Time-restricted eating on inflammation and oxidative stress in overweight, older adults

by

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B.S. Azad University, 2013

M.S., University of Leeds, 2018

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Food, Nutrition, Dietetics, and Health

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Abstract

The aim of this dissertation was to provide innovative insights into the effects of various dietary interventions on inflammation, oxidative stress, and metabolic health. Chapter 1 presents a comprehensive review discussing the rationale for assessing inflammation and oxidative stress in the context of time-restricted eating (TRE). This review suggests the potential effects of TRE on pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), IL-6, IL-8, and IL-10, as well as oxidative stress related to Alzheimer's disease in humans. Chapter 2 reviews the importance of different types of intermittent fasting (IF) regimens, including alternate-day fasting, the 5:2 diet, time-restricted eating, and Ramadan fasting, in mitigating inflammation and lowering oxidative stress. It highlights the potential of IF regimens in attenuating inflammatory responses. Chapter 3 presents a systematic review comparing the effects of isocaloric IF versus daily caloric restriction (DCR) on weight loss and metabolic risk factors for chronic noncommunicable diseases in adults with overweight and obesity. This review synthesized findings from randomized controlled trials and found that the effects of IF regimens on plasma lipid, glucose, insulin levels, HbA1c, and inflammatory markers were highly variable but generally comparable with DCR. IF (4:3 and 5:2 diets) was superior to DCR for improving insulin sensitivity in two studies. Reductions in body fat were significantly greater with IF (5:2 diet and TRE) than with DCR in two studies. Given the limited number of long-term studies using isocaloric or eucaloric IF interventions compared with control groups, future studies should incorporate more rigorous controls across intervention arms, as well as controls for energy intake and balance, nutrient composition, and weight loss. In Chapter 4, we conducted another systematic review on whether TRE confers additional benefits through mechanisms beyond calorie restriction (CR) alone or whether the benefits of TRE are related to unintentional reductions in calorie intake. The

reviewed outcomes included anthropometric and metabolic outcomes (lipid levels, fasting blood glucose, HOMA-IR, and blood pressure). A novel finding of this review was that longer daily fasting periods (≥16 hours), combined with moderate CR, resulted in significant improvements in measures of insulin resistance (HOMA-IR) in five out of six studies where it was assessed. In contrast, CR alone did not improve this marker in short- or mid-term interventions (8 to 52 weeks) without a specific dietary intervention (e.g., Mediterranean diet). Greater weight loss and reductions in diastolic blood pressure were noted with CR + TRE compared with CR alone after 8 to 14 weeks, whereas one study reported greater improvements in triglycerides and glucose tolerance with CR + TRE (3 days/week) compared with CR alone following 26 weeks. Other metabolic outcomes did not significantly differ between the two interventions. Chapter 5 presents a secondary analysis of a pilot study investigating the effects of TRE on inflammation (TNF-alpha, IL-1beta, IL-6, high-sensitivity C-reactive protein) and oxidative stress (8-isoprostane) in older adults with overweight. No significant differences were found in the outcomes of interest compared to baseline after four-week TRE (16:8) intervention. A short-term TRE intervention produced reductions in TNF-α [43.2 (11.2) pg/ml to 39.7 (10.0) pg/ml ($p = 0.101$)] with a Cohen's d effect size of 0.33 and IL-1β levels [1.4 (0.8) pg/ml to 1.3 (0.6) pg/ml ($p = 0.499$)] with a Cohen's d effect size of 0.23, indicating potential anti-inflammatory benefits. Consistent with previous studies, IL-6 and hs-CRP levels showed no significant changes. Oxidative stress marker 8-isoprostane levels decreased slightly from 39117.6 (12787.5) pg/ml to 38694.7 (11631.4) pg/ml ($p = 0.919$), with a Cohen's d effect size of 0.07.

Collectively, these findings provide initial insights into the potential effects of TRE on inflammatory and oxidative stress markers in older adults. Well-powered larger trials with longer durations are warranted to elucidate the potential of TRE in aging populations.

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Collectively, these findings provide initial insights into the potential effects of TRE on inflammatory and oxidative stress markers in older adults. Well-powered larger trials with longer durations are warranted to elucidate the potential of TRE in aging populations.

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Dedication

I dedicate this dissertation to all those who are in search of answers to improve public health and longevity, not for prestige or respect, but for the betterment of humanity. Like the gambler in Sting's song "**Shape of My Heart**," who plays the cards to discover the hidden patterns and laws of probability, a true scientist gambles their life in pursuit of deeper truths, not for personal gain, but to make a meaningful impact on the world.

Preface

This dissertation explores the effects of time-restricted eating (TRE) on inflammation and oxidative stress, two critical factors in health and aging. As a dietary intervention, TRE has gained significant attention for its potential to improve metabolic outcomes. The primary aim of this work is to investigate the effects of TRE on inflammatory markers and oxidative stress levels in the aging population.

While the core focus of this dissertation is on TRE, it is important to place this regimen within the broader context of intermittent fasting (IF) strategies. To this end, two review articles included in this dissertation compare various types of IF, such as alternate-day fasting (ADF), the 5:2 diet, and TRE, with a specific emphasis on their effects on metabolic health, inflammation, and oxidative stress.

These comparative analyses provide a comprehensive framework for understanding how different IF regimens affect health outcomes, allowing for a more informed assessment of TRE's unique benefits. By juxtaposing TRE with other IF interventions, this dissertation aims to highlight the specific benefits of TRE in reducing inflammation and oxidative stress, thereby contributing to the optimization of dietary interventions for improved health and longevity.

Chapter 1 – Introduction

Rationale for Assessment of Inflammation and Oxidative Stress

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The percentage of older adults is increasing rapidly worldwide, and it is expected that the number of older people globally will double by 2050 (United Nations Department of Economic and Social Affairs, Population Division, 2020). The unprecedented growth of the aging population has increased the incidence of age-related neurodegenerative diseases like dementia (GBD Neurology Collaborators, 2019). AD is the most prevalent neurodegenerative disease affecting over 50 million aging people worldwide (Blaikie et al., 2022). MCI is a pre-stage for dementia that is reversible (Shimada et al., 2019). The incurable nature of dementia has thus led to an urgent need for lifestyle interventions that can delay the onset and progression of the disease. Accumulating data from clinical and animal studies indicate that intermittent fasting (IF)—an approach in which individuals fast periodically—may be useful for the prevention and treatment of neurodegenerative diseases (Hadem et al., 2019; Fontana et al., 2021). Recently, time-restricted eating (TRE)—a form of IF—has gained much attention as it does not involve calorie counting. TRE involves limiting the eating window to 8- to 12-h with fasting—drinking only water and calorie-free coffee/tea— for 12 to 16 h within a 24-h cycle (Anton et al., 2021). Yet, the impact of TRE on human brain, specifically in people with neurological disorder is still unclear. The potential benefits of TRE on age-related cerebromicrovascular dysfunction—which has a pivotal role in vascular cognitive impairment—has already been investigated (Balasubramanian et al., 2020). TRE is suggested to restore endothelial function and neurovascular coupling response through attenuation of oxidative stress and inflammation (Hatori et al., 2012; Chaix et al., 2014). In addition, TRE may improve blood-brain barrier disruption which has a role in cognitive function improvement (Balasubramanian et al., 2020). In this review, we examine the possible underlying mechanisms of potential neuroprotective effects of TRE and relevant existing human research on the effects of TRE on markers of mild cognitive impairment (MCI) and AD.

The mechanisms underlying the potential neuroprotective benefits of TRE

The mechanism underlying the potential health benefits of TRE remains to be fully understood (see Fig. 1). One possible mechanism through which TRE may induce cognitive improvement arose from findings that suggests TRE regulates circadian rhythm and autophagy through aligning food intake with circadian rhythm (Hatori et al., 2012; Jamshed et al., 2019; Ulgherait et al., 2021; Zeb et al., 2021). The circadian clock coordinates metabolism and physiological functions including glucose, insulin sensitivity, lipid levels, energy expenditure, inflammation, sleep and cognitive function (Wyatt et al., 1999; Poggiogalle et al., 2018; Stenvers et al., 2019; Wang and Li, 2021); thereby circadian rhythm dysregulation is common in sleep disorders and Alzheimer's disease (Wu et al., 2006; Coogan et al., 2013; Hoyt and Obrietan, 2022). TRE also activates the metabolic switch, which occurs 12–36 h after fasting is initiated and free fatty acids are released into the blood (Anton et al., 2021). Results of animal and human pilot studies indicate that the metabolic switch may optimize brain health by increasing the levels of ketone, brain-derived neurotrophic factor (BDNF), fibroblast growth factor-2 (FGF2), Sirtuin1&3, autophagy ad DNA damage (Mattson et al., 2018; Jamshed et al., 2019) which results in enhanced cerebrovascular and cognitive function. Moreover, it may reduce glucose, leptin, insulin, inflammatory cytokines, and the mammalian target of rapamycin (mTOR) pathway activity, which results in improving cognition and preventing dysfunction and degeneration of neurons, thus protecting the brain from neurodegenerative diseases like Alzheimer's disease. β-hydroxybutyrate (BHB)—a ketone which is the product of the metabolic switch—serves as a signaling molecule that upregulates BDNF expression in neurons and thereby improves synaptic plasticity (Hood et al., 2016; Mattson et al., 2018; Hu et al., 2018). In addition, IF upregulates autophagy in cerebral cortical and cerebellar neurons (Alirezaei et al., 2010), through inhibiting mTOR protein synthesis pathway, and activation of adaptive cellular stress response signaling

pathways that promote mitochondrial health, and DNA repair (Longo and Mattson, 2014; Mattson et al., 2017). In laboratory animals, TRE induced BHB, reduced blood glucose, and improved sleep-phase in AD mice compared to the control (Whittaker et al., 2021). Similarly, TRE combined with 30–40 % calorie restriction lowered the accumulation of Aβ plaques in App-mutant mice (Wang et al., 2005; Patel et al., 2021), and IF improved cognitive impairment in the triple-transgenic mouse model of AD (Halagappa et al., 2007). Additionally, TRE (with ad libitum intake) produces weight loss (1–4 % with ad libitum intake and ≥ 5 % when combined with CR) in adults with obesity (Gabel et al., 2018; Ezzati et al., 2022). Accumulating evidence from systematic reviews and meta-analysis suggest the association of overweight and obesity with cognitive decline and increased risk of AD and vascular dementia (Pedditizi et al., 2016; Tang et al., 2021), while weight loss improves cognitive function in adults with overweight and obesity (Veronese et al., 2017). Therefore, weight loss induced by TRE may be another pathway which may play a role in cognitive improvement. 3. The effects of TRE on sleep and cognitive decline Sleep disorders are common in both MCI and AD (Pak et al., 2020). Similarly, insomnia and sleep apnea are associated with AD (Osorio et al., 2011; Kuo et al., 2020). Sleep disturbances are known as a major risk factor for AD and linked with inflammation (Hansson et al., 2006; Alvarez et al., 2007). We note that in examining existing studies on TRE on sleep and cognitive decline, the studies conducted have solely focused on one or the other, and not considered both together. To the best of our knowledge, no study has yet investigated the effects of TRE on sleep disturbances in humans; however, the effects of TRE on sleep in healthy individuals have been examined in nine studies (Gill and Panda, 2015; Hutchison et al., 2019; Gabel et al., 2018; Parr et al., 2020; Kesztyüs et al., 2020; Cienfuegos, 2021; Lowe et al., 2020; Wilkinson et al., 2020; Xie et al., 2022) (see Table 1). The Pittsburgh Sleep Quality Index (PSQI) was carried out to assess sleep quality and disturbances in six trials (Gabel et al., 2018; Parr et al., 2020; Cienfuegos, 2021; Lowe et al., 2020; Wilkinson et al., 2020; Xie et al., 2022). No trial reported significant improvement in sleep quality using the PSQI survey. In the most

recent trial, a 5-week randomized controlled trial (RCT) by Xie et al. (2022) showed no significant change in sleep quality between early TRE (fasting between 6 a.m.–3 p.m.), mid-day TRE (11 a.m.–8 p. m.) and control (ad lib intake) in 82 healthy subjects without obesity but the sleep quality improvement was greater in early TRE group (PSQI: $\Delta = -1.08 \pm 1.78$ vs. $\Delta = -0.22 \pm 2.19$ and $\Delta = -0.36 \pm 1.73$, respectively). In addition to the PSQI survey, Wilkinson et al. (2020) assessed sleep quality with the myCircadianClock app and reported significant improvement in sleep quality (23 %) following a 12-week single arm intervention of 10-h TRE (Wilkinson et al., 2020). Following a 16-week TRE intervention, Gill and Panda (2015) reported improved sleep duration and weight loss of 4 % in eight overweight and obese subjects; however, the study used a self-assessment survey which is not considered a validated tool for measuring sleep duration. Evidence suggests that >5 % weight loss can improve sleep quality in obese individuals (Verhoef et al., 2013; Martin et al., 2016). Conversely, achieving 10 % weight loss can be detrimental and increases the risk of obstructive sleep apnea (Peppard et al., 2000). None of the aforementioned studies reported >5 % weight loss versus baseline; however, TRE is believed to be associated with sleep quality independent of weight loss (Kesztyüs et al., 2020). Furthermore, in a cross-sectional study of 1226 older adults (≥64 years), a longer fasting period in TRE approach (≥12 h fasting) was associated with significantly higher sleep duration which was measured with questionnaires (Estrada-deLeon ´ et al., 2021). To date, no trial has tested the effects of TRE on cognitive decline in human AD subjects. However, the potential neurocognitive benefits of TRE in people with AD have been investigated in preliminary observations studies (Ooi et al., 2020; Currenti et al., 2021) (see Table 1). Ooi et al., 2020 tested the effects of a unique form of TRE in which fasters practiced fasting from dawn to sunset only two days per week (Monday and Thursday; no food or drink) on cognitive function among elderly people (>60 years old) with MCI in a cohort study of 3-year duration. Elderly people with MCI who regularly practice IF (2-d TRE/week) for 12 months demonstrated a significant improvement in cognitive scores (using five types of cognitive tests) as compared to irregular

and non-fasters. Moreover, a significant improvement in the antioxidant superoxide dismutase levels, inflammatory markers, and DNA damage was reported in regular faster groups versus baseline following 36 months period follow up. In a recent cross-sectional study conducted by Currenti et al. (2021), the association between TRE and cognitive status in a cohort of elderly Italian adults above 50 years old (n = 916) was investigated. Elderly individuals who practiced TRE (<10 h eating window) had lower rates of cognitive impairment versus individuals with ad libitum intake [odds ratio (OR) = 0.28; 95 % confidence intervals (CI): 0.07–0.90]. Similarly, breakfast eaters demonstrated lower risk of cognitive impairments (OR = 0.37, 95 % CI: 0.16–0.89). In contrast, those having dinner did not show such association. These results indicate the importance of timing of eating in TRE interventions and its relationship with cognition in humans.

The effects of TRE on inflammation & oxidative stress

Plasma interleukins and TRE have been explored in six RCTs in those with age ranges between 44 and 67 years (see Table 1) (Sutton et al., 2018; Martens et al., 2020; Cienfuegos, 2021; Zouhal et al., 2020; Moro et al., 2021; Xie et al., 2022). Only one study (Moro et al., 2021) tested IL-1β levels and plasma IL-6 levels were measured in five TRE interventions (Sutton et al., 2018; Martens et al., 2020; Cienfuegos, 2021; Zouhal et al., 2020; Moro et al., 2021); Of which two trials showed meaningful changes in IL-6 levels following TRE interventions (Zouhal et al., 2020; Moro et al., 2021). Significant improvements in IL-1β and IL-6 levels were shown in the TRE group (3 meals;1 p.m.,4 p.m. and 8 p. m.) compared to normal diet control (3 meals; 8 a.m., 1 p.m., and 8 p.m.) in 20 healthy subjects after 12 months (Moro et al., 2021). Similarly, Zouhal et al. (2020) carried out a one-month RCT of Ramadan TRE (from down to sunset; no drinking) in 28 obese men. The study reported a significant reduction in IL-6 levels in Ramadan TRE group vs Control. Following a 5-week intervention, IL-8 reduced significantly in early TRE group who fasted after 3 pm until 6 am compared to control (Xie et al., 2022). TNF-α was tested

in four RCTs which reported inconsistent results (Cienfuegos, 2021; Zouhal et al., 2020; Moro et al., 2021; Xie et al., 2022). While TNF-α levels remained unchanged in the study by Cienfuegos (2021), it reduced significantly in studies by Moro et al. (2021), Zouhal et al. (2020) and Xie et al. (2022). The latter study reported significant improvement in only early TRE arm compared to control and no meaningful change in mid-day TRE vs control (Xie et al., 2022). Oxidative stress has been thought to play a role in neurodegenerative diseases (Jensen et al., 2021). Levels of oxidative damage correlate significantly with the neurodegenerative impairment in various populations (Jensen et al., 2021). 8-Isoprostane is a marker of oxidative stress that may be a surrogate biomarker of the mitochondrial health in AD. To date, only two trials examined plasma levels of 8-Isoprostane in relation to TRE (Sutton et al., 2018; Cienfuegos, 2021) and 8-Isoprostane significant declined in both studies. Sutton et al. (2018) examined early TRE (e-TRE) intervention (6-h eating window, last meal before 3 pm) in 12 prediabetic men for 5 weeks and reported significant reduction (14 %) in 8-Isoprostane in e-TRE arm as compared to the control. In consistent with these findings, another TRE trial compared the effects of two forms of TRE [4-h TRE (3–7 p.m.) vs 6-h TRE (1–7 p.m.)] vs control and demonstrated significant reductions in both interventions group vs control [37 % and 34 %, respectively] (Cienfuegos, 2021).

Gaps in Current Knowledge

Neuroinflammation plays a critical role in the pathogenesis of AD (Heneka et al., 2015). Evidence indicates that inflammation promotes pathological processes that lead to AD (McCaulley and Grush, 2015; Tarkowski et al., 2003). Accumulating data suggest that proinflammatory cytokines including TNF-α, IL-1β, IL-6, IL-8 and IL-10 increase in individuals with AD and MCI versus healthy subjects (Bettcher et al., 2018; Guzman-Martinez et al., 2019; Angiulli et al., 2021; Ogunmokun et al., 2021). Interleukins contribute with complex intercellular interactions in neurons, microglia, astrocytes, and intracellular signal transduction events

(Stamouli and Politis, 2016). Elevated levels of pro-inflammatory markers including TNF-α, IL-1β, and IL-6, is linked with amyloid beta (Aβ) in brains of people with AD (Wang et al., 2015). Thus, the abnormal accumulation of Aβ induces excessive release of inflammatory cytokines resulting in neuronal and synaptic dysfunction and cognitive decline. Oxidative stress which occurs in the brain with aging is also associated with inflammation and believed to have a key role in MCI and AD (Nunomura and Perry, 2020).

Previous studies in both animal and humans suggest that CR and TRE reduces DNA damage by attenuating telomere erosion through regulating nuclear factor kappa B and the production of proinflammatory markers like C-reactive protein (Fann et al., 2014; Ooi et al., 2020). TRE has been reported to improve cognitive decline by downregulating inflammatory responses (Ooi et al., 2020). Although one of the limitations of included studies is that some TRE trials did not indicate the time that fasting initiated, preliminary evidence from short-term trials suggest that early- TRE can improve TNF-α, oxidative stress, BDNF and sleep quality which are the markers of MCI and AD (Jamshed et al., 2019; Sutton et al., 2018; Xie et al., 2022). Another limitation is that most trials have been conducted in healthy young people, which limits the generalizability of their findings to other age groups. Given that TRE was reported to improve cognition in animal models (Hernandez et al., 2022), the impact of TRE on cognition in humans, specifically in people with cognitive decline needs to be investigated further. Future clinical studies are needed to investigate the role TRE can play in relation to sleep disturbances and cognition decline in AD.

Conclusion

Conclusion TRE appears to be promising for its potential to reduce the markers of aging and neurodegenerative disease. Nevertheless, the mechanisms for these benefits are poorly understood. Moreover, the optimal time to initiate fasting needs to be elucidated in future trials. The potential benefits of TRE in neurodegenerative diseases like MCI and AD in the context of sleep should be further investigated.

