.

Study protocol

Each participant in the study received individualized care provided by the obesity unit team, comprising nutritionists and dietitians.

The protocol consisted of several phases. Initially, a baseline evaluation (T0) was conducted, followed by the prescription of a therapeutic diet accompanied by educational counseling. Regular follow-up appointments were scheduled every 15 days to monitor progress and reinforce adherence to the prescribed diet. After a 3-month follow-up period (T1), a re-evaluation was performed, including the assessment of anthropometric measurements and biological parameters.

The baseline evaluation included an interview and a physical examination, which comprised anthropometric assessments. These assessments involved measurements of height, weight, body composition, and waist circumference. BMI was calculated using the formula weight (kg) / height (m²) and interpreted according to the WHO classification. Obesity classes I, II, and III were defined based on BMI ranges of 30–34.9, 35–39.9, and ≥ 40 kg/m², respectively (11). Abdominal obesity was defined as a waist circumference ≥ 80 cm in women (12). Body composition parameters, including fat mass and lean mass, were measured at baseline and after the 3-month intervention using a TANITA BC-418M bioelectrical impedance analyzer.

Dietary Intervention

Each patient received personalized nutritional guidance tailored to their specific needs and preferences, as determined by their dietary assessment. All patients were provided with dietary prescriptions that offered a choice between intermittent fasting (IF) and continuous calorie restriction (CCR). The dietary prescriptions for all participants included:

- Reducing spontaneous caloric intake by 25%.
- Moderately increasing protein intake to 1–1.2 grams per kilogram of body weight per day.
- Limiting saturated fat intake to 7% of total energy intake (TEI).

- Prioritizing the consumption of carbohydrates with a low or medium glycemic index.
- Ensuring adequate dietary fiber intake of 25–30 grams per day.
- Restricting the consumption of high-energy-density foods, particularly those rich in fats or sugars, and avoiding sugary beverages.
- Encouraging the selection of low-energy-density foods, such as fruits and vegetables, while maintaining adequate hydration by consuming sufficient water.
- Controlling portion sizes and promoting mindful eating by encouraging slower meal consumption. Furthermore, patients following intermittent fasting were provided with the following guidelines:
- Limiting the daily eating window to 8 hours, thereby extending the overnight fasting period to 16 hours.
- Distributing energy intake across two meals and one snack within the designated eating window.
- Prohibiting the consumption of food or caloric beverages during the fasting period while allowing water, tea, and black coffee without sugar.

Adherence to intermittent fasting was defined as maintaining the food intake window for at least 5 days per week. To promote adherence, participants were contacted weekly by phone and met in person every two weeks. During each check-in, patients underwent an evaluation of anthropometric parameters, and their adherence to intermittent fasting (IF) and dietary recommendations provided during the initial consultation was assessed to address any dietary errors.

All patients were advised to engage in moderate physical activity daily, such as a 30 to 60-minute walk or a physical activity of their choice

Biological Assessment

A fasting blood sample was collected after an 8-hour fast to assess metabolic parameters, including fasting blood glucose and glycated hemoglobin. Insulin resistance was evaluated using the HOMA-IR (Homeostasis Model Assessment of Insulin Resistance) index, calculated by multiplying fasting insulin ($\mu\text{U/dL}$) by fasting blood glucose (mmol/L) and dividing the result by 22.5. Insulin resistance was defined as a HOMA-IR index of 2.4 or higher (13).

The lipid profile analysis included measurements of total cholesterol (TC), triglycerides (TG), and HDL-cholesterol (HDL-C).

High-sensitivity C-reactive protein (hs-CRP), and interleukin-6 (IL-6), both markers of systemic inflammation, were measured using high-sensitivity assays.

Oxidative stress markers, including 8-hydroxyguanosine (8-OHdG) (pg/mL) and 8-isoprostaglandin $\text{F}_2\alpha$ (8-iso-PGF $_{2\alpha}$) (pg/mL), were analyzed using the ELISA method before and three months after the dietary intervention. 8-OHdG is a biomarker of oxidative DNA damage, while 8-iso-PGF $_{2\alpha}$ reflects lipid peroxidation and is considered a reliable indicator of systemic oxidative stress.