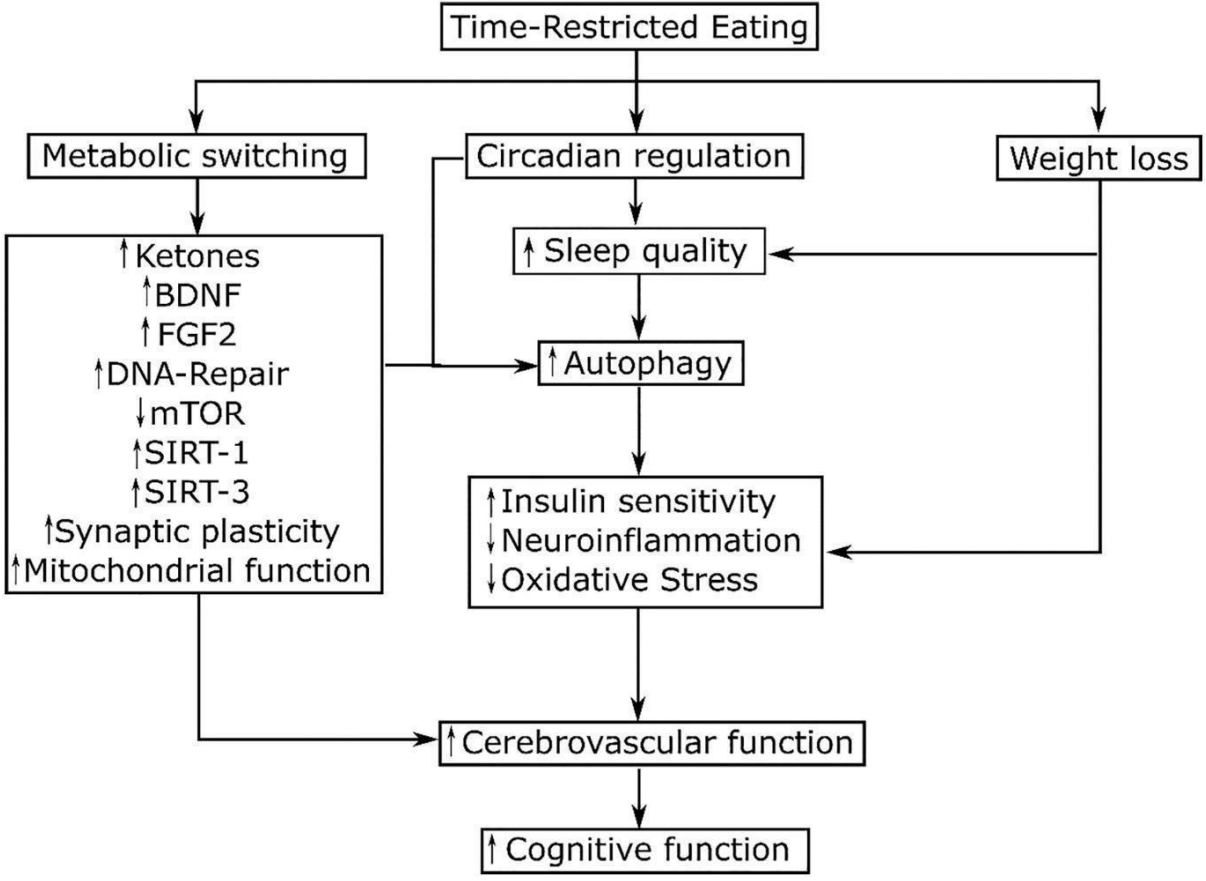

Fig. 1.1. The proposed mechanisms through which time-restricted eating may induce neuroprotective benefits.

Table 1-1: Summary of human studies on the effects of time-restricted eating on sleep, cognitive decline, and neuroinflammation.

Study	Intervention	Participants	Sleep measurement	Cognitive measurement	Markers (plasma/CSF)	Results
Cienfuegos et al. (2020)	**8-week RCT** 4-h TRE (3–7 p.m.) vs. 6-h TRE (1–7 p.m.) and control	49 obese adults	-	-	Plasma 8-Isoprostane, TNF-α, IL-6	↓8-Isoprostane levels in both intervention groups (4-h and 6h-TRE) No significant changes in TNF-α, and IL-6 levels
Cienfuegos et al. (2021)	**8-week RCT** Delayed TRE: 4-h TRE (3–7 p.m.) vs. 6-h TRE (1–7 p.m.) and control	58 obese adults	PSQI; Sleep quality, duration, insomnia severity and obstructive sleep apnea	-	-	No significant changes in sleep quality, duration, insomnia severity, or the risk of obstructive sleep apnea.
Currenti et al. (2021)	**Cross-sectional** TRE (<10h eating window) vs >10 h eating period	883 older adults	-	Short Portable Mental Status Questionnaire (SPMSQ)	-	Those practicing TRF were less likely to have cognitive impairment vs non-TRE; Similarly, breakfast consumption was associated with lower cognitive impairment prevalence
Gabel et al. (2019)	**12-week single arm** 8-h TRE (10 a.m.–6 p.m.)	23 obese adults	PSQI; Sleep quality, duration	-	-	No significant changes in sleep quality, and duration
Gill and Panda. (2015)	**16-week Single-arm:** 10-h TRE (self-selected eating window)	8 overweight and obese	Sleep duration Using self-assessment survey	-	-	↑sleep duration
Hutchison et al. (2019)	**1-week RT-Crossover** 9-h TRE (8 a.m.–5 p.m.) vs. 9-h TRE (12–9 p.m.)	15 overweight and obese males	Sleep duration was assessed by accelerometry	-	-	No significant change in sleep duration
Jamshed et al. (2019)	**4-day RT-Crossover** e-TRE (8 a.m. and 2 p.m. vs control (8 a.m. and 8 p.m.)	11 overweight adults	-	-	Ketones, BDNF, and gene expression in whole blood cells	↑ Ketones, and the expression of the stress response and aging gene *SIRT1* and the autophagy gene *LC3A* (all $p < 0.04$), in e-TRE in the morning ↑ BNDF ($p = 0.10$) and the expression of *MTOR* ($p = 0.007$) in the evening in e-TRE

Kesztyus et al. (2020)	**12-week Single-arm** 8–9-h TRE (self-selected eating window)	99 overweight and obese adults	Sleep quality was assessed by VAS Sleep duration self-reported survey	-	-	↑sleep quality (↑10 points) No change in sleep duration
Martens et al. 2020	**6-week RCT:** TRE (16/8) without weight loss vs control	22 healthy non-obese older adults	-	NIH Cognition Battery	Plasma IL-6, oxidized-LDL or the oxidized-to-total LDL ratio, acetoacetate and β-hydroxybutyrate	No significant changes in IL-6, oxidized-LDL or the oxidized-to-total LDL ratio, acetoacetate and β-hydroxybutyrate No improvement in cognitive performance
Moro et al. (2021)	**12-month RCT** TRE (3 meals;1 p.m.,4 p.m. and 8 p.m.) vs ND (8 a.m-8 p.m.)	20 healthy athletic males	-	-	Plasma TNF-α, IL-1β**,** IL-6 and 8-Isoprostane	↓TNF-α, IL-1β**,** IL-6 and 8-Isoprostane vs baseline and control
Lowe et al. (2020)	**12-week RCT** 8-h delayed TRE (12–8 p.m.) vs. Control	116 overweight and obese adults	Sleep quality was assessed by PSQI Sleep duration was assessed by Oura ring	-	-	No significant changes in sleep quality, and duration
Ooi et al. (2020)	**36-month longitudinal study** regularly practicing down to sunset TRE 2-d/w (r-IF), vs irregularly practicing TRE (i-IF), and non-fasters (n-IF)	99 older adults with MCI	-	Five types of cognitive tests: the Malay version of the Mini Mental Examination State (MMSE), Malay version of Montreal Cognitive Assessment (MoCA), Rey Auditory Verbal Learning Test (RAVLT), Digit Span Test, and Digit Symbol.	superoxide dismutase activity, malondialdehyde, DNA damage, plasma CRP	↑ cognitive improvements in older people with MCI in r-IF group vs i-IF and n-IF ↓superoxide dismutase activity, malondialdehyde, DNA damage, and CRP levels in r-IF vs i-IF and n-IF
Parr et al. (2020)	**4-week single-arm** 9-h TRE (10 a.m.– 7 p.m.)	19 overweight and obese adults With type 2 diabetes	Sleep quality and duration was assessed by PSQI	-	-	No significant change in sleep quality/duration

Sutton et al. (2018)	**5-week RCT** early-TRE (6-hr, last meal before 3 pm) vs control (12-hr. eating window)	12 prediabetic men	-	-	Plasma 8-Isoprostane, and IL-6	↓8-Isoprostane vs control No significant changes in IL-6
Xie et al. (2022)	**5-week RCT** e-TRE (6 a.m.–3 p.m.) vs mid-day TRE (11 a.m.–8 p.m.) vs control	82 healthy adults	Sleep quality was assessed by PSQI	-	Plasma TNF-α, IL-8	↓TNF-α, IL-8 in e-TRE group vs control ↑ sleep quality in early TRE vs mid-day TRE and control
Zouhal et al. (2020)	**30-day RCT** Ramadan TRE (down to sunset) vs control	28 obese men	-	-	Plasma TNF-α, IL-6	↓TNF-α, and IL-6 in Ramadan-TRE vs control ↓TNF-α in control vs baseline

Abbreviations: RCT: Randomized controlled/comparative trial; TRE: Time restricted eating; TNF-α: Tumor necrosis factor α; IL-6: Interleukin-6; PSQI; Pittsburgh Sleep Quality Index; e-TRE: Early-time-restricted eating; BDNF: brain-derived neurotrophic factor; MTOR: Mammalian target of rapamycin; LDL: Low-density lipoprotein; IL-1β: Interleukin-1β; CRP: C-reactive protein; IL-8: Interleukin-8.

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Chapter 2 - Importance of Intermittent Fasting Regimens and Selection of Adequate Therapy on Inflammation and Oxidative Stress in SARS-CoV-2 Infection

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Abstract

The unpredictable nature of new variants of coronavirus 2 (SARS-CoV-2)-highly transmissible and some with vaccine-resistance, have led to an increased need for feasible lifestyle modifications as complementary therapies. Systemic inflammation is the common hallmark of communicable diseases like severe coronavirus disease 2019 (COVID-19) and non-communicable chronic diseases (NCDs) such as obesity, cardiovascular diseases (CVD), diabetes mellitus, and cancers, all for which mitigation of severe outcomes is of paramount importance. Dietary quality is associated with NCDs, and intermittent fasting (IF) has been suggested as an effective approach for treatment and prevention of some NCDs, similar to that of caloric restriction. There is a paucity of high-quality data from randomized controlled trials regarding the impact of IF and the intake of specific nutrients on inflammation and post-infection outcomes in patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The current review of recent literature was performed to explore the immunomodulatory roles of IF regimens and supplements involving the intake of specific nutrients including vitamins (A, B, C, D, and E), zinc, and nutraceuticals (n-3 polyunsaturated fatty acids, quercetin, and probiotics) on inflammatory and oxidative stress markers, with consideration of how they may be related to SARS-CoV-2.

Introduction

A new wave of a subvariant of concern of the “omicron” variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)—which poses very high risk of infection—is spreading worldwide, continuing the evolving pandemic that is now in its third year [1]. The efficacy of

current vaccines against this heavily mutated variant is still unclear [2]. The unpredictable nature of new variants of SARS-CoV-2 and the poorly characterized and poorly understood post-acute sequelae (PASC) of coronavirus disease 2019 (COVID-19) have led to an increased need for feasible lifestyle modifications as complementary therapies to vaccines to reduce disease severity, particularly given that those effects wane over a period of months [3]. Identification of effective and feasible complementary therapies is critical to provide protection against the severity of both acute and PASC outcomes, since vaccinating a majority of the world population every 6 months or even more frequently is unlikely to be financially or logistically possible.

A key characteristic of severe presentations of acute SARS-CoV-2 infection involves overactive host inflammatory responses, with a substantial proportion of severe outcomes such as hospitalizations and deaths from COVID-19 linked to hyper-inflammation [4]. Inflammation and oxidative stress play pivotal roles in the progression of infectious diseases including COVID-19 [5]. Evidence suggests that high sensitivity (hs) C-reactive protein (CRP), interleukin (IL)-6, and matrix metalloproteinases (MMPs) are among the most important biomarkers of COVID-19 severity, similar to the chronic conditions involved in vascular aging [6,7,8,9]. Lactate dehydrogenase (LDH) and hsCRP are also biomarkers of respiratory failure in patients with COVID-19 [10]. Furthermore, it is well established that elevated levels of other inflammatory markers are common in COVID-19 patients. These markers include IL-1β, IL-7, IL-8, IL-18, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, procalcitonin (PCT), serum ferritin, and erythrocyte sedimentation rate (ESR) [11,12].

Interestingly, hs-CRP, IL-6, and many of the other inflammatory biomarkers mentioned above, are also markers of risk in NCDs such as coronary artery disease and diabetes [13,14,15,16]. A wealth of evidence indicates that treatments that reduce inflammation, assessed using these biomarkers, also protect against major adverse cardiovascular events [17,18,19]. Some of these

treatments may include simple nutritional therapies such as vitamin D supplementation in patients with vitamin D deficiency [20]. Many studies suggest that a high-quality diet, calorie restriction-induced weight loss (>5%), and intermittent fasting (IF), lower the risk of NCDs including diabetes and coronary artery disease [21,22,23,24,25,26,27,28,29,30,31,32], which may be due in part to reduction of chronic inflammation. If so, it may also be that IF and some simple specific nutrients may play a role in boosting immunity in response to SARS-CoV-2 infection, as has been hypothesized in previous reviews [33,34,35,36]. Yet, there is a paucity of comprehensive data from randomized controlled trials (RCTs) regarding the impact of IF and other simple nutritional therapies on inflammation generally, and specifically related to their effects on responses to SARS-CoV-2. While weight loss is known to reduce inflammation and to control risk factors related to both COVID-19 outcomes and NCDs, some dietary regimens, including IF, may impact inflammation and other pathways independently of weight loss [30,37,38]. The current review of recent literature was performed to explore the immunomodulatory roles of dietary supplements (including some vitamins, minerals, and nutraceuticals) and IF on inflammatory and oxidative stress markers that may be related to SARS-CoV-2 or host responses to infection, and also have some connection to reduction of chronic inflammation linked to NCDs.

Methodology of Literature Search

A literature search of the PubMed and Google Scholar databases was conducted to identify peer-reviewed articles published from January 2017 to March 2022 that involved assessments of markers of inflammation or oxidative stress. The search terms for dietary supplementation articles included the following: “vitamin”, “nutrient”, “mineral”, “antioxidant”, “nutraceuticals”, “polyphenols”, “resveratrol”, “quercetin”, “probiotic”, “omega-3”, combined with “infection”,

"inflammation", "inflammatory markers", "immune response", "immunity", "oxidative stress", "CVD", "obese adults", "obesity", "overweight", "'SARS-CoV-2′", and "COVID-19." The search terms for intermittent fasting articles included the following: "intermittent fasting", "alternate-day fasting", "time-restricted eating", "5:2 diet", "Ramadan fasting", combined with "infection", "inflammation", "inflammatory markers", "immune response", "immunity", "oxidative stress", "CVD", "obese adults", "obesity", "overweight", "SARS-CoV-2", and "COVID-19." Filters were set to allow only "human" studies, and studies written in the "English" language.

The Impact of Dietary Supplements on Inflammatory Markers and in Response to SARS-CoV-2

Vitamins and Minerals

Supplementation with vitamins such as A, B, C, D, and E is thought to play a significant role in the severity of COVID-19 infection by reducing inflammation, time to recovery, and preventing lung fibrosis [39,40]. To our knowledge, no RCTs have tested the effects of supplementation with vitamin A, B, or C alone, on COVID-19 severity; however, in a 7-day randomized placebo-controlled trial of 60 COVID-19 patients admitted to intensive care unit (ICU), those who received a combination of vitamins A (25,000 IU daily), D (600,000 IU; one dose), E (300 IU twice daily), C (500 mg four times daily), and a B-complex ampule (daily), demonstrated significant reductions in the duration of hospitalization, TNF-α, Il-6, erythrocyte sedimentation and hs-CRP, but not IFN-γ, as compared to a placebo [41]. In contrast, 10-days of standard treatment plus high doses of vitamin C (2 g), Melatonin (6 mg), and Zinc (50 mg), was not effective for lowering inflammatory markers or length of hospitalization in 20 patients with severe COVID-19 [42].

Accumulating data highlight the immunomodulatory role of vitamin D, and link hypovitaminosis D (25 OHD ≤ 20 ng/mL) with hyperinflammation (i.e., the so-called "cytokine storm") and elevated risk of mortality in patients with COVID-19 [43,44,45,46]. Therefore, supplementation with vitamin D is suggested to attenuate the risk of the cytokine storm, and the severity of COVID-19 [43,47]. The impact of vitamin D on inflammatory markers and in response to SARS-CoV-2 infection has been explored in several RCTs (see Table 1). The current state of evidence from RCTs indicates that higher doses of daily intake of vitamin D can reduce the levels of inflammatory markers like IL-6, hsCRP, and time to recovery in COVID-19 patients [48,49]. Ten days of supplementation with 60,000 IU/day of vitamin D in combination with standard treatment significantly reduced IL-6, hsCRP, LDH, ferritin, and Neutrophil/Lymphocyte ratio, in 87 hospitalized COVID-19 patients with vitamin D deficiency (D < 30 ng/m) as compared with the controls, who received standard treatment for 8 to 10 days [49]. Similarly, in a 2-week trial, oral intakes of two different doses of vitamin D3 (5000 IU vs. 1000 IU) resulted in significant decreases in plasma IL-6 versus baseline, with no between group differences, while hsCRP levels remained unchanged in both groups [48]. Furthermore, time required for resolving cough symptoms with D3 supplementation (5000 IU) was significantly shorter compared to the comparison group [48]. Rastogi et al. investigated the effects of high-dose vitamin D supplementation (60,000 IU) as compared with control in 40 SARS-CoV-2 RNA positive individuals. Patients with vitamin D deficiency (25(OH) D < 20 ng/mL) who were positive for SARS-CoV-2 RNA, with mild or no symptoms, significantly improved with regard to viral SARS-CoV-2 RNA clearance (62.5% vs. 20.8%) and fibrinogen levels [50]. In contrast, a single dose of vitamin D was ineffective for lowering inflammatory markers and for the treatment of patients with severe COVID-19 [51,52,53].

Evidence for the potential role of zinc supplementation for COVID-19 infection is inconclusive [54,55,56]; with only one 28-day RCT of 191 patients with COVID-19 having investigated the effects of zinc supplementation (50 mg of zinc twice daily) combined with chloroquine/ hydroxychloroquine (CQ/HCQ) on inflammatory markers. The study results indicated no significant changes in hs-CRP levels or clinical recovery time [57].

n-3 Polyunsaturated Fatty Acids (PUFAs)

It is well known that n-3 PUFAs including eicosatetraenoic acid (EPA) and docosahexaenoic acid (DHA) have anti-inflammatory properties [64,65,66]. The current state of evidence from RCTs suggests that supplementation with n-3 fatty acids appears to be effective for alleviating clinical symptoms and inflammatory responses in patients with COVID-19 (see Table 1). Three of the trials included in this review investigated the efficacy of n-3 PUFAs for COVID-19 outcomes [59,60,62]. Supplementation with daily omega-3 capsules (1000 mg; 400 mg EPAs and 200 mg DHAs) for 2 weeks resulted in significantly higher 1-month survival rates in 128 ICU patients with COVID-19 when compared to the control patients [59]. The study also reported meaningful improvements in respiratory and renal function parameters such as arterial PH, bicarbonate, and other base excess in the treatment group. Similarly, DHA and EPA supplementation (2 g) plus hydroxychloroquine, significantly reduced hs-CRP, body pain, and fatigue compared to hydroxychloroquine alone in 30 patients with COVID-19 [60]. In agreement with those results, a 7-day trial in 43 patients with COVID-19 who were treated with oral immuno-nutrient supplements containing arginine, omega-3 fatty acids, and nucleotides (Two 200 mL units over 24 h), indicated meaningful reductions in hs-CRP when compared with the patients who were given only high-protein nutritional supplements (Two 200 mL units) [62].

Quercetin

Quercetin—a polyphenol with antioxidant and anti-inflammatory properties—is widely known to have favorable effects on inflammation and infection [67,68,69]. Di Pierro et al. reported that in 42 outpatients with COVID-19, two weeks of quercetin therapy (500–1000 mg daily) significantly diminished the rate (−68.2%) and length (−76.8%) of hospitalization, the need for non-invasive oxygen therapy (−93.3%), and the mortality rate (although events were very limited: non vs. 3 people) compared to the control (standard of care) [58]. Furthermore, significant reductions in LDH and ferritin levels were reported in the treatment group vs. control, while hs-CRP remained unchanged [58]. Unlike the 2-week trial by Di Pierro et al., a shorter 7-day RCT including 60 patients with severe COVID-19, treated with daily quercetin (1000 mg) along with antiviral drugs, compared with control (only antiviral drugs), did not alter mortality or ICU-admission duration significantly. Notably, significant reductions in inflammatory markers such as TNF-α, IL-1β, IL-6, hs-CRP, ALP, and LDH were shown in the treatment group as compared to the control or baseline [61].

Probiotics

High quality evidence from systematic reviews and meta-analyses of RCTs, indicates that probiotics may have a favorable role for responses to infections [70]. In a recent one-month RCT of 293 outpatients with COVID-19, supplementation with four-strain probiotics consisting of Lactiplantibacillus plantarum KABP033 (CECT30292), L. plantarum KABP022 (CECT7484), L. plantarum KABP023 (CECT7485), and Pediococcus acidilactici KABP021 (CECT7483), produced significant reductions in remission rates vs. placebo (53.1% in probiotic group vs. 28.1% in placebo [63].