Ethics Statement

This project was approved by the Ethics Committee of the National Institute of Nutrition and Food Technology in Tunis (02/2021). Prior to inclusion, each participant was provided with detailed information regarding the study protocol and signed an informed consent form. All procedures were conducted in compliance with applicable guidelines and regulations, ensuring the ethical integrity of the research.

The trial was registered in the Pan African Clinical Trial Registry under the number PACTR202409898073796.

Statistical analysis

Statistical analysis was carried out using SPSS statistical package version 27.0 (IBM SPSS Inc., New York, NY, USA). Comparison between CCR and IF groups were made by independent-samples T test for continuous variables (Values are expressed as mean $\pm$ standard deviation). The differences in the distribution between groups were evaluated by Pearson Chi-square test for categorical variables (Values are expressed as percentage). Comparisons within each group (Baseline vs. 3 months) and Cohen's d (measures the size of the difference between two groups) for tested variables were evaluated using paired-samples t-test. Interaction (Time $\times$ Group) was tested using two-way repeated measures ANOVA that determined the F-statistic value for the interaction test between time and group and calculated Partial eta-squared (η^2p) was as a measure of effect size. Furthermore, the interaction (Time $\times$ Group) using two-way repeated measures ANOVA was tested while adjusting on confounding factor (professional status marital status and socioeconomic status)

RESULTS

The mean age was 35.5 ± 9.13 years in the IF group and 36.3 ± 9.27 years in the CCR group. The initial BMI was $38.3 \pm 4.8 \text{ kg/m}^2$ in the IF group and $36.8 \pm 4.3 \text{ kg/m}^2$ in the CCR group.

In the IF group, two-thirds of the patients had a middle or low socio-economic status, compared to 94.7% in the CCR group. The table 1 shows the characteristics of the population.

Weight change was characterized by a statistically significant weight loss of $-7.76 \pm 4.24 \text{ kg}$ in the IF group ($p = 0.01$) and $-3.48 \pm 4.02 \text{ kg}$ in the CCR group ($p = 0.01$). This weight reduction was significantly greater in the IF group than in the CCR group ($p = 0.001$).

Assessment of the impact of IF on metabolic parameters revealed a statistically significant decrease in the HOMA-IR index in the IF group ($p < 10^{-3}$), whereas no significant change was observed in the CCR group.

The evolution of inflammatory markers showed no significant changes in IL-6 and hs-CRP in either group. Similarly, no significant changes were observed in plasma concentrations of 8-OHdG and 8-iso-PGF $_{2\alpha}$ between T0 and T3 months in both groups (Table 2).

Table 1. Baseline characteristics of the population.

Characteristic	CCR group (n=19) n(%)	IF group (n=26) n(%)	p
professional status			
In profession	2 (10,5)	10 (38,5)	0,036
Housewife	17 (89,5)	16 (61,5)	
Physical activity			
Low	9 (47,4)	13 (50,0)	0,984
Moderate	7 (36,8)	9 (34,6)	
High	3 (15,8)	4 (15,4)	
Marital status			
Single	13 (68,4)	7 (26,9)	0,017
Married	6 (31,6)	17 (65,4)	
Widowed	0 (0)	2 (7,7)	
Socioeconomic status			
Low	2 (10,5)	8 (30,8)	0,004
Middle	16 (84,2)	9 (34,6)	
High	1 (5,3)	9 (34,6)	
Educational level			
Illiterate	0 (0)	1 (3,8)	0,753
Primary	3 (15,8)	6 (23,1)	
Secondary	6 (31,6)	7 (26,9)	
University	10 (52,6)	12 (46,2)	
Obesity class			
I	9 (47,4)	6 (23,1)	0,206
II	5 (26,3)	12 (46,2)	
III	5 (26,3)	8 (30,8)	

IF=Intermittent Fasting ; CCR= Continuous Caloric Restriction.

DISCUSSION

Our study evaluated the effects of 16:8 IF on anthropometric parameters, metabolic markers, and selected inflammatory and oxidative stress markers compared to Continuous Caloric Restriction (CCR) over a 3-month period. The results showed that while both groups experienced weight loss and improved insulin resistance, these effects were significantly greater in the IF group than in the CCR group. However, no significant changes were observed in inflammatory markers (hs-CRP and IL-6) or oxidative stress status (8-Iso-PGF2 α and 8-OHdG levels) in both groups. Our results align with those of several authors who suggest that intermittent fasting not only contributes to better weight management but also improves glycemic control by reducing insulin resistance and enhancing lipid profiles (8, 14).

Despite the weight loss and improvement in insulin sensitivity observed in our study, inflammatory markers showed no significant changes in hs-CRP in either group and a non-significant decrease in IL-6 in the IF group. Studies indicate that normal levels of IL-6 do not exclude ongoing low-grade inflammation, complicating the interpretation of these biomarkers in clinical settings (15). Research has shown that IL-6 and CRP levels can be influenced by various factors, including chronic diseases and lifestyle, which may obscure their role as specific indicators of low-grade inflammation.

Table 2. Comparative Analysis of Changes in Anthropometric and biological parameters between CCR and IF Groups over Time

	CCR group (n=19)				IF group (n=26)				Interaction (Time Group)		
	Baseline	Three months	p	Effect size	Baseline	Three months	p	Effect size	F	η^2_p	p
Weight, Kg	93.9 $\pm$ 13.3	90.5 $\pm$ 13.9	0.001	0.87	102 $\pm$ 10.8	94.8 $\pm$ 9.7	<0.001	1.82	11.5	0.211	0.001
Waist Circumference, cm	107.0 $\pm$ 10.7	105 $\pm$ 10.5	0.003	0.78	117 $\pm$ 11.6	110 $\pm$ 10.7	<0.001	2.21	34.4	0.444	<0.001
BMI, Kg/m²	35.8 $\pm$ 4.3	34.0 $\pm$ 4.9	0.003	0.79	38.3 $\pm$ 4.8	35.4 $\pm$ 4.5	<0.001	1.88	3.89	0.083	0.055
Fat mass, Kg	40.2 $\pm$ 10.1	38.6 $\pm$ 10.3	0.031	0.54	47.5 $\pm$ 8.0	42.7 $\pm$ 7.8	<0.001	1.42	10.6	0.198	0.002
Lean mass, kg	53.7 $\pm$ 4.1	52.0 $\pm$ 4.7	0.004	0.76	51.1 $\pm$ 5.4	49.5 $\pm$ 4.8	0.082	0.36	0.01	0.001	0.945
Total cholesterol, mmol/l	5.14 $\pm$ 1.4	4.59 $\pm$ 0.7	0.051	0.48	4.87 $\pm$ 0.8	4.40 $\pm$ 0.7	<0.001	1.08	0.09	0.003	0.761
Triglycerides, mmol/l	1.05 $\pm$ 0.4	0.96 $\pm$ 0.4	0.059	0.46	1.17 $\pm$ 0.5	0.88 $\pm$ 0.4	<0.001	0.94	4.69	0.098	0.036
HDL-C, mmol/l	1.33 $\pm$ 0.3	1.22 $\pm$ 0.2	0.025	0.60	1.22 $\pm$ 0.2	1.32 $\pm$ 0.2	0.033	-0.44	8.49	0.172	0.006
FBG, mmol/L	5.02 $\pm$ 0.3	4.93 $\pm$ 0.4	0.270	0.26	5.70 $\pm$ 0.5	5.37 $\pm$ 0.5	<0.001	1.08	6.61	0.133	0.014
Hba1C, %	5.33 $\pm$ 0.2	5.26 $\pm$ 0.3	0.040	0.51	5.55 $\pm$ 0.4	5.32 $\pm$ 0.4	<0.001	0.98	6.34	0.128	0.016
Insulinemia, μU/dl	16.0 $\pm$ 6.2	13.1 $\pm$ 4.6	0.009	0.67	21.1 $\pm$ 8.6	12.18 $\pm$ 4.9	<0.001	1.41	13.1	0.234	<0.001
HOMA-IR	3.57 $\pm$ 1.4	2.89 $\pm$ 1.2	0.008	0.69	5.39 $\pm$ 2.4	2.92 $\pm$ 1.3	<0.001	1.44	16.5	0.278	<0.001
Hs-CRP, mg/L	3.58 $\pm$ 2.9	4.82 $\pm$ 5.36	0.127	0.37	6.86 $\pm$ 6.3	6.81 $\pm$ 6.0	0.490	0.14	2.6	0.057	0.114
IL-6, pg/ml	1.25 $\pm$ 0.4	1.31 $\pm$ 0.3	0.9	0.15	0.94 $\pm$ 0.3	0.68 $\pm$ 0.2	0.35	0.13	2.5	0.097	0.214
8-OHdG, pg/ml	78.3 $\pm$ 50.2	85.7 $\pm$ 39.3	0.149	-0.35	81.7 $\pm$ 26.8	92.3 $\pm$ 36.3	0.148	-0.29	0.26	0.006	0.615
8-iso-PGF2α, pg/ml	240 $\pm$ 176	194 $\pm$ 146	0.340	0.23	194 $\pm$ 153	184 $\pm$ 176	0.540	0.12	0.25	0.006	0.623