The Potential Impact of IF on Inflammatory Markers

To date, no RCTs have investigated the effects of IF on SARS-CoV-2 infection or severity of COVID-19 outcomes, although epidemiologic evidence regarding periodic fasting suggests that fasting in some forms may prevent severe COVID-19 outcomes such as hospitalization and death [71], perhaps in part, through the control of hyper-inflammation. The impacts of IF on markers of inflammation and oxidative stress are summarized below:

Interluekins

Current evidence from RCTs reveals that time-restricted eating (TRE) may be effective for reducing interleukins IL-1β, IL-6 and IL-8 (see Table 2.) Of the 11 TRE trials included in this review, only one trial assessed IL-1β levels. This 12-month trial of 20 healthy athletes reported significant reductions in both IL-1β and IL-6 levels in the TRE group (3 meals; 1 p.m., 4 p.m., and 8 p.m.) compared with normal diet control group (3 meals; 8 a.m., 1 p.m., and 8 p.m.) [72]. However, IL-6 levels have remained unchanged in other TRE studies [30,32,73,74]. Xie et al. conducted a 5-week trial examining plasma IL-8 levels in 82 healthy participants who had normal weight, when following early TRE (6 a.m.–3 p.m.), mid-day TRE (11 a.m.–8 p.m.), and as compared with an ad libitum intake control group, and showed a significant reduction in IL-8 levels vs. control when following early TRE [75]. Similarly, results of observational and clinical trials from Ramadan IF (fasting from sunrise to sunset) studies suggest that this form of IF may be effective for counterbalancing inflammatory interleukins such as IL-1β, IL-2, Il-6, IL-8, and IL-10 [76,77,78,79,80,81,82,83,84,85] (see Table 3). In a one-month RCT in 28 men with obesity conducted by Zouhal et al., Ramadan IF produced meaningful reductions in plasma IL-6 levels vs. control. Unlike the results from TRE and Ramadan IF trials, other popular types of IF, including

alternate-day fasting (ADF) and twice-weekly 24-h fasting (commonly called "5:2 diets") have not shown effects on inflammatory interleukins including IL-1β, IL-6, and IL-10 [28,86,87,88,89,90,91,92] (see Table 4). The effects of 24-h prolonged fasting on inflammatory markers has been tested in two single-arm trials by Han et al. with results indicating significant improvements in IL-1β, IL-2, IL-4, IL-5, IL-13, IL-17 and IL-22 as compared to postprandial responses measured 3 h after refeeding with isocaloric breakfast meal (500 kcal) [93,94].

TNF-α

Studies of the impact of different IF regimens on TNF-α have reported inconsistent results (see Table 2, Table 3 and Table 4). Early TRE and Ramadan IF have been shown to significantly improve TNF-α levels vs. control and/or baseline, while mid-day TRE has not shown such improvements in two out of the three TRE trials included in this review [32,72]. Likewise, ADF appears to be ineffective for reducing plasma TNF-α, as two ADF trials in adults with overweight or obesity and metabolic syndrome, which tested changes in TNF-α have not demonstrated significant improvements following ADF regimens [86,91]. Of the two twice-weekly fasting studies which assessed TNF-α, one RCT in 43 adults with central obesity showed significant decreases (−18%) vs. baseline, but no between group differences when compared with a caloric restriction arm [90].

C-Reactive Protein

There has also been inconsistency in the results of various trials of IF regimens on hs-CRP levels, with most studies indicating no effects (see Table 2, Table 3 and Table 4). Of the seven TRE studies included in this review, only two trials (4 to 5 week durations) reported significant improvements in hs-CRP levels (−45% and −42%) in overweight and obese participants vs. a control or as compared with baseline [96,97]. In two TRE studies, only hs-CRP was examined and

was unchanged [95,98]. Hs-CRP levels were not altered in short-term (8 weeks) ADF studies, whereas longer duration trials (17 and 24 weeks; with a high protein diet) have significantly decreased hs-CRP (−48% and −19%) versus CR or baseline, respectively [87,89,91]. In addition, twice-weekly fasting did not appear to affect hs-CRP levels [28,38,88,90]. Furthermore, only one observational Ramadan IF study in 83 patients with non-alcoholic fatty liver disease, has shown significant decreases in hs-CRP levels vs. control and baseline following Ramadan IF after 30 days [76].

IGF-1

IGF-1 levels remain unchanged with most IF trials that recruited participants with overweight and obesity. In athletes, however, two RCTs conducted by Moro et al. reported significant reductions in IGF-1 levels with TRE as compared to control and baseline after both 4 weeks and 12 months [72,74]. IGF-1 significantly increased (34%) in a single arm trial of early TRE (8 a.m.–4 p.m.) in 15 overweight and obese women with Polycystic ovary syndrome [97]. There is lack of consistency in IGF-1 results of observational Ramadan IF studies (see Table 3).

Interferon Gamma and Other Inflammatory Markers

Interferon gamma (IFN-γ), a proinflammatory cytokine that plays a significant role in immune responses, has been investigated in only two 24-h prolonged fasting studies by Han and colleagues. These studies indicate that IFN-γ levels were significantly reduced when compared with 3 h after refeeding with isocaloric breakfast meal (500 kcal) in a total of adults who fasted for 24 h [93,94]. Matrix metalloproteinase 9 (MMP-9) was assessed in one observational Ramadan IF study in 34 healthy adults [85]. The levels MMP-9 were determined at five different time periods within 27–29 days of fasting during the month of Ramadan. While MMP-9 levels remained unchanged from mid-month (days 14–16) to one week after Ramadan, they were significantly lower vs. baseline at

the one month after Ramadan timepoint (see Table 3). Changes in CD40 ligand have also been tested in two studies, with only one 8-week RCT of twice-per-week fasting in 39 adults with metabolic syndrome, demonstrating a meaningful reduction in CD40 ligand vs. control after 8 weeks [92].

Oxidative Stress

8-Isoprostane is a marker of oxidative stress that is associated with COVID-19 [99]. To date, only two trials have examined 8-Isoprostane levels following TRE [30,32], with significant decreases in 8-Isoprostane reported in both studies compared to both control and baseline. Early TRE (eTRE; 6-hr eating window, last meal before 3 pm) showed a 14% reduction in 8-Isoprostane in the eTRE arm versus control (12-hr. eating window) in 12 prediabetic men after 5 weeks. In addition, restricting eating windows to four (3–7 p.m.) and six hours (1–7 p.m.) significantly reduced 8-Isoprostane levels in both groups (37% and 34%, respectively) vs. control (ad libitum intake) in adults with obesity following 8 weeks [32]. In contrast, short-term (6 weeks) eucaloric TRE with self-selected eating windows did not significantly enhance plasma oxidized LDL or the oxidized total LDL ratio in non-obese healthy older adults [73].

Conclusions and Future Directions

Evidence suggests that intermittent fasting and supplementation with vitamin D, and nutraceuticals including quercetin, n-3 fatty acids, and probiotics, impact inflammation in canonical pathways related to both NCDs and infectious diseases including COVID-19 and, thus, should reduce COVID-19 severity. A lack of high-quality evidence exists for the support of such efficacy of supplementation with vitamins A, B, and C, as well as zinc in COVID-19 patients. Further investigation of these fasting and supplementation effects is indicated. Observational studies report that some diets (e.g., the Mediterranean diet, plant-based diets) may reduce SARS-CoV-2 severity,

potentially—at least in part—because the foods contain antioxidants and other components with anti-inflammatory properties [100,101,102,103], but further research is needed on such diets.

Ultimately, the impact of intermittent energy restriction methods on inflammation during energy restriction may be acute but transient, contrasting with longer-term persistent changes to basal levels of inflammation. Future randomized controlled trials are needed to elucidate the effects of intermittent fasting on NCDs and on inflammatory host responses to infection.

Table 2-1: Summary of Randomized controlled trials (RCTs) on the impact of dietary supplements on inflammatory markers and in response to SARS-CoV-2

Authors/Year / Country	Duration	Participants	Study design	TNF-α	IL-1β	IL-4	IL-6	IL-10	CRP	IFN-γ	Other outcomes
Rastogi et al. 2020 [50] India	7-d	40 SARS-CoV-2 RNA positive individuals	**RCT:** 1. Daily oral cholecalciferol (60 000 IU) with therapeutic target 25(OH)D>50 ng/ml 2. Control	-	-	-	-	-	Ø	-	↓† Fibrinogen ↑† Negative conversion of SARS-CoV-2 RNA (62.5% vs 20.8%)
Murai et al. 2021 [53] Brazil	-	237 patients hospitalized for moderate to severe COVID-19	**RCT:** 1. A single oral dose of 200,000 IU of vit. D3 2. Placebo	-	-	-	-	-	Ø	-	Ø Same LOS (median of 7.0 vs 7.0 days)
Lakkireddy et al. 2021 [49] India	8-10-d	87 Patients hospitalized for COVID-19 Vit. D < 30 ng/m	**RCT:** 1. 60,000 IU/day of oral vitamin D +standard treatment 2. Only standard treatment	-	-	-	1.↓† 2.Ø		1.↓*† 2.↓*	-	↓*† LDH, ferritin, N/L ratio
Beigmohammadi et al. 2021 [41] Iran	7-d	60 ICU-admitted COVID-19 patients	**RCT:** 1. Oral Vit. A (25,000 IU) daily, vit.D (600,000 IU; one dose), vit. E (300 IU twice daily), vit. C (500 mg four times daily), and one amp daily of B complex 2. Placebo	1.↓*† 2.↓*	-	-	1.↓*† 2.↓*	-	1.↓*† 2.↓*	1.↓* 2.↓*	↓† Hospitalization rate and ESR in intervention group
Sabico et al. 2021 [48] Saudi Arabia	14-d	69 patients with mild to moderate COVID-19 and sub-optimal vit. D status	**RCT:** 1. Oral vit. D3 (*5000 IU*) 2. Oral vit. D3 (1000 IU)	-	-	-	1.↓* 2.↓*	-	1.Ø 2.Ø	-	↓† Time to recovery in resolving cough with D3 (*5000 IU*) vs D3 (1000 IU)
Abd-Elsalam et al. 2021 [57] Egypt	4 weeks	191 patients with COVID-19	**RCT:** 1. CQ/HCQ + 220 mg of zinc sulfate twice daily 2. HCQ only	-	-	-	-	-	Ø	-	Ø Clinical efficacy of HCQ

Di Pierro, et al. 2021 [67] Italy	2 weeks	42 COVID-19 outpatients	**RCT:** 1.Quercetin (500 mg/day (first week) and of 1000 mg/day (second week) 2. Standard of care	-	-	-	-	-	Ø	-	↓† LOS, virus clearance, symptoms frequency, LDH, ferritin
Doae et al. 2021 [61] Iran	2 weeks	128 critically ill COVID-19 patients	**RCT:** 1. 1000 mg omega-3 daily (400 mg EPAs and 200 mg DHAs) 2.Control	-	-	-	-	-	-	-	↑† 1-month survival rate, pH, HCO_3, and Be ↓† Levels of BUN, Cr, and K in the intervention group
Darban et al. 2021 [42] Iran	10-d	20 patients with severe Covid -19	**Pilot RCT:** 1. Standard care + oral zinc sulfate (220 mg containing 50 mg zinc) , oral melatonin (6 mg, q6hr), and intravenous vit. C (2g) 2.Standard care alone	-	-	-	-	-	1.↓* 2.↓*	-	Ø LOS
Sedighian, et al. 2021 [62] Iran	2 weeks	30 patients with COVID-19	**Single blind RCT:** 1.Hydroxychloroquine + 2 g DHAs and EPAs 2. Hydroxychloroquine	-	-	-	-	-	1.↓†	-	↓† Body pain and fatigue in the intervention group Ø Olfactory
Cannata-Andía et al. 2022 [52] Spain	-	543 patients with moderate to severe COVID-19	**RCT:** 1.A single-oral bolus of 100,000 IU of cholecalciferol 2.Control	-	-	-	Ø	-	Ø	-	Ø Hospitalization rate and death
Shohan et al., 2022 [68] Iran	7-d	60 patients with severe COVID-19	**RCT:** 1.Quercetin (1000 mg daily) + antiviral drugs 2. Antiviral drugs	↓*	↓*	-	↓†	-	↓†	-	↓† ALP, LDH Ø Mortality, duration of ICU-admission
Pimentel et al. 2022 [63] Brazil	7-d	43 adult patients with COVID-19	**RCT:** 1.Two 200 mL units of high-protein nutritional supplement (arginine, omega-3 fatty acids and nucleotides) over 24h	-	-	-	-	-	1.↓†	-	↑Lymphocytes in the intervention group

			2. Two 200 mL units of high-protein nutritional supplement alone						2.Ø		↓Lymphocytes in the control group
Fernandes et al. 2022 [51] Brazil	-	200 patients with moderate to severe COVID-19	**RCT:** 1. Single oral dose of vit. D_3 (200 000 IU) 2. Placebo	Ø	Ø	Ø	Ø	Ø	-	Ø	-
Gutiérrez-Castrellón et al. 2022 [70] Spain	30-d	293 COVID-19 outpatients	**RCT:** 1. Probiotic (Lactiplantibacillus plantarum stains KABP022, KABP023 and KABP033 =Pediococcus acidilactici strain KABP021) 2. Placebo	-	-	-	-	-	1.↓† hs-CRP Only on day 15	-	↓† Complete remission (53.1% in probiotic group vs. 28.1% in placebo; P < .001)

-: Not measured. Ø: Non-significant difference between groups. * $p < 0.05$, Significantly different from baseline (within group effect). † $p < 0.05$, Significantly different from the control or comparison group. TNF-α: tumor necrosis factor -α ; IL-1β: Interleukin-1β; IL-4: Interleukin-4; IL-6:Interleukin-6 ; IL-10 : Interleukin-10; IFN-γ: Interferon gamma; CQ/HCQ:Chloroquine/ hydroxychloroquine; HCQ: Hydroxychloroquine; ALP: Alkaline phosphatase; Lactate dehydrogenase; Be: Base excess; BUN: Blood urea nitrogen; Cr: creatinine; LOS: Length of hospital stay*;* LDH*:* Lactate dehydrogenase; N/L ratio: Neutrophil/Lymphocyte ratio; ESR: Erythrocyte sedimentation rate; DHA: Docosahexaenoic acid; EPA: eicosapentaenoic acid.

Table 2-2: Summary of clinical trials on the impact of time-restricted eating (TRE) on inflammatory markers and oxidative stress.

Authors/Year/Country	Duration	Participants	Study design	8-Isoprostane	TNF-α	L-1β	IL-6	IL-8	CRP	IGF-1
Sutton et al. 2018 [30] US	5 weeks	12 prediabetic men	**RCT crossover:** 1. Eucaloric eTRE (6-hr eating window, last meal before 3 pm) without weight loss 2. Control (12-hr. eating window)	1.↓14%† 2.Ø	-	-	Ø	-	Ø hs-CRP	-
Jamshed et al. 2019 [37] US	4-day	11 overweight adults	**RCT crossover:** 1. eTRE (8 a.m.–2 p.m.) 2. Control (8 a.m.–8 p.m)	-	-	-	-	-	-	Ø
Martens et al. 2020 [73] US	6 weeks	22 healthy non-obese older adults	**RCT:** 1. Eucaloric TRE (16/8)-without weight loss 2. Control	-	-	-	Ø	-	Ø	
Wilkinson et al. 2020 [99] US	12 weeks	19 adults with MetSyn	**Single arm:** 10-h TRE (self-selected eating window)	-	-	-	-	-	Ø hs-CRP	-
Cienfuegos et al. 2020 [32] US	8 weeks	49 obese adults	**RCT:** 1. 4-h TRE (3–7 p.m.) 2. 6-h TRE (1–7 p.m.) 3. Control	1. ↓37% *,† 2. ↓34% *,† 3. Ø	Ø	-	Ø	-	-	-
McAllister et al. 2020 [97] US	4 weeks	22 men BMI: 28.5 ± 8.3	**RCT:** 16:8 TRE 1. Isocaloric TRE 2. *Ad libitum* TRE	-	-	-	-	-	1. ↓45† hs-CRP 2. ↓14	-
Moro et al. 2020 [74] Italy	4 weeks	16 elite under - 23 cyclists	**RCT:** 1. TRE (10 a.m.–6 p.m.) 2. Control (7 a.m.–9 p.m.)	-	Ø	-	Ø	-	-	1.↓14%* 2.Ø

Moro, et al. 2021 [72] Italy	12 months	20 healthy resistance-trained males	**RCT:** 1. mTRE (3 meals;1 p.m.,4 p.m. and 8 p.m.)	-	1.↓*†	1.↓*†	1.↓*†	-	-	1.↓*†
			2. Control (8 a.m., 1 p.m., and 8 p.m.)		2.Ø	2.Ø	2.Ø			2.↑†
Li et al. 2021 [96] China	5 weeks	15 overweight and obese women with PCOS	**Single arm:** eTRE (8 am–4 pm) 1-week baseline weight stabilization + 5-week trial	-	-	-	-	-	↓42% * hs-CRP	↑34%*
Kotarsky et al. 2021 [100] US	8 weeks	21 overweight and obese adults	**RCT:** 1. mTRE (12–8 p.m.) 2. Normal eating	-	-	-	-	-	Ø hs-CRP	-
Xie et al. 2022 [75] China	5 weeks	82 healthy adults	**RCT:**							
			1.e-TRE (6 a.m.–3 p.m.)	-	1. †	-	-	1. †	Ø	-
			2.mTRE (11 a.m.–8 p.m.)		2. Ø			2.Ø		
			3. Control (ad lib intake)		3. Ø			3. Ø		

-: Not measured. Ø: Non-significant difference between groups. * $p < 0.05$, Significantly different from baseline (within group effect). † $p < 0.05$, Significantly different from the control or comparison group; TNF-α: tumor necrosis factor -α; IL-1β: Interleukin-1β; IL-6: Interleukin-6; IL-8: Interleukin-8; CRP: C-reactive protein; IGF-1: Insulin-like growth factor 1TRE: Time-restricted eating; eTRE: Early-TRE; mTRE: Mid-day TRE; MetSyn: Metabolic syndrome; PCOS: Polycystic ovary syndrome.

Table 2-3: Summary of studies on the impact of Ramadan fasting on inflammatory markers

Authors/Year/ Country	Duration	Participants	Study design	MMP-9	TNF-α	IL-1β	IL-2	IL-6	IL-8	IL-10	CRP	IGF-1
Aliasghari et al. 2017 [76] Iran	30-d	83 patients with NAFLD	Observational Ramadan fasting 1.Ramadan fasting (n=42) 2.Control (n=41)	-	-	-	-	1.↓*† 2.↓*			1.↓*† Hs-CRP 2. ↓*	-
Mohammad zade et al. 2017 [77] Iran	30-d	23 healthy men	Observational Ramadan fasting	-	-	-	-	Ø	-	-	Ø	-
Mushtaq et al. 2019 [78] Pakistan	29-d	110 normal, overweight, and obese men (n=55) and women (n=55)	Observational Ramadan fasting (1st vs 29th day of Ramadan just before Iftar) + dietary recommendation (oily foods prohibited at Iftar (breaking of fast time) , plus white oats provided (bran diet) for Sahar (onset of fasting time) meal	-	↓16%* Ψ men ↓11%* Ψ women	-	-	-	-	-	-	-
Almeneessier et al. 2019 [79] Saudi Arabia	14-d	12 healthy men	Single arm Ramadan fasting (from dawn to sunset)	-	-	↓*	-	↓*	↓*	-	-	-
Rahbar et al. 2019 [80] Saudi Arabia	30-d	34 healthy men	Observational Ramadan fasting	-	-	-	↓*	-	-	-	-	↓*
Faris et al. 2019 [81] UAE	30-d	57 overweight and obese adults	Observational Ramadan fasting	-	↓*	-	-	↓*	-	↑*	-	↓*
Mansoor et al. 2020 [81] Iraq	21-d	20 healthy young women aged 19-20y	Observational Ramadan fasting	-	↓*	-	-	↓*	-	-	-	-

Lubis and Pase. 2020 [83] Indonesia	30-d	30 obese adults	Observational Ramadan fasting	-	-	-	-	↓*	-	-	-	-
Zouhal et al. 2020 [84] Tunisia	30-d	28 obese men	**RCT:** 1.Ramdan fasting 2. Control	-	1.↓† 2.↓*	-	-	1.↓† 2.Ø	-	-	1.Ø 2.Ø	-
Riat et al. 2021 [85] Germany	27-29-d	34 healthy adults	Observational Ramadan fasting (17-18h) T1: baseline: 6-8d before Ramadan T2: Mid of Ramadan (day 14-16) T3: End of Ramadan (day 27-29) T4: One week after Ramadan T5: One month after Ramadan	T2:Ø T3:Ø T4:Ø T5:↓*	-	-	-	-	T2:↑* T3:↓¥ T4:↓¥ T5:↓¥			T2:↑* T3:↓¥ T4:Ø T5:↑*

-: Not measured. Ø: Non-significant change between groups. * $p < 0.05$, Significantly different from baseline (within group effect). † $p < 0.05$, Significantly different from the control or comparison group. ¥ $p < 0.05$, Significantly different from T2. MMP-9: Matrix metalloproteinase ; TNF-α: tumor necrosis factor -α ; IL-1β: Interleukin-1β; IL-2: Interleukin-2; IL-6 : Interleukin-6 ; IL-8: Interleukin-8; IL-10 : Interleukin-10; CRP: C-reactive protein; IGF-1: Insulin-like growth factor 1; NAFLD: Non-alcoholic fatty liver disease. Ψ: Significant reduction in only obese group (30 men, 30 women)