IF=Intermittent Fasting; CCR= Continuous Caloric Restriction, HOMA-IR= Homeostatic Model Assessment of Insulin Resistance; hs-CRP= High-sensitivity C-reactive protein; 8-OHdG= 8-hydroxyguanosine; 8-iso-PGF2=8-isoprostaglandin F2 α . F-statistic value for the interaction test between time and group. η^2_p : Partial eta squared, a measure of effect size. It represents the proportion of total variance attributable to a particular factor.

Intermittent fasting (IF) has gained significant attention for its potential effects on inflammation in obese individuals, but findings remain inconsistent across studies. While some clinical trials have reported reductions in inflammatory markers following IF interventions, others have found no significant changes. This discrepancy may be attributed to variations in IF protocols, study durations, sample sizes, and participant characteristics. Liu et al. reported that after eight weeks of IF, levels of TNF- α , IL-6, and IL-10 showed no significant changes (16). Similarly, changes in CRP levels have been assessed in several trials, but most studies did not observe significant differences (9, 10). Lauridsen et al. also found no significant changes in TNF- α , IL-6, and IL-10 following an IF intervention (17). However, Mao et al. reported that after five weeks of IF, lower levels of TNF- α and IL-8 were observed (18). A systematic review analyzing IF studies in different populations suggests that IF may have immunomodulatory effects in obese individuals (19). Several mechanisms may contribute to these effects. In obesity, IF may exert anti-inflammatory effects by regulating macrophages, reducing oxidative stress through hormone secretion, and modulating the expression of oxidative stress-related genes. These mechanisms highlight the potential benefits of IF beyond weight loss, particularly in inflammation and immune regulation.

Regarding oxidative stress markers, 16:8 IF had no effect on oxidative stress. The changes in plasma concentrations of 8-OHdG and 8-iso-PGF2 α were not statistically significant. We observed a non-significant increase in plasma 8-OHdG levels and a non-significant decrease in plasma 8-iso-PGF2 α concentrations between T0 and T3 months in both groups. Few studies have assessed the impact of nutritional interventions on oxidative stress in obese subjects as 8-OHdG has renal elimination and interindividual variability (20). A study by Johnson et al. (21) investigated the effects of an alternate-day caloric restriction diet on obese asthmatic patients, focusing on their symptoms, lung function, and markers of oxidative stress and inflammation. Ten subjects with a BMI > 30 kg/m² followed an 8-week diet, consuming unlimited food on one day and less than 20% of their usual caloric intake on the next (320 Kcal/day for women and 380 Kcal/day for men). The authors reported significant reductions in 8-isoprostane levels, explaining that 8-iso-PGF2 α is a marker of lipid oxidation, and intermittent fasting can reduce lipid peroxidation, thereby decreasing oxidative stress. Lipid peroxidation induced by oxidative stress alters the structure and fluidity of biological membranes, ultimately affecting their function. Commonly studied markers of lipid peroxidation include isoprostanes such as 8-iso-prostaglandin F2 α (8-iso-PGF2 α), malondialdehyde (MDA), thiobarbituric acid-reactive substances (TBARS), and 4-hydroxynonenal (HNE) (20). The 8-iso-PGF2 α , with a structure similar to prostaglandins, is primarily produced from esterified arachidonic acid through non-enzymatic reactions catalyzed by free radicals. It can contribute to platelet activation (22).