Table 2-4: Summary of Randomized controlled trials (RCTs) on the impact of intermittent calorie restriction on inflammatory markers

Author/Year/ Country	Duration	Participants	Study design	TNF-α	IL-1β	IL-2	IL-4	IL-5	IL-6	IL-13	IL17	IL-22	CRP	IFN-γ	CD40 ligand	IGF-1
Trepanowski et al. 2018 [86] US	24 weeks	69 overweight and obese	**RCT:** 1. ADF (25% energy needs on fast days;125% on fed days) 2. CR (75% energy needs daily) 3. Control (100% energy needs daily)	1.Ø 2.Ø 3.Ø	-	-	-	-	1.Ø 2.Ø 3.Ø	-	-	-	-	-	-	-
Bowen et al.,2018 [87] Australia	24 weeks	162 overweight and obese adults	**RCT:** 1. High protein, ADF + CR (1 Ad lib intake d/wk) 2. CR	-	-	-	-	-	-	-	-	-	1.↓19* hs-CRP 2.Ø	-	-	-
Schübel et al. 2018 [28] Germany	12 weeks	144 overweight and obese adults	**RCT:** 12-wk trial 5:2 vs CR and control +38wk (12 wk maintenance +26 wk follow up)	-	-	-	-	-	-	-	-	-	Ø	-	-	Ø
Sundfor et al. [88] 2018 Norway	52 weeks	112 obese adults	**RCT:** 5:2 diet vs CR + Med diet	-	-	-	-	-	-	-	-	-	Ø at 6 months	-	-	-
Cho et al. 2019 [89] South Korea	8 weeks	31 overweight and obese adults	**RCT:** ADF vs usual diet with or without exercise	-	-	-	-	-	-	-	-	-	Ø hs-CRP	-	-	-

Pinto et al. 2019 [90] UK	4 weeks	43 adults with central obesity	**RCT:** 1.5:2 diet 2.CR	1.↓18%* 2.Ø	1.Ø 2.Ø	-	-	-	1.Ø 2.Ø	-	-	-	-	-	-	-
Razavi et al. 2021 [91] Iran	17 weeks	75 adults with MetSyn	**RCT:** 1.ADF (400-600kcal on fast days) 2. CR (75% energy restriction daily)	1.Ø 2.Ø	-	-	-	-	1.Ø 2.Ø	-	-	-	1.↓48%* † hs-CRP 2.↓26%*	-	-	-
Han et al. 2021 [94] US	1-day	20 adults	**Single arm:** 24-h prolonged fasting vs. post-prandial response, 3h after isocaloric breakfast (500 kcal)	-	↓*	↓*	↓*	↓*	-	-	↓*	-	-	↓*	-	-
Han et al. 2021 [93] US	1-day	21 adults	**Single arm:** 24-h prolonged fasting vs post-prandial response, 3h after isocaloric breakfast (500 kcal)	-	-	-	Ø	↓*	-	↓*	↓*	↓*	-	↓*	-	-
Bartholomew et al. 2021 [38] US	26 weeks	103 adults; ≥1 MetSyn component or T2D	**RCT:** 1.5:2 diet for 4 weeks; followed by fasting once a week for 22 weeks 2. Ad libitum control	-	-	-	-	-	-	-	-	-	Ø	-	-	-
Guo et al. 2021 [92] China	8 weeks	39 adults with MetSyn	**RCT:** 1. 5:2 diet (two days fasting per week) 2. Control	Ø	-	-	-	-	Ø	-	-	-	-	-	1.↓† 2.Ø	-

-: Not measured. Ø: Non-significant change between groups. * $p < 0.05$, Significantly different from baseline (within group effect). † $p < 0.05$, Significantly different from the control or comparison group. TNF-α: tumor necrosis factor -α ; IL-1β: Interleukin-1β; IL-2: Interleukin-2 IL-4: Interleukin-4 IL-5 : Interleukin-5 ; IL-6 : Interleukin-6 ; IL-13 : Interleukin-13; IL-17 : Interleukin-17 ; IL-22 : Interleukin-22 ; CRP: C-reactive protein ; IFN-γ: Interferon gamma; IGF-1: Insulin-like growth factor 1**;** ADF: Alternate-day fasting. CR: Caloric restriction; MetSyn: Metabolic syndrome; Med diet: Mediterranean diet; T2D: Type 2 diabetes.

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Chapter 3 - The Effects of Isocaloric Intermittent Fasting vs Daily Caloric Restriction on Weight Loss and Metabolic Risk Factors for Noncommunicable Chronic Diseases: A Systematic Review of Randomized Controlled or Comparative Trials

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Abstract

Background

Intermittent fasting (IF) has gained favor as an alternative regimen to daily caloric restriction (DCR). Therefore, there is a need for systematic reviews of randomized controlled/comparison trials examining the effects of isocaloric IF vs DCR on metabolic risk factors for noncommunicable chronic diseases.

Objective

To systematically investigate the effects of isocaloric IF vs DCR on metabolic risk factors for noncommunicable chronic diseases in adults with overweight and obesity.

Methods

Five online databases (PubMed, Scopus, Web of Science, Cochrane Central Register of Controlled Trials, and Google Scholar) were searched for articles published from January 2000 through April 2022. The updated Cochrane Risk of Bias Assessment tool for randomized controlled/comparison trials was used to assess risk of bias in the included studies. This review includes randomized controlled/comparison trials with matched energy intakes (isocaloric) between IF and DCR among adults with overweight and obesity with ≥8-week durations, that assessed risk factors related to obesity and for diabetes, cardiovascular diseases, and cancers.

Results

Thirteen randomized controlled/comparison trials with matched energy intakes (isocaloric) between IF and DCR were identified. The effects of IF on weight loss and metabolic risk markers of diabetes, cardiovascular diseases, and cancers were varied but generally comparable with DCR. IF (4:3 and 5:2 diets) was superior to DCR for improving insulin sensitivity in two studies. Reductions in body fat were significantly greater with IF (5:2 diet and time-restricted eating) than DCR in two studies of isocaloric diets.

Conclusions

With matched energy intakes, IF interventions produced similar beneficial effects for weight loss and chronic disease risk factors compared with DCR. Very limited evidence suggests that IF may be more effective vs DCR for fat loss and insulin sensitivity, but conclusions cannot be drawn based on the current evidence. Future clinical studies with larger populations and longer durations are needed for further elucidation of any potential effects of IF regimens for prevention of noncommunicable chronic diseases.

Introduction

Noncommunicable chronic diseases (NCDs) can be defined as diseases that last 1 year or more and demand persistent medical care, resulting in 41 million deaths worldwide annually (71% of all deaths globally).[1] In the United States, obesity, cardiovascular diseases (CVDs), cancers, and type 2 diabetes mellitus (T2DM) are among the most prevalent chronic diseases, and six in 10 adults have at least one chronic disease.[2]

Overweight and obesity are considered to be metabolic risk factors for NCDs, and therefore clinically meaningful weight loss (≥5%) can improve markers of diabetes, CVDs, and some cancers.[3] Furthermore, serum lipid, fasting glucose, and insulin, adiponectin, leptin, C-reactive protein (CRP), and insulin-like growth factor 1 levels and high blood pressure are considered to be common risk markers of T2DM, CVDs, and cancers.[4, 5, 6, 7, 8, 9, 10, 11, 12, 13] Thus, any dietary interventions that can improve those markers have the potential to prevent or reduce the risk of NCDs.

Daily caloric restriction (DCR), which involves consistent daily reduction in energy intake, is believed to improve chronic disease incidence and is supported by 2013 American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society Guidelines for the Management of Overweight and Obesity in Adults.[3] Because adherence to DCR has been shown to be challenging, intermittent fasting (IF), an approach in which individuals fast periodically, has gained favor in recent years. Numerous research studies have examined different ways to implement IF and have

determined potential effects on chronic disease risk factors. The most well-studied IF approaches include: time-restricted eating (TRE),[14] and intermittent calorie restriction (ICR).[15] TRE typically involves limiting the eating window to 8 to 12 hours with fasting, drinking only water and calorie-free coffee/tea, for 12 to 16 hours within a 24-hour cycle.[14] ICR involves cycling between calorie restriction and more typical dietary intake. Alternate-day fasting (ADF) and 4:3 and 5:2 diets are examples of ICR regimens.[15] ADF includes recommendations for consumption of 25% of energy needs or less on the fasting day, followed by a day of ad libitum eating without restriction of caloric intake.[16] Unlike the ADF approach in which the number of fasting days switches between 3 to 4 days weekly, in the 4:3 diet, fasting is practiced on 3 nonconsecutive days per week.[17] The 5:2 diet involves fasting for two nonconsecutive days of the week, and on the remaining 5 days, a typical dietary intake is followed.[15] This fasting regimen allows for the consumption of 20% to 25% of energy needs on fasting days.[15]

Several animal[18,19] and human[20,21] studies report that some benefits of IF are independent of weight loss and may be due to metabolic switching.[22] Metabolic switching occurs 12 to 36 hours after fasting is initiated, when glycogen stores are depleted, and free fatty acids are released into the blood.[22] Generally, IF is believed to upregulate autophagy through inhibiting the mammalian target of rapamycin protein synthesis pathway, and activation of adaptive cellular stress response signaling pathways that promote mitochondrial health, and DNA repair.[23] The role of metabolic switching in fasting is unknown, as autophagy cannot yet be measured in human beings. Therefore, IF interventions that compare IF with DCR using matched energy intakes, which would allow analysis of independence from weight loss are of great importance. Moreover, it may also be that benefits derived from IF interventions as compared with DCR are due to differences in energy intakes over time when interventions are not matched, thereby making interpretation of many previous studies difficult.

For the current review, the effects of isocaloric IF diet approaches on weight loss and metabolic risk factors for NCDs compared with DCR were investigated to elucidate whether or not IF can produce clinically significant improvements in risk biomarkers of T2DM, CVDs, and cancers. In addition, the

current systematic review examined whether or not different types of IF produce clinically meaningful weight loss compared with DCR when energy intakes are matched between diet interventions.

Methods

Methods of analysis and inclusion and exclusion criteria were determined *a priori*. Registration of this systematic review was completed through PRSOPERO International prospective register of systematic reviews (CRD42020197053). The Cochrane Handbook for Systematic Reviews of Interventions was used as a guide for developing the methods for the review.[24] For reporting methods and results, the preferred reporting items for systematic reviews and meta-analyses guidelines were followed.[25]

Eligibility Criteria

Randomized controlled/comparison trials (RCTs) with matched energy intakes (isocaloric) between IF and DCR using one of the IF approaches: TRE, or ICR (ADF, 4:3 diet, and 5:2 diet) in adults with overweight or obesity, were included when the trial was at least 8 weeks in duration, and assessed metabolic risk factors that were related to at least one of the relevant NCDs or associated metabolic risk factors (eg, obesity, T2DM, CVDs, and cancers) (Table 1). Articles written in English, published between January 2000 and April 2022 were included. Any RCT that was based on athletes, religious fasting, or animal studies, and studies of shorter durations (<8 weeks), or sample sizes <20 participants, were excluded from this review.

Search Strategy

The final search strategy was developed in consultation with a subject specialist librarian (C.L.). The initial systematic search for articles occurred on January 15, 2020. The search was repeated on January 30, 2021, August 4, 2021, and again on May 4, 2022, to retrieve newly published articles through April 2022.

The online databases searched were PubMed, Scopus, Web of Science, Cochrane Central Register of Controlled Trials, and Google Scholar. The following keywords were searched: *intermittent fasting* or *intermittent calorie restriction* or *intermittent energy restriction* or *alternate-day fasting* or *time-restricted feeding* or *time-restricted eating* or *5:2 diet* and *chronic disease* or *obesity* or *cardiovascular disease OR CVD* or *diabetes* or *cancer*. Both sets of keywords were also searched with *AND* between the two different concepts. Keywords were searched in the title, abstract, or subject/keywords for each electronic database. The detailed search strategy used for PubMed is shown in Figure 1 (available at www.jandonline.org). Other relevant publications were identified through hand searching the reference lists of included articles, as well as from related previously published review articles.

Selection Process

Two independent reviewers assessed each record for inclusion or exclusion based upon the initial review of the title and abstract. Articles that fulfilled all of the inclusion criteria (Table 1) were then retrieved for review of the full text of the article. The same independent reviewers assessed the full-text articles. Discrepancies regarding inclusion of an article were resolved via subsequent discussions that yielded a mutual decision by the independent reviewers.

Risk of Bias Assessment

Two independent reviewers used the Cochrane Risk of Bias Tool to determine the risk of bias of each full-text article.[26] Discrepancies regarding risk of bias assessment were resolved via subsequent discussions that yielded a mutual decision by the independent reviewers. The updated Cochrane Risk of Bias Assessment Tool for RCTs was used to evaluate five domains of bias: arising from the randomization process, deviation from the intended intervention, missing outcome data, measurement of the outcome, and selection of the reported results. Evaluation of these five criteria resulted in overall judgments of low

risk of bias, some concerns, or a high risk of bias.[26] The overall risk of bias judgment is determined by the highest risk of bias of the five domains.

Data Extraction

Two independent reviewers extracted relevant data from all included articles. The data extracted were assessed, and any differences were investigated and resolved through discussions between the reviewers. The following information was extracted for all studies: study design, recruitment setting, eligibility criteria, and population characteristics. Intervention groups, duration, outcomes, content, and compliance were also extracted.

Data Synthesis and Analysis

Due to heterogeneity across studies with respect to different IF regimens, study designs, protocols, and outcomes of interest, it was not possible to combine studies for meta-analysis that would yield meaningful information. Instead, results indicating significance and direction of the observed effects were summarized for each study.

Results

The initial literature search resulted in 456 relevant articles (Figure 2). After removing duplicates, 337 articles remained. Based on the inclusion criteria, reviewers excluded 295 articles during the title and abstract screening. Forty-two remaining research articles were examined for eligibility, and 29 additional studies were removed that either did not match the energy intakes between IF and DCR, or due to the population/outcome of interest or the study design. The reasons for exclusion of articles are shown in Figure 2.

Study Characteristics

Thirteen studies, including four TRE,[27, 28, 29, 30] one ADF,[31] two 4:3 diet,[32,33] and six 5:2 diet studies,[20,34,35, 36, 37, 38] in which energy intakes were matched between intervention arms, were included in the current systematic review. The durations of included studies were between 8 and 52 weeks, with six of the 13 studies having short durations (≤26 weeks) and seven studies having longer durations (39 to 52 weeks).[27, 28, 29,34, 35, 36, 37] One study examined the effects of IF in patients with T2DM,[34] one examined the effects of IF in women with previous gestational diabetes,[35] and two included adults with metabolic syndrome.[30,36] Three studies only recruited women,[20,28,35] whereas all participants in one study (n = 23) were men.[38] Foods were provided in three trials.[27,31,38] Dietary protocols and macronutrient compositions varied among the included studies. Healthfulness of diets was considered in 11 studies, whereas consideration of healthfulness of diets was unclear in two trials.[28,33] IF combined with a Mediterranean diet (30% to 35% fat, 20% to 25% protein, and 45% to 50% carbohydrates) was used in three studies,[20,30,36] and one study imposed high-protein intake (>30% of energy intake) with the intervention.[37]

Risk of Bias Assessment

Of the included 13 studies, six studies were judged as low-risk,[20,28, 29, 30,32,37] five were judged as some concern,[27,33,34,35,38] and two were rated as high-risk (Figure 3).[31,37] With regard to bias arising from the randomization process, 13 studies, seven were low risk,[20,28,30,32,]35, 36, 37 whereas six studies were rated as some concern due to lack of the randomization information.[27,29,31,33,34,38] With respect to bias due to deviations from intended interventions, three studies were rated as low risk,[20,29,37] nine were rated as some concern,[27,28,]30, 31, 32, 33, 34,[35,38] and one study rated as high risk.[36]

The Effects of Isocaloric IF vs DCR on weight loss

Of the 13 trials that compared isocaloric IF interventions with DCR, 11 reported weight and fat loss percentages that were comparable with DCR.[27,29, 30, 31, 32, 33, 34,35, 36, 37, 38] In contrast, there were two

interventions (5:2 diet and 12 hour TRE) in which significant reductions in body fat vs DCR were reported.[20,28] A total of five of the 13 included studies recruited only adults with obesity (body mass index ≥30k) (see Table 2).[28,32,34,36,38]

TRE

A total of four TRE studies with 12- to 52-week durations were included in this systematic review. These studies examined body weight and composition in adults with overweight and obesity.[27, 28, 29, 30] (Table 2). Two isocaloric TRE studies implemented early-TRE (early-TRE combined with DCR) vs DCR alone.[27,29] These long-term isocaloric early-TRE trials restricted eating windows to 8:00 am to 4:00 pm,[27] or 10 hours within 3 hours of waking,[29] and produced comparable clinically meaningful weight loss with DCR (at least 5% of total body weight), following 52 and 39 weeks, respectively. Unlike the results of early-TRE studies, a 12-month TRE intervention (12 hours) that used a self-selected eating window, reported no effects for TRE for weight loss; however, reductions in body fat and waist circumference were statistically, but not clinically, greater in TRE group compared with DCR.[28] This study reported high dropout rates for both intervention arms.

ADF

Only one isocaloric ADF study with matched energy intakes vs DCR was identified.[31] The study reported similar clinically meaningful weight loss (5% of total body weight) with ADF compared with DCR after 12 weeks.

4:3 Diet

Two 4:3 diet studies were included in this review that reported similar weight and fat loss when comparing the 4:3 diets with DCR in adults with overweight or obesity following 8- to 12-week interventions[32,33] (Table 2).

5:2 diet

A total of six 5:2 diet studies with matched energy intakes between intervention arms, ranging from 12- to 52-week durations, were included. Results indicated similar weight loss between 5:2 and DCR diets.[20,34,35, 36, 37, 38] No significant between-group differences were indicated for weight loss or waist circumference in all studies comparing 5:2 diets vs DCR.[20,34,35, 36, 37, 38] However, fat loss was significantly greater in the 5:2 diet group compared with DCR in a 12-week study by Harvie and colleagues.[20] In this study, two types of 5:2 diets (with and without ad libitum protein and fat intake) produced greater reductions in body fat vs high protein DCR (with similar overall 25% energy restriction) in women with overweight and obesity.[20]

The Effects of Isocaloric IF vs DCR on metabolic risk factors for T2DM, CVDs, and Cancers

TRE

Fasting blood glucose levels improved vs baseline in two of four studies that examined TRE in adults without T2DM vs DCR; however, there were no between group differences reported.[27,29] The effects of TRE on fasting insulin and glycated hemoglobin (HbA1c) levels were inconsistent and similar to that of DCR. In the longest duration TRE trial (52 weeks), isocaloric early-TRE (25% energy restriction; 8-hour eating window: 8:00 am to 4:00 pm) and DCR regimens showed similar improvements in insulin sensitivity in 115 adults with overweight and obesity.[27] In contrast, a 12-week trial of isocaloric early-TRE with a longer eating window (10-hour eating window within 3 hours of waking; combined with DCR) and DCR alone, did not improve HbA1c levels in 63 participants with overweight and obesity.[29]

Of the four TRE studies included in the current review, three examined blood lipid levels,[27,29,30] and blood pressure levels were also measured in three studies.[27, 28,30] Similar reductions in total cholesterol, low-density lipoprotein cholesterol, and triglyceride level, and blood pressure were shown when comparing TRE (plus DCR) with DCR in adults with overweight and obesity[27,28] or metabolic syndrome.[30] In contrast, no significant within- or between-group differences were observed in blood lipid levels or blood

pressure with both early-TRE (10-hour eating window within 3 hours of waking) and DCR in 63 adults with overweight and obesity following a 12-week intervention.[29] No TRE studies examined adiponectin, leptin, CRP, or insulin-like growth factor 1 concentrations.