The impact of dietary approaches on 8-OHdG remains controversial. However, it is important to note that 8-OHdG levels can be influenced by factors other than oxidative stress, such as inflammation and nucleic acid

metabolism (23). Therefore, the interpretation of results should be made with caution, considering these potential confounding factors. IF can improve inflammatory profiles but it depends on duration and individual metabolic status (24-25). More research is required to understand the long-term effects and optimal fasting durations for sustained inflammatory benefits (25).

The study by Bosch-Sierra et al. (5), published in 2024, aimed to assess the impact of weight loss through caloric restriction on metabolic profiles, inflammatory markers, and oxidative stress parameters in metabolically healthy (MH) and metabolically unhealthy (MUH) obese individuals. A total of 74 participants (34 MS and 40 MNS) followed two cycles of dieting: a very low-calorie diet (654 Kcal/day) alternating with a hypocaloric diet (500 Kcal/day reduction) for 24 weeks. Oxidative stress markers were measured at the beginning and end of the intervention. After the intervention, improvements in insulin resistance, a significant reduction in inflammatory markers, and an increase in antioxidant capacity and glutathione were observed, with more pronounced effects in the MUH group. However, 8-OHdG levels significantly decreased only in the MH group, while superoxide dismutase (SOD) levels showed no significant changes.

Heilbronn et al. (27) conducted a randomized controlled trial involving healthy, sedentary men and women (N = 48). Participants were randomly assigned to one of four groups and followed for 6 months (M6): control group (weight maintenance diet); caloric restriction group (CR: 25% caloric restriction from baseline energy needs); caloric restriction with exercise group (CR: 12.5% caloric restriction plus 12.5% increase in energy expenditure from physical activity); very low-calorie diet group (890 kcal/day until 15% weight loss, followed by weight maintenance). Results showed that DNA damage was significantly reduced from baseline in the CR, CR with exercise, and very low-calorie diet groups (-0.56, -0.45, and -0.35, respectively, at M6, $p < 0.005$), but not in the control group.

Oxidative stress leads to the accumulation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in atherosclerotic plaques. This process may contribute to the development of vasculopathy and atherosclerosis by inducing cellular senescence through DNA damage, both telomeric and non-telomeric. Additionally, RNA can undergo significant oxidation, resulting in the formation of 8-hydroxyguanosine (8-oxoGuo). This may cause ribosomal dysfunction, the formation of non-functional or truncated proteins, and reduced levels of functional proteins. These processes contribute to complications associated with oxidative stress, particularly in vasculopathy and atherosclerosis (20).

Study Strengths and Limitations:

This study is the first in Tunisia to compare the effects of 16:8 intermittent fasting and continuous caloric restriction on inflammatory and oxidative stress markers in a group of obese women. It was conducted in a specialized obesity management unit with a sample size (42 obese women) larger than that of most studies. Participants received personalized, multidisciplinary care involving a physician, a nutritionist, and a psychologist, with regular follow-ups

every 15 days to monitor and reinforce adherence to dietary prescriptions.

This study has some limitations. It was a non-randomized clinical trial, as each participant freely chose the dietary approach that best suited her lifestyle. Nutritional intake was not reassessed to verify adherence to dietary prescriptions. Additionally, the study population consisted exclusively of women, which may limit the generalizability of the findings. The selection of oxidative stress markers is crucial in designing and interpreting clinical trials assessing the effects of different dietary approaches in obese individuals. Redox reactions in the body are complex and involve numerous biochemical processes. Therefore, the use of inappropriate or insufficiently sensitive markers may lead to incorrect conclusions about the actual impact of nutritional interventions on oxidative stress. Since some research look into the IF for six months or longer, the three-month period might be considered as a limit.

CONCLUSION

Even though obese women in our study lost a substantial amount of weight, intermittent fasting (16:8) had less effect on oxidative stress or inflammation markers. Long-term studies with larger sample sizes, careful selection of appropriate inflammatory and oxidative stress markers, and a focus on the effect of the eating window may be needed to address gaps in the literature, better understand the true impact of intermittent fasting on inflammation and oxidative stress, and more effectively guide clinical recommendations for optimal dietary approaches to weight management and cardio-metabolic health in obese individuals.

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