ADF

One ADF study was included in the current review that examined ADF compared with a DCR, an exercise arm, and control with matched energy intakes, set at a level intended to achieve 5% weight loss.[31] The study included 49 adults with overweight and obesity, and results indicated improved Low-density lipoprotein, increased low-density lipoprotein particle size with 5% weight loss in the ADF and DCR groups, whereas triglyceride levels significantly improved only with ADF. In addition, no effects on high-density lipoprotein size were shown in ADF and DCR arms. Conversely, exercise increased large high-density lipoprotein particles following the same levels of weight loss.[31]

4:3 Diet

Of the two 4:3 diet studies included in the current systematic review, one reported similar improvements in fasting and postprandial insulin levels following 12 weeks of a matched energy intake 4:3 diet vs DCR in adults with obesity.[32] One 4:3 diet trial examined insulin sensitivity and reported that the 4:3 diet was more effective for improving insulin sensitivity compared with DCR in 80 participants with mild-to-moderate hypertriglyceridemia, whereas weight loss was similar between groups.[33] The study reported comparable significant reductions in triglyceride levels with both diets after 8 weeks. Macronutrient distribution was not controlled between intervention groups and there was significantly lower carbohydrate intake and higher cholesterol and protein consumed in the IF group, with no between-group differences in blood lipid levels.[33] Adiponectin levels were tested in one of the two 4:3 diet trials and were unchanged with both the 4:3 diet and DCR at 8 weeks.[33]

5:2 Diet

Of the six 5:2 diet interventions, one exclusively recruited participants with T2DM (body mass index ≥30.0),[34] and one included women with previous gestational diabetes.[35] Four of six 5:2 diet studies examined fasting glucose and reported no significant changes in both IF and DCR arms.[35, 36, 37, 38] With matched energy intakes, significant reductions in HbA1c were shown compared with baseline for both the 5:2 diet and DCR in adults with T2DM after 52 weeks,[34] but HbA1c rose to above baseline in both diets at the 52-week follow-up.[39] Similar significant reductions in HbA1c compared with preintervention, with a 5:2 diet and DCR were shown in a 6-month study of 112 adults with metabolic syndrome.[36] Of the two 5:2 IF trials that tested insulin sensitivity, one reported that the 5:2 diet was superior to DCR for improving insulin sensitivity following 12 weeks.[20]

The effects of 5:2 diets on lipid profiles and blood pressure levels were inconsistent with most studies demonstrated no effects. In one 52-week isocaloric intervention, the 5:2 diet did not improve plasma lipid levels (with the exception of meaningful improvement in high-density lipoprotein cholesterol), whereas triglyceride levels were significantly reduced in the DCR arm.[37] Two studies examined the 5:2 diet approach combined with a Mediterranean diet following 12 to 52 weeks[20,36] and reported relatively comparable improvements for lipid and blood pressure levels between 5:2 and DCR diets in individuals with overweight and obesity[20] and/or adults with metabolic syndrome.[20]

One isocaloric 5:2 diet study examined leptin, adiponectin, and inflammatory markers, and demonstrated comparable improvements in leptin, leptin to adiponectin ratio, and proinflammatory marker interleukin 6 vs DCR, but no significant changes were observed in adiponectin, insulin-like growth factor 1, tumor necrosis factor alpha, or oxidative stress following a 12-week matched energy intake intervention.[20] Similarly the only study that examined CRP levels reported no significant changes in both the 5:2 diet and DCR groups in adults with metabolic syndrome at 6 months.[36]

Discussion

The present systematic review investigated the effects of IF on metabolic risk factors for NCDs in adults with overweight and obesity, and is the only systematic review, to our knowledge, that has focused specifically on isocaloric interventions to address the efficacy of IF compared with DCR when energy intakes are matched between groups.

Results of the current systematic review indicate that IF interventions produced clinically meaningful weight loss when prescribed with calorie restriction, similar to that of DCR. Of the 13 isocaloric studies with matched energy intakes between IF and DCR, only two isocaloric IF (4:3 and 5:2 diets) intervention showed superiority for improving insulin resistance when energy intake was matched between groups.[20,33] The 5:2 diet and (12-hour) time-restricted eating were more effective than DCR for fat loss in two isocaloric studies,[20,28] whereas other TRE interventions produced comparable effects vs DCR. Overall, IF interventions produced similar beneficial effects for weight loss and chronic disease risk factors compared with DCR.

Interpretation

Findings from previous systematic reviews of RCTs have consistently shown that IF produced similar weight loss compared with DCR,[40], [41], [42] and one systematic review and meta-analysis of ICR RCTs, including the 5:2 diet and ADF, demonstrated greater weight loss compared with DCR in short-term studies (2 to 3 months).[43] To date, no systematic reviews have evaluated the effects of isocaloric IF interventions on risk biomarkers of chronic diseases vs DCR. Given that there is uncertainty regarding the effectiveness of IF vs DCR in the body of evidence from systematic reviews, investigating IF vs DCR interventions under isocaloric conditions is essential for interpretation. Accordingly, the current systematic review exclusively investigated isocaloric IF interventions in comparison with DCR.

Overall, results from the current systematic review demonstrated that the effects of IF regimens on plasma lipid, glucose, and insulin levels; HbA1c; and inflammatory markers were highly variable. These

variations may be explained by the heterogeneity of included studies in terms of types of IF regimens, participants, fasting initiating times and durations, as well as control of diet and physical activity. Nevertheless, results from the included studies indicated that IF may be effective for improving some risk factors of NCDs similarly to DCR when energy intakes are matched between IF and DCR.

The mechanisms underlying the potential health effects of IF are unclear. Although several studies have shown that some of the benefits of IF may be due to metabolic switching,[20,21,33] some additional studies have demonstrated that these benefits are solely a result of energy restriction and weight loss.[17,27] Weight loss (≥5%) is associated with improved blood lipid levels, glycemic control, blood pressure, and proinflammatory markers.[44, 45, 46, 47] However, results of RCTs demonstrate inconsistent results for these risk factors following modest weight loss produced by IF and DCR. The differences in the study populations, adherence to diet interventions, and baseline levels of risk markers may explain some inconsistencies in results. Likewise, of the two isocaloric IF studies which reported greater improvements in insulin sensitivity with IF vs DCR,[20,33] one[20] reported better adherence in the IF groups (70% with IF groups on restricted days, vs 39% with DCR), whereas nutrient compositions were not matched between groups in both trials.[20,33]

Despite the accumulating evidence indicating the potential importance of time of eating in alignment with circadian clocks, due to regulation of metabolic functions and autophagy,[48,49] the optimal timing of eating for IF protocols has not been established. In one year-long trial, comparable weight loss and metabolic improvements were shown with early-TRE (8-hour eating window until 4:00 pm) plus 25% energy restriction daily vs DCR.[27] However, body fat and metabolic risk markers of CVDs, and T2DM decreased significantly in both groups, with no significant differences between groups. Nonetheless, participants were generally healthy, and on average, baseline eating windows in the early-TRE group were already relatively short (10 hours and 23 minutes). Other TRE interventions have included only participants with metabolic syndrome, and/or prolonged eating windows (≥14 hours/day).[50,51] The two long-term TRE

studies[27,28] included in the current review indicated that timing of eating did not play a significant role in health benefits associated with IF. However, a recent relevant short-term 5-week trial in healthy adults without obesity, showed significant improvements in body mass, fasting glucose, insulin sensitivity, and the pro-inflammatory markers tumor necrosis factor alpha and interleukin 8, in an early-TRE group (eating window: 6:00 am to 3:00 pm) vs control, but not with mid-day TRE (11:00 am to 8:00 pm). In this short-term study, energy reductions were similar between TRE groups.[52] Fully powered trials comparing early- vs late-TRE are needed to elucidate the role of the time of the eating window.

Strengths and Limitations

The strengths of the current systematic review are that it is comprehensive and includes relevant RCT studies with sample sizes of at least 20 participants and durations of at least 8 weeks. The present systematic review also has some limitations. One such limitation is the heterogeneity between studies in terms of the types of IF, study designs, intervention durations, comparison groups, and sample sizes, all of which made combining studies difficult for interpretation. Of the 13 included RCTs, six studies were of short- or moderate-term durations (8 to 24 weeks), whereas only seven studies had longer durations. In addition, the majority of the included studies recruited healthy and overweight or obese participants, which may have limited the magnitude of the effect of IF interventions regarding significant improvements in risk factors for T2DM, CVDs, and cancers. Likewise, most participants in the included studies were women, which limits the generalizability of findings. Although this systematic review sought to determine whether or not the benefits of IF are independent of dietary quality, there were insufficient studies to examine this question.

Implications for Future Research

Given that there are few long-term studies using isocaloric or eucaloric IF interventions compared with control groups, future studies should be conducted with more rigorous controls across intervention arms, as well as controls for energy intake and balance, nutrient composition, and weight loss.

Future randomized controlled trials with larger populations, populations at higher risk for NCDs, better dietary controls, and longer durations are needed to better understand the role of IF on chronic disease risk factors. In addition, IF research that seeks to elucidate the effects that weight loss and dietary quality may have on the relationship between IF and NCD risk factors, is needed.

Conclusions

Intermittent fasting may be an effective approach for the primary and secondary prevention of NCDs. The current systematic review of energy matched IF vs DCR RCTs, indicated that overall, IF interventions produced similar beneficial effects for weight loss and chronic disease risk factors compared with DCR, with limited evidence suggesting that IF may be more effective vs DCR for fat loss and insulin sensitivity.

Table 3-1. Population, intervention, comparison, outcomes and study (PICOS) framework for formulation of the research question for a systematic review on the effects of isocaloric intermittent fasting vs daily caloric restriction on weight loss and metabolic risk factors of noncommunicable chronic disease.

Category	Result
Population	Overweight or obese adults
Intervention/exposure	At least an 8-wk regimen of intermittent fasting
Comparison/control	Daily caloric restriction
Outcome	Obesity and markers of diabetes, cardiovascular diseases, and cancers
Study designs	Randomized controlled or comparative trials

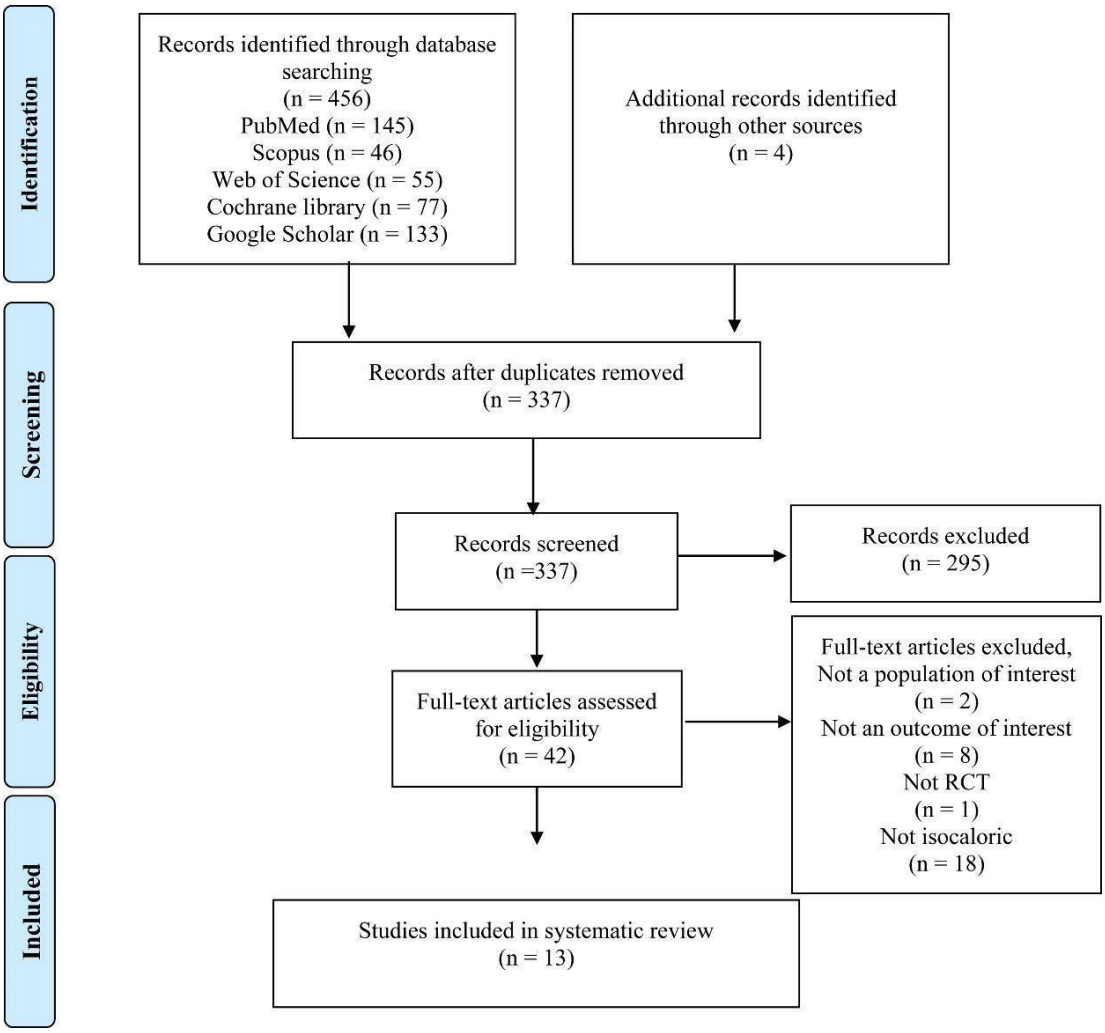

Figure 3.1 Preferred reporting items for systematic reviews and meta-analyses flow chart for the process of selecting articles for the systematic review of the effects of isocaloric intermittent fasting vs daily caloric restriction on weight loss and metabolic risk factors of noncommunicable chronic disease

Figure 3. Risk of bias assessment[26] for each randomized controlled trial included in the systematic review on the effects of isocaloric intermittent fasting vs daily caloric restriction on weight loss and metabolic risk factors of noncommunicable chronic disease outcomes (N = 13). Domains (D): D1 = bias arising from the randomization process. D2 = bias due to deviations from intended intervention. D3 = bias due to missing outcome data. D4 = bias in measurement of the outcome. D5 = bias in selection of the reported result. Judgment: High Some concerns Low

Figure 3-2. Risk of bias assessment

Authors, year; country	N(completers), inclusion criteria	Duration (weeks)	IF [a] intervention	Health of diet considered?	Outcomes	Key findings and considerations
TRE						
Liu and colleagues, 2022; [27] China	N:118 Age:18–75 (years) BMI [b] ≥28.0 kg/m^2	52	e-TRE [c] (8:00am–4:00pm) plus DCR [d] (25% energy restriction) vs DCR alone (25% energy restriction)	Yes	Body weight and composition, plasma glucose, HOMA-IR,[e] blood lipids, BP [f]	e-TRE was not superior compared to DCR; e-TRE and DCR produced similar weight/fat loss and improvements in markers of diabetes and CVD in adults with obesity; baseline eating window in the e-TRE group was relatively short (10 h and 23 min)
de Oliveira and colleagues, 2021; [28] Brazil	N:58 women Age:18–75 (years) BMI≥ 30 kg/m^2 waist circumference ≥ 88 cm ; and/or body fat ≥ 35%	52	12h-TRE [g] (self-selected window) plus DCR (mean energy restriction: 637.16 Kcal) vs DCR only (mean energy restriction: 651.85 Kcal)	--	Body weight and composition, BP	12h-TRE showed no effects on weight loss; however, reductions in body fat suggest that 12h-TRE is viable approach in the long-term management of obesity; pre-and post- intervention eating windows were not provided
Thomas and colleagues, 2022; [29] United States	N:63 Age:18–50 (years) BMI ≥27.0 kg/m^2	39	e-TRE plus DCR (10h eating window starting 3h of waking) vs DCR alone +behavioral weight-loss intervention	Yes	Body weight and composition (at 12 and 39 wk), $HbA1_c$,[h] blood lipids (at 12 wk)	Comparable weight loss with 14h-e-TRE+ DCR vs DCR alone; no improvements in CVD [i] and diabetes markers in groups; both groups decreased their eating windows
Kunduraci and colleagues, 2020; 30 Turkey	N:65 MetS [j] Age 18–65 (years) BMI≥27.0 kg/m^2	12	16:8 TRE (25% energy restriction, vs DCR (25% energy restriction) +Med [k] diet	Yes	Body weight and composition, BP, blood lipids fasting glucose, fasting insulin, HOMA-IR, $HbA1_c$	TRE is a feasible and well-tolerated weight loss strategy to improve metabolic syndrome, diabetes and CVD markers with similar to DCR; pre- and post- intervention eating windows were not provided
ADF [l]						

Varady and colleagues, 2011; [31] United States	N:49 Age:35–65 (years) BMI:25–40 kg/m^2	12	ADF (25% of energy need), vs DCR, vs moderate intensity exercise or control	Yes	Body weight, blood lipids, LDL[m] and HDL[n] particle size	ADF and DCR increased LDL particle size with 5% weight loss, while no effect on HDL size; conversely, exercise increased large HDL particles with the same levels of weight loss
4:3 Diet						
Coutinho and colleagues, 2017; [32] Norway	N:24 Age≥18 (years) BMI≥30 kg/m^2	12	4:3 diet:3-d non-consecutive fast/wk (25% of energy needs; matched energy intake on fed days) vs DCR	Yes	Body weight and composition, insulin	4:3 diet and DCR produced similar weight loss and significantly reduced postprandial insulin levels
Maroofi and colleagues, 2020; [33] Iran	N:80 Age:35–65(years) BMI≥ 25 kg/m^2 mild-to-moderate high triglyceride (HTG)	8	4:3 diet:3-d fast/wk (30% energy need) + four days of eucaloric diet vs DCR (70% of energy needs daily)	--	Body weight and composition, blood lipids, glucose, insulin, adiponectin	4:3 diet is comparable to DCR diet for weight loss, and triglyceride reduction in adults with HTG, and more effective than DCR in improving insulin resistance
5:2 diet						
Carter and colleagues, 2018; [34] Carter and colleagues, 2019; [35] Australia	N:137 T2D[o] Age≥18 (years) BMI ≥30 kg/m^2	52	5:2 diet:2-d fast (500–600kcal) vs DCR (1200–1500kcal/d) +52wk follow-up	Yes	Body weight and composition, HbA_{1c}, blood lipids	5:2 diet is comparable to DCR diet for glycemic control in patients with type 2 diabetes; HbA_1c rose to above baseline in both diets following 52wk follow-up
Gray and colleagues, 2021; [36] Australia	N:62 women with previous GDM [p] Age≥18 (years) BMI ≥25 kg/m^2	52	5:2 diet (2-d 500 kcal) vs DCR (1500kcal daily)	Yes	Body weight, fasting glucose, fasting insulin, HOMA-IR	5:2 diet produced comparable weight loss and improvements in markers of diabetes to DCR at 12- month in women with previous GDM
Sundfor and colleagues, 2018; [37] Norway	N:112 MetS Age:21–70 (years) BMI:30–45 kg/m^2	52	5:2 diet (28% energy restriction; 400–600 kcal on fasting days) vs DCR (26% energy restriction) + Med diet	Yes	Body weight and composition, glucose, HbA_{1c}, blood lipids, BP, CRP [q]	Similar weight loss and improvements in risk markers of CVD with IF (5:2) and DCR after one year

Headland and colleagues, 2019; [38] Australia	N:146 Age:18–72 (years) BMI:25–40 kg/m^2	52	5:2 diet:2-d fast (25% of usualenergy intake) and 5-d usual diet vs vs weekly IF (alternating between one week DCR one week habitual diet) vs DCR	Yes	Body weight and composition, glucose, blood lipids	Similar weight loss and improvements in HDL levels with two forms of IF and DCR after one year
Conley and colleagues, 2018; [39] Australia	N:23 men Age:55–75 (years) BMI≥ 30 kg/m^2	24	5:2 diet (600 kcal for 2 days of the week) vs DCR	Yes	Body weight, WC, BP, glucose, blood lipids, BP	5:2 diet was as effective as DCR for weight loss
Harvie and colleagues, 2013; [20] United Kingdom	N:115 women Age:20–69 (years) BMI:24–45 kg/m^2 family history of breast cancer	12	5:2 diet with low carbohydrate (<40 g) +Med diet vs 5:2 diet with low carbohydrate, ad lib [r] protein and fat+ Med diet vs high protein DCR+ Med diet + 1 month weight maintenance (1 d/wk fasting)	Yes	Body weight and composition, HOMA-IR, oxidative stress markers, leptin, adiponectin, IGF-1 [s], blood lipids, BP, IL-6,[t] TNF-α [u]	5:2 diet was superior to DCR for improvement of insulin sensitivity and body fat reduction; both diets decreased CVD and cancer risk factors; higher adherence in 5:2 diet groups and higher protein intake in DCR

Table 3-2: Summary of randomized controlled/comparative trials on the effects of isocaloric intermittent fasting on chronic disease biomarkers as compared with daily calorie restriction

[a] IF=Intermittent fasting.[b] BMI=Body mass index.[c] e-TRE=early-Time-restricted eating.[d] DCR=Daily calorie restriction.[e] HOMA-IR= Homeostatic Model Assessment for Insulin Resistance.[f] BP=Blood pressure.[g] TRE=Time-restricted eating.[h] HbA1c=Hemoglobin A1c.[i] CVD=Cardiovascular disease.[j] Mets=Metabolic syndrome.[k] MD diet= Mediterranean diet.[l] ADF=Alternate-day fasting.[m] LDL=Low-density lipoprotein.[n] HDL=High-density lipoprotein.[o] T2DM=Type 2 diabetes mellitus.[p] GDM= Gestational diabetes mellitus.[q] CRP=C-reactive protein.[r] Ad lib=Ad libitum.[s] IGF1= Insulin-like growth factor 1.[t] IL6=Interleukin-6.[u] TNF-α= Tumor necrosis factor α.

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Chapter 4- Does time-restricted eating add benefits to calorie restriction? A systematic review

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Abstract

Objective: A growing body of evidence has supported the health benefits of extended daily fasting, known as time-restricted eating (TRE); however, whether the addition of TRE enhances the known benefits of calorie restriction (CR) remains unclear.

Methods: PubMed, Scopus, the Cochrane Library, and Google Scholar were searched through April 2023. This systematic review includes randomized controlled trials (RCTs) that compared CR + TRE with CR alone in energy-matched conditions of at least 8 weeks in duration that assessed changes in body weight and cardiometabolic disease risk factors in adults with overweight and/or obesity.

Results: Seven studies were identified (n = 579). Two studies reported greater weight loss and reductions in diastolic blood pressure with CR + TRE compared with CR alone after 8 to 14 weeks, whereas one study reported greater improvements in triglycerides and glucose tolerance with CR + TRE (3 d/wk) compared with CR alone following 26 weeks. One study reported significant increases in Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) levels with CR + TRE versus CR alone after 8 weeks. There were no statistically significant differences in any other outcome variable between the two interventions.

Conclusions: The addition of TRE to CR regimens resulted in greater weight loss and improvements in cardiometabolic risk factors in some studies; however, the majority of studies did not find additional benefits.

Introduction

Chronic diseases, including obesity, cardiovascular diseases (CVD), cancers, and diabetes, are a major global public health concern resulting in 41 million deaths worldwide annually, which represents 71% of all deaths globally [(1)]. In the United States, six in ten adults have at least one chronic disease [(2)]. The increased risk of chronic disease

incidence can be attributed to many aspects of the modern American lifestyle, particularly increased sedentary behaviors and frequent consumption of high-calorie, low-nutrient-dense foods [(3, 4)]. The increasing prevalence of s

Such chronic diseases in middle-aged and older adults has led to an urgent need for lifestyle interventions that can potentially delay the onset and progression of these diseases.

Calorie restriction (CR), which is currently recommended for the management of overweight and obesity in adults [(5)], is a dietary approach involving consistent daily reduction in calorie intake, typically by 20% to 40% of baseline levels [(6)]. Although the benefits of CR on markers of diabetes, CVD, and cancer have been well-established, particularly in individuals with obesity [(6-9)], many individuals find it difficult to sustain CR over the long-term [(10)] and thereby ultimately regain lost weight [(11, 12)]. Given the challenges of long-term adherence to CR, there has been increased scientific interest in alternative dietary approaches such as intermittent fasting (IF) that may produce similar health benefits as CR.

IF is an umbrella term that describes an eating pattern in which an individual fasts for an extended time period, typically longer than 12 h, on a repeated basis [(13)]. Of the various types of IF approaches, time-restricted eating (TRE), i.e., the pattern of extending the daily fasting period and thereby reducing the daily eating period, has gained popularity over the last few years [(14)]. Findings from our past reviews [(13, 15-17)] have suggested that TRE is a viable dietary approach to improve body composition [(13, 15)], some metabolic parameters [(15)], proinflammatory markers [(16, 17)], and oxidative stress markers [(16, 17)], which are known risk factors for several chronic diseases including diabetes, CVD, Alzheimer's disease [(16)], and some cancers [(17)]. Furthermore, promising results from short-term and relatively small clinical trials have indicated that TRE improves fat oxidation [(18)], insulin sensitivity, and blood pressure, even in the absence of weight loss [(19)].

Emerging literature has suggested that restricting the timing of food intake and aligning food intake with circadian rhythms can improve metabolic health parameters and activate cellular pathways similar to that of CR, without calorie counting [(20, 21)]. However, recent findings from randomized controlled trials (RCTs) that have compared energy-matched TRE interventions with CR have suggested that the metabolic benefits of TRE can be accounted for solely by

the observed reduction in energy intake [(22-24)]. Thus, it is currently unclear whether the benefits of TRE are related to unintentional reductions in calorie intake or are due to other mechanisms associated with the fasting period and circadian rhythm alignment.

Given the question of whether the effects of TRE are independent of CR, the current systematic review of RCTs evaluated the effects of CR + TRE interventions compared with CR alone, in which calorie intakes were matched between groups, on anthropometric and cardiometabolic risk factors in adults with overweight and/or obesity.

METHODS

For reporting methods and results, the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed [(25)]. The Cochrane Handbook for Systematic Reviews of Interventions was used as a guide for developing the methods for the review [(26)]. This systematic review has been registered with the PROSPERO, an international prospective register of systematic reviews (CRD42023412344).

Data sources and searches

The authors (Armin Ezzati and Carly Bohlman) performed two separate searches of the online databases including PubMed, Scopus, Cochrane Central Register of Controlled Trials, and Google Scholar between September 2022 and April 2023. The search terms included the following: “time-restricted eating” and “time-restricted feeding” combined with “weight loss,” “obesity,” “overweight,” “metabolic risk factors,” “CVD,” “cardiovascular disease,” “cardiometabolic health,” “type 2 diabetes,” “cancer,” and “isocaloric” (online Supporting Information). These terms were combined using the “OR” Boolean operator with “AND” between the two different concepts. Through PubMed, filters were set to allow only “clinical trials,” “human” studies, and studies in the “English” language.

Study selection/synthesis

Studies were included if they met the following criteria: 1) RCTs with matched energy prescriptions between CR + TRE (TRE ≥ 14-h fasting) and CR alone (no time restriction or >12 h eating window); 2) adults with overweight

and/or obesity; 3) at least an 8-week intervention; 4) assessment of anthropometric and/or metabolic outcomes; and 5) publication in English. Because it is currently unknown how many days per week of TRE are required for cardiometabolic health benefits, we did not limit our inclusion criteria by the number of days per week that TRE was practiced. Studies were excluded if they prescribed <14 h of fasting or compared TRE versus CR with 12-h eating windows. Two independent reviewers (Armin Ezzati and Christian McLaren) assessed each record for inclusion or exclusion based on the initial review of the title and abstract. Articles that fulfilled all of these criteria were retrieved for review of the full text. The same independent reviewers assessed the full-text articles. Discrepancies regarding the inclusion of an article were resolved via subsequent discussions that yielded a mutual decision by the independent reviewers.

Quality assessment

Two independent reviewers (Armin Ezzati and Christian McLaren) used the Cochrane risk-of-bias tool to determine the risk of bias in each full-text article [(27)]. Discrepancies regarding the risk-of-bias assessment were resolved via subsequent discussions that yielded a mutual decision by the independent reviewers. The updated Cochrane risk-of-bias assessment tool for RCTs was used to evaluate five domains of bias: arising from the randomization process; deviation from the intended intervention; missing outcome data; measurement of the outcome; and selection of the reported results. Evaluation of these five criteria resulted in overall judgments in the following three categories: 1) low risk of bias; 2) some concerns; or 3) high risk of bias [(27)].

RESULTS

The initial literature search resulted in 109 relevant articles (Figure 1). After removing duplicates, 62 articles remained. Based on the inclusion criteria, reviewers excluded 44 articles during the title and abstract screening. Eighteen remaining research articles were examined for eligibility, and eleven additional studies were removed that either did not match the calorie intakes between TRE and CR or because other aspects of the study design did not meet our criteria (e.g., CR with 12-h eating window). Only seven studies (n = 579) were included in the analysis.

Risk-of-bias assessment

Risk-of-bias assessments were carried out for all included RCTs. Overall risk-of-bias assessments were judged as low to moderate risk for the included studies (Figure 2). Five out of seven studies were rated as low risk of bias. Two studies were rated as having some risk of bias due to either the limited information on the randomization process or deviations from the interventions [(22, 28)]. With regard to bias arising from the randomization process, only one study [(28)] was rated as high risk for baseline group differences, three studies [(22, 24, 31)] were rated as some concern due to lack of randomization information, and three other studies [(23, 29, 30)] were rated as low risk. Considering bias due to deviations from intended interventions, five studies [(22, 23, 28-30)] were rated as some risk due to potential performance bias, and two [(24, 31)] were rated as low risk.

All studies were rated as low risk for missing outcomes. With respect to the bias in the measurement of the outcome, only one study [(30)] was deemed as having some risk of bias, whereas other studies were rated as low risk. Similarly, all studies were rated as low risk concerning selective reporting.

Study characteristics

Table 1 summarizes the key characteristics of the seven RCTs included in this systematic review. The eligible studies recruited a range of 63 to 209 participants with a duration of 8 to 52 weeks. The age of participants ranged from 31.6 ± 9.3 to 57 ± 10 years for the CR + TRE arms and 31.7 ± 8.3 to 58 ± 10 years for the CR alone arms. Only one study [(31)] exclusively recruited adults with obesity, whereas the other six studies included adults with both overweight and obesity. One study [(28)] only enrolled women with overweight and obesity. Five studies implemented CR + 16:8 TRE (16-h fasting), one [(24)] tested CR + 14:10 TRE (14-h fasting), and one study [(29)] designed IF plus early TRE (iTRE; 3 nonconsecutive fasting days per week; 8:00 a.m. to 12:00 p.m.; ad libitum eating on other days) compared with a CR alone comparison group. Five studies compared CR + early TRE (eTRE) versus CR alone [(22, 24, 29-31)], whereas two other studies [(23, 28)] allowed for self-selected eating windows for the TRE group. One study [(23)] recruited patients with metabolic syndrome, whereas two studies included adults with a high

risk of developing type 2 diabetes [(29)] or patients with nonalcoholic fatty liver disease (NAFLD) [(30)]. Body composition was measured using dual-energy x-ray absorptiometry (DXA) in five out of seven included studies [(22, 24, 29-31)], whereas two studies utilized bioelectrical impedance analysis (BIA) [(23, 28)]. Dietary intake was measured using food records in five studies [(22, 28-31)]. Of five studies that utilized food records, four studies used photographs [(22, 28, 30, 31)]. The other two studies assessed either 24-h dietary recall administered by a registered dietitian [(23)] or photographic food records [(24)]. The amounts of reductions in calorie intake ranged from 12% to 32.4% with CR + TRE interventions and 9.2% to 29.8% with CR alone. Most studies instructed participants to maintain their usual levels of physical activity [(22, 23, 29, 30)], two studies counseled participants to engage in 75 to 150 min/wk of moderate exercise [(24, 31)], and one [(28)] incorporated exercise into the intervention with eight weekly 30-min moderate exercise sessions. All studies assessed physical activity for comparability among groups throughout the interventions.

The effects of CR + TRE versus CR alone on anthropometric and body composition outcomes

Body weight

Of the seven included studies, five [(22-24)] found no significant differences in weight loss between CR + TRE and CR alone. By contrast, two studies [(28, 31)] reported significantly greater reductions in body weight with CR + TRE versus CR alone. Lin and colleagues [(28)] reported significantly greater weight loss with CR + 16:8 TRE compared with CR alone (4.1% with TRE vs. 2.4% with CR) in 63 women with overweight and obesity after 8 weeks. Likewise, Jamshed and colleagues [(31)] reported significantly greater weight loss with CR + eTRE versus CR alone (5.6% with CR + TRE vs. 3.8% with CR). The study prescribed a hypocaloric diet (500 kcal/d below resting energy expenditure) and only enrolled participants with >12-h eating windows at baseline in both intervention groups [(31)]. Overall, both CR + TRE and CR alone interventions produced significant weight loss compared with baseline in all included studies (4.1%–9.9% with CR + TRE vs. 2.4%–8.5% with CR alone). However, there were no significant differences in the changes induced by CR + TRE compared with CR alone in most studies (Table 2, Figure 3).

Body fat and lean mass

Body fat reductions of 1.9% to 18.0% were observed with CR + TRE which were not significantly different from body fat reductions of 1.4% to 16.6% with CR alone arms in all included studies (Table 2). Four studies [(22, 24, 28, 29)] also evaluated changes in lean mass with reductions in lean mass observed in the CR + TRE arms (2.2%–4.1%) that were comparable with those in the CR alone arms (2.8%–3.4%). Both CR + TRE and CR alone interventions produced significant reductions in body fat and lean mass compared with baseline in all included studies in which they were measured.

Waist circumference

Changes in waist circumference were assessed in five studies [(22, 28-31)] with no significant differences in the changes induced by CR + TRE compared with CR alone (2.2–9.3 cm with CR + TRE vs. 1.9–8.2 cm with CR alone), following short- to long-term trials of 8- to 52-week durations (Table 2). Of note, both CR + TRE and CR alone interventions produced significant reductions in waist circumference compared with baseline in all five studies.

The effects of CR + TRE versus CR alone on metabolic risk factors

Total cholesterol

Of the seven included studies, four [(22, 23, 29, 30)] reported significant reductions in total cholesterol (TC) in both the CR + TRE and CR alone interventions following 12 and 52 weeks, with no significant differences between groups (−5.2% to 12.9% with CR + TRE vs. −3.9% to 12.8% with CR alone; Table 3). One study [(31)] noted a significant reduction in TC (−7.4%) compared with baseline only in the CR alone control arm in 59 participants with obesity at 14 weeks. By contrast, TC levels increased or remained unchanged in two other studies [(24, 28)] that enrolled metabolically healthy adults.

High-density lipoprotein

In three [(22, 29, 30)] out of seven studies, both CR + TRE and CR alone demonstrated significant improvements in high-density lipoprotein (HDL) levels compared with baseline (3.8%–15% with CR + TRE vs. 6.4%–8.5% with CR alone), with no significant differences between groups (Table 3). Conversely, a 14-week study [(31)] comparing CR + TRE with CR alone noted significant reductions in HDL levels versus baseline levels in both arms (−4 mg/dL [−6.5%] with CR + eTRE vs. −4 mg/dL [−6.6%] with CR alone). CR + TRE and CR alone did not alter HDL levels in the other three studies after 8, 12, and 39 weeks [(23, 24, 28)].

Low-density lipoprotein

Of the seven included studies, there were no significant differences in the changes in low-density lipoprotein (LDL) induced by CR + TRE compared with CR alone (Table 3). Four [(22, 23, 29, 30)] studies reported significant differences in LDL with both CR + TRE and CR alone compared with baseline, in which participants had high levels of LDL at baseline (−6.4% to 11.5% with CR + TRE vs. −6.2% to 10.8% CR alone). However, one study [(31)] noted a significant reduction in LDL only in the CR alone group, but not in the CR + TRE group, following 14 weeks (−5 mg/dL [−4.2%] with CR + TRE versus −9 mg/dL [−7.7%] with CR alone). The two other studies [(24, 28)] reported no significant changes in LDL levels at 8 and 39 weeks.

Triglycerides

Of the seven included studies, only one [(29)] reported significant improvements in triglyceride (TG) levels induced by CR + TRE compared with CR alone (Table 3, Figure 3). The study imposed CR + iTRE (eating window 8:00 a.m. to 12:00 p.m. followed by 20-h fasting on 3 nonconsecutive days per week) versus CR alone at month 6 [(29)] and recruited 137 adults with overweight and obesity who were at increased risk of developing type 2 diabetes. Both CR + TRE and CR alone showed significant improvements in TG levels compared with baseline in four out of seven studies (−12% to 25% with CR + TRE vs. −2.1% to 25.4% with CR alone).

Fasting blood glucose

Six of the seven included studies measured fasting blood glucose (FBG) and reported no significant differences between CR + TRE and CR alone (Table 3). However, CR + TRE produced significant improvements compared with baseline in four [(23, 29-31)] out of six studies, whereas CR alone led to statistically significant improvements in only two of these studies (−3.1% to 13% with CR + TRE vs. −5.8% to 11.4% with CR alone; Table 3) [(23, 31)]. Specifically, two studies [(29, 30)] found significant improvements only with CR + TRE versus baseline, but not with CR alone, in adults with overweight and obesity who were at high risk of developing type 2 diabetes [(29)] or NAFLD [(30)] following 26 and 52 weeks, respectively. Moreover, two studies [(23, 31)] found improvements in FBG with both CR + TRE and CR alone in adults with overweight and obesity and/or metabolic syndrome. In one of these studies, the intervention was combined with a Mediterranean diet [(23)], and in the other, only participants with ≥12-h eating windows at baseline were enrolled [(31)]. In contrast, one study [(28)] reported a significant increase in FBG only with CR + TRE compared with baseline in 63 women at 8 weeks. Of note, glucose tolerance significantly improved with CR + iTRE compared with CR alone in adults with increased risk of type 2 diabetes at week 26 (−10.10 [95% CI: −14.08 to −6.11] vs. −3.57 [95% CI: −7.72 to 0.57] mg dL−1 min−1; p = 0.03) [(29)].

Homeostatic Model Assessment of Insulin Resistance

Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was tested in five studies, of which three [(22, 30, 31)] showed significant improvements in HOMA-IR levels only with CR + TRE versus baseline (−26.6% to 43.2%), but not CR alone (with no between-group differences), at 14 to 52 weeks. (Table 3). Furthermore, one study [(23)], which included a Mediterranean diet in both arms, noted improvements in HOMA-IR with CR + TRE and CR alone in 65 patients with metabolic syndrome (with no between-group differences) following 12 weeks. Conversely, another study [(28)] reported a significant increase in HOMA-IR only with CR + TRE compared with baseline in women with overweight and obesity, with no other metabolic risk factors, at week 8. However, the baseline HOMA-IR levels were significantly lower in the CR + TRE group compared with the CR alone group, which may have contributed to this

finding [(28)]. Overall, of the five studies that assessed HOMA-IR, most CR + TRE interventions resulted in improvements in HOMA-IR levels versus baseline, whereas CR alone did not improve HOMA-IR in the majority of studies.

Systolic blood pressure

Six [(22, 23, 29-31)] of the seven included studies tested blood pressure with CR + TRE versus CR alone. No significant between-group differences in systolic blood pressure (SBP) were observed with CR + TRE compared with CR alone in all six studies in which it was measured; however, CR + TRE improved SBP significantly compared with baseline in five of six studies [(22, 23, 29-31)], and CR alone significantly decreased SBP in four of six studies (Table 3) [(22, 23, 29, 30)]. By contrast, one study [(31)] reported significant improvements in SBP only in the CR + TRE group (−6.3% with CR + TRE vs. −2.5% with CR alone).

Diastolic blood pressure

Two [(28, 31)] of six trials that assessed blood pressure levels observed significantly greater improvements in diastolic blood pressure (DBP) with CR + TRE versus CR alone following 8 and 14 weeks. One study [(28)] showed a significant reduction in DBP with CR + TRE compared with CR alone (−4.8 mm Hg [−5.2%] vs. +3.4 mm Hg [+6.1%]), in which both groups were prescribed 1400-kcal/d calorie intakes. In line with these findings, another study [(31)] reported a significant reduction in DBP with CR + TRE (eating window: 7:00 a.m. to 3:00 p.m.) versus CR alone in 59 adults with obesity following 14 weeks. Of note, this study prescribed a dietary intake of 500 kcal/d below the resting energy expenditure and only enrolled participants with >12-h eating windows at baseline in both intervention groups [(31)]. Other studies that either recruited participants with short eating windows [(22, 30)] or did not report eating windows [(23, 29)] observed similar reductions in DBP with CR + TRE versus CR alone after 12 and 52 weeks. Of the six studies that assessed DBP, both CR + TRE and CR alone improved DBP versus baseline (−5.2% to 10% with CR + TRE vs. −1.3 to 9.2% with CR alone); however, there were no significant differences in the changes induced by CR + TRE compared with CR alone in the majority of studies (Table 3, Figure 3).

DISCUSSION

The purpose of the current systematic review was to examine the effects of combining TRE with CR compared with CR alone under energy-matched conditions on anthropometric and cardiometabolic risk factors in adults with overweight and obesity. In the majority of studies included in this systematic review, the addition of TRE to CR interventions did not produce statistically significant improvements in anthropometric or metabolic outcomes compared with CR alone. However, in two studies [(28, 31)], greater weight loss and improvements in DBP were observed with CR + TRE compared with CR alone after 8 to 14 weeks. Additionally, one study [(29)] reported superior improvements in glucose tolerance and TG levels with CR + TRE (3 d/wk) compared to the CR alone arm following 26 weeks.

The reasons for these mixed findings are not entirely clear; however, we believe that variations in the fasting protocols in the TRE interventions may have been a contributing factor. Unfortunately, only one [(31)] of the two studies [(28, 31)] that reported significantly greater weight loss and reductions in DBP with CR + TRE interventions assessed pre-post intervention eating windows. Of note, this study reported greater reductions in daily eating windows compared with the studies that prescribed CR + eTRE but showed no effect (4.8-h/d change in baseline eating window vs. 2.3 h vs. 2.4 h, respectively) [(31, 24, 22)]. The extended fasting periods in the CR + TRE conditions may have contributed to greater weight loss through a few potential mechanisms, including increased fat oxidation [(20, 35)], daily energy expenditure [(37, 38)], and/or increased fecal energy loss [(34)]. Additionally, other studies have found that intermittent energy restriction attenuates the reduction in resting metabolic rate associated with continuous CR [(37, 38)].

In addition to extended fasting periods, the timing of the fasting interval may also contribute to greater weight loss. For example, previous studies have shown that TRE increases fat oxidation [(20, 35)], particularly when practiced earlier in the day [(20, 32, 33)]. Another recent finding [(34)] was that eTRE resulted in a 22.7% greater fecal energy loss compared with the control, which was equal to 2.6% negative energy balance, in healthy adults of normal weight,

translating to 0.7 kg of weight loss in an individual weighing 84 kg over 8 weeks [(36)]. In line with these findings, the study by Jamshed et al. [(31)] showed that CR + eTRE may be more effective in improving weight loss and some metabolic risk factors in individuals with obesity compared with CR alone when participants have relatively large eating windows at baseline (≥12 h).

Furthermore, the significant reductions in DBP observed in the two aforementioned studies [(28, 31)] were a novel finding. The precise mechanism underlying this effect is unknown, but we believe the reduction is related to the degree of weight loss, which, as aforementioned, was significantly greater in the CR + TRE conditions versus CR alone in these two studies [(28, 31)]. In line with this finding, one study has attributed the reduction in DBP to weight loss [(41)]. However, it is suggested that IF (including TRE) can lower blood pressure through other mechanisms, including improved insulin sensitivity, increased brain-derived neurotrophic factor [(46)], and thereby improved endothelial function [(48)] and activity of autophagy [(39, 47)] as a result of metabolic switching [(39)] and eating earlier in the day in line with circadian rhythms [(19, 31)].Another noteworthy finding was that the CR + TRE intervention produced greater improvements in both glucose tolerance and fasting TG levels compared with CR alone in the study by Teong and colleagues [(29)]. This study prescribed CR + 20 h of fasting only 3 d/wk; therefore, these superior effects also may be attributed to improved insulin sensitivity and insulin secretion or delayed gastric emptying [(29, 42)] as a result of the longer fasting hours [(43, 44)] in the iTRE group and/or the early eating plan during the TRE intervention [(40, 45)]. In line with these findings, we observed that CR + TRE produced significant reductions in HOMA-IR from baseline in the four [(22, 23, 30, 31)] of five studies [(22, 23, 28, 30, 31)] in which it was measured. Surprisingly, one study reported an increase in HOMA-IR and FBG levels following CR + TRE in female participants with normal baseline HOMA-IR values. In contrast to CR + TRE, CR alone improved HOMA-IR in only one study [(23)]. In this study, patients with metabolic syndrome were also instructed to follow the Mediterranean diet. Similarly, the CR + TRE interventions decreased FBG in four [(23, 29-31)] out of six studies [(22, 23, 29-31)] compared with baseline, whereas CR alone improved FBG in only two [(23, 31)] out of six studies [(23, 28-31)]. Taken together, these results suggest that CR + TRE is effective for improving HOMA-IR and FBG in adults

with overweight and obesity. For plasma lipid profile, the result of our systematic review is consistent with previous studies [(19, 49-51)] that have shown that short-term TRE has no effect on lipid profile. Furthermore, one study [(31)] reported improvements in TC and LDL levels only with CR alone despite the significantly greater weight loss in the CR + TRE intervention.

We believe that key aspects of the study design may have played a role as to why significant results were observed in some studies [(28, 29, 31)], but not in others [(22-24, 30)]. For example, only four [(22, 24, 30, 31)] out of the seven included trials assessed pre-post intervention eating windows. In the two [(22, 24)] of the four studies [(22-24, 30)] in which no between-group differences were observed, participants with shorter baseline eating windows (<12 h) were included in both study arms, which may have led to similar interventions in both conditions. Another possible explanation for the null results in some studies is that some of the metabolic benefits observed in CR alone may have been due to an unintentional reduction in eating windows of participants assigned to the CR intervention arms (Table 1). Thus, the daily fasting periods may have been similar in both the CR + TRE and CR alone conditions, which may have negated the potential independent effects of TRE and calorie intake.

The results of this systematic review should be interpreted in the context of its limitations. The main limitation of this review is the limited number of RCTs that compared energy-matched CR + TRE interventions with CR alone at the present time. Another limitation of the current review is the heterogeneity among studies in terms of the fasting durations/times. We included studies that imposed TRE with 14, 16, and 20 h of fasting (3 d/wk) and different fasting initiating times (early or self-selected eating windows). As aforementioned, TRE protocols implemented different designs, with the majority of the studies (six out of seven) enrolling participants with relatively short eating windows (<12 h) at baseline. The short intervention durations and small sample sizes in the studies included are also notable limitations. Furthermore, the majority of the included studies recruited individuals who were metabolically healthy with overweight and obesity, which may have blunted the magnitude of the effect of TRE interventions. Finally, physical activity remains an important factor that may influence the efficacy of TRE because there may be benefits to adding activity to CR + TRE regimens, but further research is needed in this area.

A strength of this review is that we included energy-matched studies with more than 8-week durations that compared CR + TRE versus CR alone (i.e., no time restriction or ≥12-h window). Additionally, studies were of relatively high

quality with a low to moderate risk of bias (Figure 2). Studies included in this review reported good adherence rates to both CR and CR + TRE (range = 73%–86%), which increases confidence in the fidelity of the interventions of the studies included in this review. Moreover, none of the studies reported a significant difference in adherence level between the CR + TRE and the CR groups.

The findings of this review have some implications for clinical practice. The addition of TRE to CR regimens appears to be an effective strategy for decreasing body weight and reducing cardiometabolic risk factors such as glycemic profile and DBP in patients with overweight and obesity and/or elevated cardiometabolic risk factors. At this point in time, there is not sufficient evidence to indicate which populations would specifically benefit from CR + TRE compared with CR alone based on between-group differences. Of note, the CR + TRE interventions improved metabolic outcomes (FBG and HOMA-IR) more than the CR alone interventions in participants with obesity and related metabolic conditions. Further research is needed to inform on the additional benefits of adding TRE to CR interventions in specific populations or phenotypes.

CONCLUSION

Although the addition of TRE to CR did not produce additional benefits in the majority of studies included in this review, three of the seven studies did find significant between-group differences in anthropometric and metabolic outcomes. This suggests that TRE may enhance the benefits of CR in some circumstances, but there is currently not adequate evidence to draw strong conclusions. Recent studies [(24, 31)] have suggested that CR interventions may lead to restricted eating windows and thereby unintentionally lengthened fasting periods, which may have decreased the potential beneficial effects of adding TRE to CR interventions. This suggests that eating windows should be carefully assessed in future studies. Therefore, longer-term, well-designed RCTs are needed to disentangle the potential independent effects of TRE from CR and determine whether TRE has the potential to enhance the known health benefits of CR.

Future studies should include pre-post eating windows of participants exploring the effects of energy-matched CR + TRE compared with CR alone in which eating periods are extended throughout the day (>12-h window).

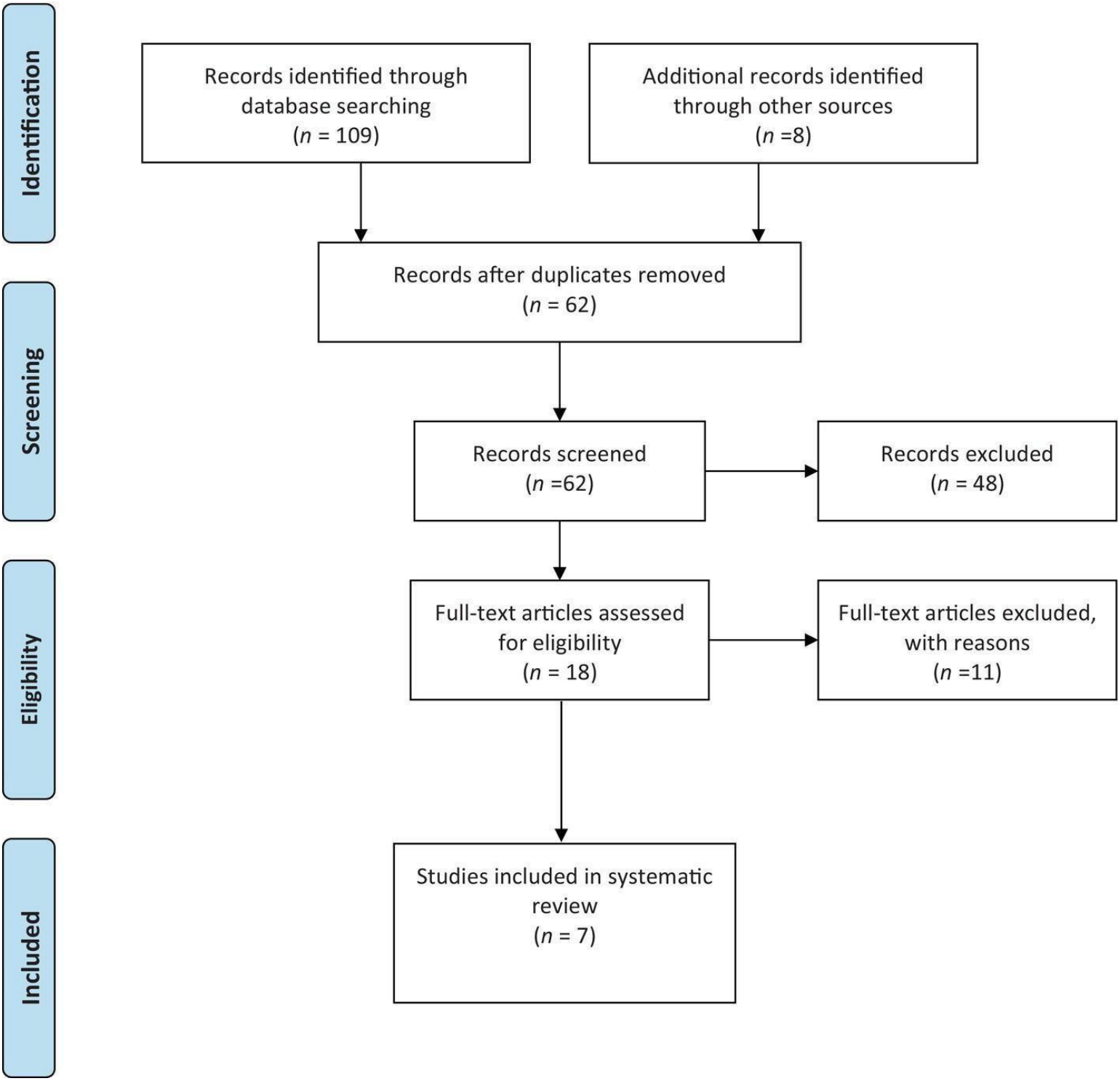

FIGURE 4-1. Flow diagram of selection of studies.

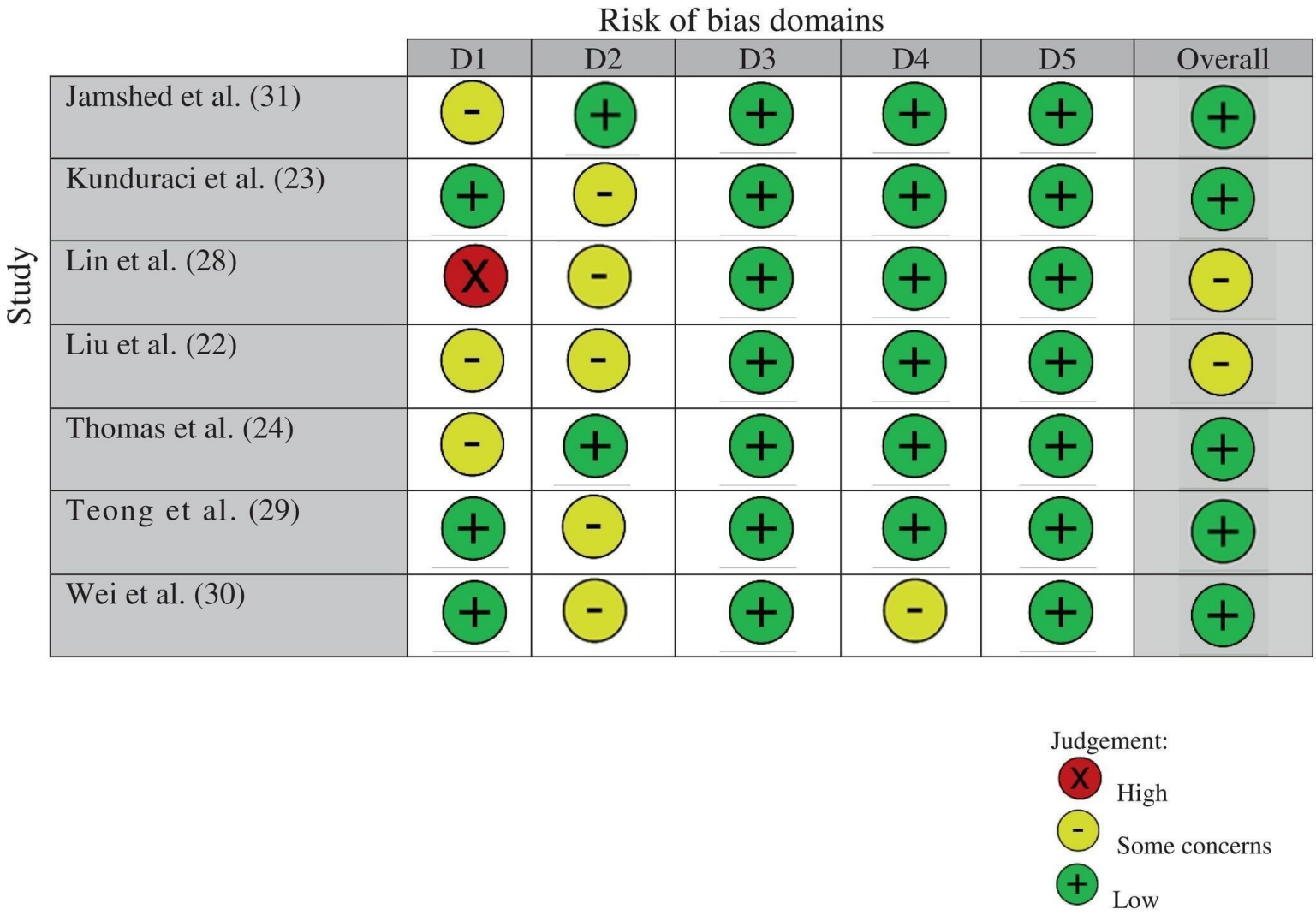

FIGURE 4-2

Risk-of-bias assessment. Domains: D1: Bias arising from the randomization process; D2: Bias due to deviations from intended intervention; D3: Bias due to missing outcome data; D4: Bias in measurement of the outcome; D5: Bias in selection of the reported result.

Table 4-1. Characteristics of included studies

Study (reference)	Country	Participants (% female)	Intervention	Age (SD), yrs	Post-intervention Eating Windows, hour: minute	Dietary assessment method	Body composition method	Quality Assessment, RoB-2
Jamshed et al., 2022 (31)	USA	59 (80% F) Adults with obesity BMI 40.1± 6.6 kg/m2	14 weeks 16:8 e-TRE (eating window: 7:00 am–3:00 pm) + CR (25% energy restriction)	43.0 (10.0)	Start of eating window: 8:00± 0:46 End of eating window: 15:30 ± 00:41 Daily eating window: -	Food record (Assisted with Photography)	DEXA	Low risk
			CR (self-selected ≥12-hour window; 25% energy restriction)	43.0 (11.0)	Start of eating window: 8:08± 1:01 End of eating window: 19:24± 01:08 Daily eating window: -			
Kunduraci et al., 2020 (23)	Turkey	65 (52.3% F) Adults with MetS, overweight & obesity BMI 36.6± 0.9 kg/m2 (CR + TRE) BMI 32.8± 0.7 kg/m2 (CR alone)	12 weeks 16:8 TRE (self-selected eating window) + CR (25% energy restriction + MD	47.4 (2.2)	-	24-hour dietary recall	BIA	Low risk
			CR (25% energy restriction) + MD	48.8 (2.1)	-			
Lin et al., 2021 (28)	Taiwan	63 (100% F) Adults with overweight & obesity BMI 25.9± 3.7 kg/m2 (CR+TRE) BMI 25.7 ±3.8 kg/m2 (CR alone)	8 weeks 16:8 TRE (either 10:00 am–6:00 pm or 12:00–8:00 pm) + CR (1400 kcal/d; ≈12 % energy restriction)	50.1 (7.5)	-	Food record (Assisted with Photography)	BIA	Some risk
			CR (1400 kcal/d; ≈ 9% energy restriction)	54.2 (9)	-			

Study (reference)	Country	Participants (% female)	Intervention	Age, mean ± SD, yrs	Post-intervention Eating Windows, hour: minute	Dietary assessment method	Body composition assessment	Quality Assessment, RoB-2
Liu et al., 2022 (22)	China	118 (48.9% F) Adults with overweight & obesity BMI 31.8± 2.9 kg/m2 (CR+TRE)	52 weeks 16:8 e-TRE (eating window: 8:00 am–4:00 pm) + CR (25% energy restriction)	31.6 (9.3)	Start of eating window: 08:27 ± 01:10 End of eating window: 16:36 ± 00:30 Daily eating window: 08:08 ± 01:11	Food records (Assisted with Photography)	DEXA	Some risk
		BMI 31.3±2.6 kg/m2 (CR alone)		32.2 (8.8)	Start of eating window: 08:32 ± 01:06 End of eating window: 19:25 ± 00:27 Daily eating window: 10:52 ± 01:04			
Thomas et al., 2022* (24)	USA	63 (85.2% F) Adults with overweight & obesity BMI 34.6±5.8 kg/m2 (CR+TRE)	39 weeks 14:10 e-TRE (10 h eating window within 3 h of waking) + CR (≈35% energy restriction)	38.3 (7.9)	Start of eating window: - End of eating window: - Daily eating window: 9.1 ± 0.93h	Photographic food records	DEXA	Low risk
		BMI 33.7±5.6 kg/m2 (CR alone)	CR (≈35% energy restriction)	37.8 (7.8)	Start of eating window: - End of eating window: - Daily eating window: 10.4 ± 1.5h			
Teong et al. 2023 (29)	Australia	137 (57% F) Adults with overweight & obesity & risk of T2D BMI 34.7±4.6 kg/m2 (CR+TRE)	26 weeks i-TRE (3 nonconsecutive days/week; 30% energy requirements between 8:00am – 12:00 pm; ad libitumeating on other days)	57.0 (10.0)	-	Food records	DEXA	Low risk
		BMI 35.0±4.6 kg/m2 (CR alone)	CR alone (70% energy requirements)	58.0 (10.0)	-			

Study (reference)	Country	Participants (% female)	Intervention	Age, mean ± SD, yrs	Post-intervention Eating Windows, hour: minute	Dietary assessment method	Body composition assessment	Quality Assessment, RoB-2
Wei et al. 2023 (30)	China	74 (51% F) Adults with obesity & NAFLD BMI 32.2± 3.4 (CR+TRE)	52 weeks TRE (eating window between 8:00 am – 4:00 pm; men: 1500 – 1800 kcal/day/d; women: 1200-1500 kcal/day/d)	32.3 (10.5)	Start of eating window: 08:15 ± 00:53 End of eating window: 16:36 ± 00:23 Daily eating window: 08:20 ± 00:51	Food records (Assisted with Photography)	DEXA	Low risk
		BMI 32.2 ±3.2 kg/m2 (CR alone)	CR (habitual meal timing; men: 1500 – 1800 kcal/day/d; women: 1200-1500 kcal/day/d)	31.7 (8.3)	Start of eating window: 08:40 ± 01:11 End of eating window: 19:32 ± 00:26 Daily eating window: 10:52 ± 01:11			

Abbreviations: CR: Calorie restriction; TRE: time-restricted eating; BMI: Body mass index; e-TRE: early time-restricted eating; Mets: Metabolic syndrome; NAFLD: Non-alcoholic fatty liver disease; MD: Mediterranean diet; iTRE: intermittent plus early time-restricted eating; T2D: Type 2 diabetes; DEXA: dual x-ray absorptiometry; BIA: Bioelectrical Impedance Analysis.

* Energy intake (photographic food records), physical activity (accelerometry), and dietary adherence (questionnaires) measured at Week 12.

Study (reference)	Participants	Intervention	Change in Calorie Intake, kcal/day (%)	ΔBody Weight, kg (%)	ΔFat Mass, kg (%)	Δ Lean Mass, kg (%)	ΔWaist Circumference, cm
Jamshed et al. 2022 (31)	N: 59 Age 25–75 yrs BMI 30-60 kg/m2	14 weeks 16:8 e-TRE (eating window: 7:00 am–3:00 pm) + CR (25% energy restriction)	↓579.0 kcal/day ‡ (-29.9%)	↓**6.3 kg** †‡ **(-5.6%)**	↓4.7 kg ‡ (-8.6%)	-	↓5.2 cm ‡
		CR (self-selected ≥12-hour window; 25% energy restriction)	↓580.0 kcal/day ‡ (-29.8%)	↓**4.0 kg** ‡ **(-3.8%)**	↓3.4 kg ‡ (-6.7%)	-	↓4.1 cm ‡
Kunduraci et al., 2020 (23)	N:65 Age 18–65 yrs BMI ≥27.0 kg/m2	12 weeks 16:8 TRE (self-selected eating window;8 am–4 pm, or 9 am–5 pm, 10 am–6 pm, or 11 am–7 pm) + CR (25% energy restriction) + MD	↓569.6 kcal/day ‡ (-27.6%)	↓8.3kg ‡‖ (≈ -7.0%)	↓5.5kg ‡ (-16.5%)	-	-
		CR (25% energy restriction) + MD	↓524.0 kcal/day ‡ (-25.6%)	↓5.8kg ‡ ‖ (≈ -7.0%)	↓4.1kg ‡ (-14.2%)	-	-
Lin et al. 2021 (28)	N: 63 Age 40–65 yrs BMI ≥24 kg/m2	8 weeks 16:8 TRE (either 10:00 am–6:00 pm or 12:00–8:00 pm) + CR (1400 kcal/day/d)	↓185.8 kcal/day ‡ (-12.0%)	↓**2.7kg** †‡ **(-4.1%)**	- (-1.9%) ‡	-	↓2.2cm ‡ (-2.1%)
		CR (1400 kcal/day/d; ≈ 9% energy restriction)	↓153.1 kcal/day ‡ (-9.2%)	↓**1.6kg** ‡ **(-2.4%)**	- (-1.4%) ‡	-	↓1.9cm ‡ (-2.1%)
Liu et al. 2022 (22)	N: 118 Age 18–75 yrs BMI ≥28 kg/m2	52 weeks 16:8 e-TRE (eating window: 8:00 am–4:00 pm) + CR (25% energy restriction)	↓613.0 kcal/day (-30.0%)	↓8.0 kg ‡ (-9.0%)	↓5.9 kg ‡ (−4.3%)	↓1.7 kg ‡ (-3.3%)	↓8.8 cm ‡ (-8.9%)
		CR (25% energy restriction)	↓504.3 kcal/day (-24.3%)	↓6.3 kg ‡ (-7.2%)	↓4.5 kg ‡ (−3.0%)	↓1.4 kg ‡ (-2.8%)	↓7.0 cm ‡ (-7.1%)
Thomas et al. 2022 * (24)	N:63 Age 18–50 yrs BMI 27–45 kg/m2	39 weeks 14:10 e-TRE (10 h eating window within 3 h of waking) + CR (≈35% energy restriction)	↓569.0 kcal/day (-30.3%)	↓4.9kg ‡ (-5.1%)	↓3.5kg ‡ (-8.5%)	↓1.2kg ‡ (-2.2%)	-
		CR (≈35% energy restriction)	↓480.0 kcal/day (-29.2%)	↓4.3kg ‡ (-4.6%)	↓2.6kg ‡ (-6.5%)	↓1.5kg ‡ (-2.9%)	-

Study (reference)	Participants	Intervention	Change in Calorie Intake, kcal/day (%)	Δ Body Weight, kg (%)	Δ Fat Mass, kg (%)	Δ Lean Mass, kg (%)	ΔWaist Circumference, cm
Teong et al. 2023 (29)	N: 137 Age 35–75 yrs BMI – kg/m2	26 weeks iTRE (3 nonconsecutive days/week; 30% energy requirements between 8:00 am – 12:00 pm; ad libitum eating on other days)	↓520.1 kcal/day‡	↓7.4kg ‡ (-7.5%)	↓6.2kg ‡ (-14.2%)	↓1.9kg ‡§ (-3.4%)	↓6.8cm ‡ (-6.2%)
		CR (70% energy requirements)	↓424.3 kcal/day ‡	↓7.0kg ‡ (-6.9%)	↓5.8kg ‡ (-12.9%)	↓1.3kg ‡§ (-2.5%)	↓6.4cm ‡ (-5.7%)
Wei et al. 2023 (30)	N:74 Age 18–50 yrs BMI 27–45 kg/m2	52 weeks TRE (eating window between 8:00 am – 4:00 pm; men: 1500 – 1800 kcal/day/d; women: 1200-1500 kcal/day/d)	↓684.7 kcal/day ‡ (-32.4%)	↓8.4kg‡ (-9.4%)	↓6.1kg ‡ (-18.0%)	↓2.1kg‡ (-4.1%)	↓9.3cm ‡ (-9.3%)
		CR (habitual meal timing; men: 1500 – 1800 kcal/day/d; women: 1200-1500 kcal/day/d)	↓586.9 kcal/day‡ (-27.2%)	↓7.8kg ‡ (-8.5%)	↓5.8kg ‡ (-16.6%)	↓1.8kg ‡ (-3.4%)	↓8.2cm ‡ (-8.0%)

Table 4-2. Effects of energy-matched CR + TRE vs. CR alone on anthropometric outcomes.

Abbreviations: CR: Calorie restriction; TRE: time-restricted eating; BMI: Body mass index; e-TRE: early time-restricted eating; iTRE: Intermittent early time-restricted eating; MD: Mediterranean diet; iTRE: intermittent plus early time-restricted eating

Key: * Body weight measured at week 39 and body composition (dual-energy x-ray absorptiometry) measured at both Week 12 and Week 39; † Significant between -group change vs CR ($p < 0.05$); ‡ Significant within-group change ($p < 0.05$); § Fat-free mass; || Imbalance between groups at baseline.
-Bold results indicate significant between group differences.

Table 4-3. The effects of energy-matched CR+TRE vs. CR alone on metabolic outcomes (14<x<24 hrs)

Study (reference)	N, inclusion criteria	Intervention	Δ TC, mg/dL (%)	Δ HDL, mg/dL (%)	Δ LDL mg/dL (%)	Δ TG, mg/dL (%)	Δ FBG, mg/dL (%)	Δ HOMA-IR (%)	Δ SBP, mmHg (%)	Δ DBP, mmHg (%)
Jamshed et al. 2022 (31)	N: 59 Age 25–75 yrs BMI: 30-60 kg/m2	14 weeks 16:8 e-TRE (eating window: 7:00 am–3:00 pm) + CR (25% energy restriction)	↓9 mg/dL (-4.4%)	↓4 mg/dL ‡ (-6.5%)	↓5 mg/dL (-4.2%)	↓3 mg/dL (-2.6%)	↓8 mg/dL ‡ (-7.5%)	↓2.00 ‡ (-33.2%)	↓8 mmHg ‡ (-6.3%)	**↓5 mmHg †‡ (-6.1%)**
		CR (self-selected ≥12-hour window; 25% energy restriction)	↓15 mg/dl‡ (-7.4%)	↓4 mg/dl‡ (-6.6%)	↓9 mg/dL‡ (-7.7%)	↓9 mg/dL (-7.6%)	↓6 mg/dL ‡ (-5.8%)	↓0.74 (-16.7%)	↓3 mmHg (-2.5%)	**↓1 mmHg†‡ (-1.3%)**
Kunduraci et al., 2020 (23)	N:65 Age 18–65 BMI ≥27.0 kg/m2	12 weeks 16:8 TRE (self-selected eating window) + CR (25% energy restriction + MD)	↓29.3 mg/dL‡ (-12.9%)	↑0.5 mg/dL (+1.2%)	↓17.0 mg/dL‡ (-11.5%)	↓41.8 mg/dL‡ (-19.7%)	↓15.5 mg/dL‡ (-13%)	↓1.3‡ (-26.6%)	↓7.4 mmHg‡ (-5.6%)	↓4.8 mmHg‡‖ (-5.7%)
		CR (25% energy restriction) + MD	↓29.4 mg/dL‡ (-12.8%)	↑0.4 mg/dL (+0.9%)	↓16.0 mg/dL‡ (-10.8%)	↓40 mg/dL‡ (-20.2%)	↓13.1 mg/dL‡ (-11.4%)	↓0.9‡ (-22.0%)	↓13 mmHg‡ (-9.2%)	↓8.2 mmHg‡‖ (-9.2%)
Lin et al. 2021 (28)	N: 63 Women Age 40–65 BMI ≥24 kg/m2	8 weeks 16:8 TRE (either 10:00 am–6:00 pm or 12:00–8:00 pm) + CR (1400 kcal/d; ≈12 % energy restriction)	↑3.1 mg/dL (+6.1%)	↑1.5mg/dL (+2.8%)	↓1.5mg/dL (-1.2%)	↓7.6mg/dL (-3.8%)	↑4.4 mg/dL‡ (+5.2%)	**↑0.4 †‡ ‖ (+27.3%)**	↓3.1mmHg (-1.8%)	**↓4.8 mmHg†‡ (-5.2%)**
		CR (1400 kcal/d; ≈9% energy restriction)	↑0.1 mg/dL (+0.4%)	↓0.2 mg/dL (-0.3%)	↑0.7 mg/dL (+2.2%)	↓10.8 mg/dL (-3.6%)	↑1.8mg/dL (+3.4%)	**↓0.1† ‖ (-8.9%)**	↑0.1mmHg (+0.4%)	**↑3.4mmHg †‡ (+6.1%)**
Liu et al. 2022 (22)	N: 118 Age 18–75 BMI ≥28 kg/m2	52 weeks 16:8 e-TRE (eating window: 8:00 am–4:00 pm) + CR (25% energy restriction)	↓7.3 mg/dL‡ (-3.8%)	↑4.6 mg/dL‡ (+10.0%)	↓8.4 mg/dL‡ (-6.4%)	↓25.5 mg/dL‡ (-19.6%)	↓3.5 mg/dL (-3.9%)	↓1.0‡ (-29.4%)	↓8.1 mmHg‡ (-6.5%)	↓5.1 mmHg‡ (-7.0%)
		CR (25% energy restriction)	↓9.3 mg/dL‡ (-4.5%)	↑2.9 mg/dL‡ (+6.4%)	↓8.9 mg/dL‡ (-6.9%)	↓19.6 mg/dL‡ (-14.3%)	↓3.0 mg/dL (-3.3%)	↓0.5 (-15.2%)	↓7.7 mmHg‡ (-6.2%)	↓3.8 mmHg‡ (-5.1%)

Study (reference)	N completers, inclusion criteria	Intervention	Δ TC, mg/dL (%)	Δ HDL, mg/dL (%)	Δ LDL mg/dL (%)	Δ TG, mg/dL (%)	Δ FBG, mg/dL (%)	Δ HOMA-IR (%)	Δ SBP, mmHg (%)	Δ DBP, mmHg (%)
Thomas et al. 2022* (24)	N:63 Age 18–50 BMI 27–45 kg/m2	39 weeks 14:10 e-TRE (10 h eating window within 3 h of waking) + CR (≈35% energy restriction)	NS	NS	NS	NS	-	OGTT NS	-	-
		CR (≈35% energy restriction)	NS	NS	NS	NS	-	-	-	-
Teong et al. 2023 (29)	N: 137 Age 35–75 BMI -	26 weeks i-TRE (3 non-consecutive days/week; 30% energy requirements between 8:00 am – 12:00 pm; ad libitum eating on other days)	↓14.6mg/dL‡ (-7.3%)	↑1.8mg/dL‡ (+3.8%)	↓13.9mg/dL‡ (-10.0%)	↓**18.4mg/dL†‡ (-12.0%)**	↓3.4mg/dL‡ (-3.1%)	-	↓8.1mmHg‡ (-6.6%)	↓5.4mmHg‡ (-6.7%)
		CR (70% energy requirements)	↓10.0mg/dL‡ (-5.1%)	↑0.5mg/dL (+1.0%)	↓9.8mg/dL‡ (-7.3%)	↓**2.8mg/dL‡‡ (-2.1%)**	↓0.5mg/dL (-0.5%)	-	↓8.8mmHg‡ (-7.0%)	↓4.8mmHg‡ (-6.0%)
Wei et al. 2023 (30)	N: 74 Age 18–75 BMI 28–45 kg/m2	52 weeks TRE (eating window between 8:00 am – 4:00 pm; men: 1500 – 1800 kcal/d; women: 1200-1500 kcal/d)	↓10.2 mg/dL‡ (-5.2%)	↑6.5 mg/dL‡ (+15.2%)	↓12.5 mg/dL‡ (-9.5%)	↓39.0 mg/dL‡ (-25.0%)	↓5.9 mg/dL‡ (-6.4%)	↓1.6‡ (-43.2%)	↓11.0 mmHg‡ (-8.8%)	↓7.4 mmHg‡ (-10.0%)
		CR (habitual meal timing; men: 1500 – 1800 kcal/d; women: 1200-1500 kcal/d)	↓7.9 mg/dL‡ (-3.9%)	↑3.7 mg/dL‡ (+8.5%)	↓8.2 mg/dL‡ (-6.2%)	↓38.0 mg/dL‡ (-25.4%)	↓0.8 mg/dL (-0.9%)	0.0 (0.0%)	↓8.5 mmHg‡ (-6.6%)	↓5.5 mmHg‡ (-7.1%)

Abbreviations: CR: Calorie restriction; TRE: time-restricted eating; BMI: Body mass index; e-TRE: early time-restricted eating; TC: Total cholesterol;; HDL: High-density protein; LDL; Low-density protein; TG: Triglycerides; FBG: Fasting blood glucose; HOMA-IR: Homeostatic Model Assessment for Insulin Resistance; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; RT: Resistance training; iTRE: intermittent fasting plus early time-restricted eating; NS: Not significant; - : Not provided ; MD: Mediterranean diet;
Symbols: * Lipids measured at week 12; † Significant between group change vs CR ($p < 0.05$) ‡ Significant within-group change ($p < 0.05$), || * Imbalance between groups at baseline.

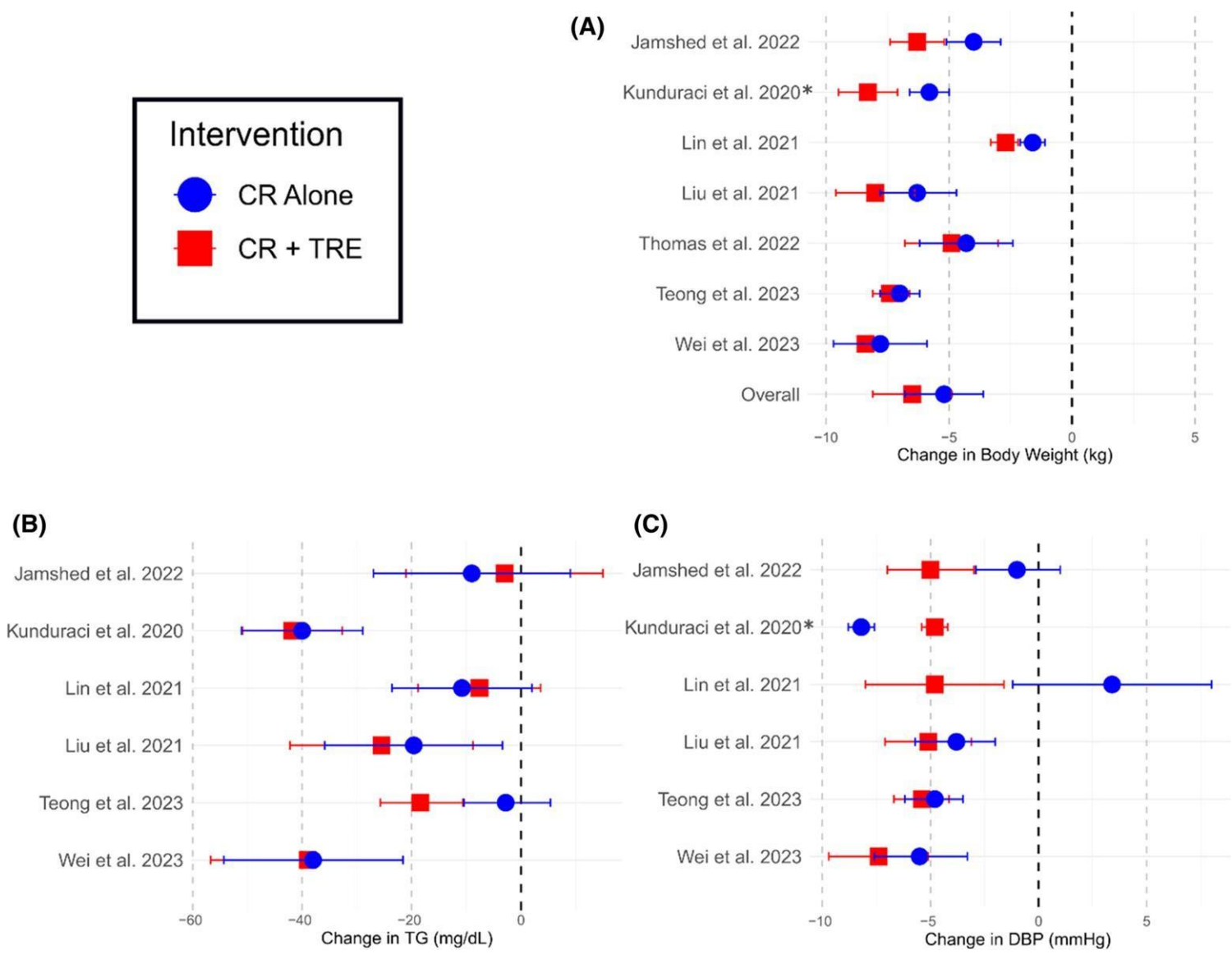

FIGURE 4-3
Forest plot depicting the effects of calorie restriction (CR) + time-restricted eating (TRE) versus CR alone on changes in the following: (A) body weight; (B) triglycerides (TG); and (C) diastolic blood pressure (DBP) in adults with overweight and obesity. Values are shown as mean changes from baseline with 95% CI. *Imbalance between groups at baseline.

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Chapter 5 - The Effects of Time-Restricted Eating on Inflammation and Oxidative Stress in Older Adults with Overweight

Abstract

Objectives: To assess the effects of time-restricted eating (TRE) on markers of inflammation and oxidative stress in older adults with overweight.

Design: Single-arm pilot trial

Participants: Ten sedentary, overweight older adults (the mean (SD) age = 77.1 ± 6.1 years; 6 women and 4 men) at risk for mobility decline.

Intervention: Participants followed a TRE protocol for 16 hours per day and consuming food ad libitum during an 8-hour window, for 4 weeks. Blood samples were collected at baseline and after 4 weeks to assess outcomes.

Measurements: TNF-alpha, IL-1beta, IL-6, high-sensitivity C-reactive protein (hs-CRP), and the oxidative stress marker 8-isoprostane were measured.

Results: There were no significant differences in the outcomes of interest compared to baseline ($p > 0.05$). TNF-α levels decreased from 43.2 (11.2) pg/ml to 39.7 (10.0) pg/ml ($p = 0.101$) with a Cohen's d effect size of 0.33, and IL-1β levels decreased from 1.4 (0.8) pg/ml to 1.3 (0.6) pg/ml ($p = 0.499$) with a Cohen's d effect size of 0.23, suggesting potential anti-inflammatory benefits. IL-6 and hs-CRP levels showed no significant changes. The oxidative stress marker 8-isoprostane levels decreased slightly from 39117.6 (12787.5) pg/ml to 38694.7 (11631.4) pg/ml ($p = 0.919$) with a Cohen's d effect size of 0.07.

Conclusion: This secondary analysis provides initial insights into the potential effects of TRE on inflammatory and oxidative stress markers in older adults. Well-powered studies with longer duration are warranted to elucidate the potential benefits of TRE in aging populations.

Introduction

Aging is accompanied by gradual biological and cellular changes that result in functional decline and significant increases in the risk of age-related diseases (1). Among these changes is chronic inflammation, which is considered a hallmark of aging (2). Similarly, oxidative stress, a key driver of age-related diseases, increases with aging (3). Calorie restriction (CR) has been shown to lower inflammation and oxidative stress (4,5). However, challenges in adherence and daily calorie monitoring necessitate alternative approaches (6).

Intermittent fasting (IF) is an umbrella term that describes an eating pattern in which an individual fasts for an extended time period, typically longer than 12 hours, on a repeated basis (7). Of the various types of IF approaches, time-restricted eating (TRE), the pattern of extending the daily fasting period and thereby reducing the daily eating period, has gained popularity over the last few years (8). Some studies reported a reduction of 200-500kcal daily with TRE without the need for calorie counting (9-12). Findings from our past reviews have suggested that TRE is a viable dietary approach to improve body composition, metabolic parameters, inflammatory markers, and oxidative stress markers, which are known risk factors for several age-related chronic diseases including diabetes, CVD, Alzheimer's disease, and some cancers (7,8,13,14). Additionally, our recent systematic review suggests that the addition of TRE to CR regimens may lead to greater weight loss and improvements in metabolic risk factors (8). The mechanisms underlying the potential benefits of TRE are not yet fully understood. However, TRE is thought to mitigate inflammation and oxidative stress through several pathways, as outlined below:

Circadian Modulation by TRE Improves Metabolic Health. Accumulating evidence indicate that meal timing has the potential to improve metabolic health including inflammation (15-17). Therefore, TRE regimen, that is based on the concept of circadian rhythm, has been proposed as a promising lifestyle intervention to improve metabolic health (8). In agreement with this, a growing number of studies suggest that aligning food intake with circadian rhythm can improve metabolic health parameters including improved insulin sensitivity, and oxidative stress and pro-

inflammatory markers (18-20). Emerging research also suggest that TRE approaches play a critical role on the expression of clock genes and several nutrient-sensing factors, including GR, CREB, FOXO, TFEB, and PPARs, in metabolic tissues (21).

TRE and Metabolic Switching. It is known that at least 12 to 14 hours of fasting is needed to deplete glycogen stores and flip the metabolic switch, leading to improved metabolic endpoints such as fasting glucose and insulin sensitivity (22,23). In agreement with this, emerging evidence suggests that a minimum fasting duration of 14 hours may be necessary to trigger certain metabolic changes in individuals with type 2 diabetes (24-27). During prolonged fasting periods, the body shifts its metabolism towards fat oxidation in response to the depletion of glycogen stores, resulting in ketone body production such as β-hydroxybutyrate (BHB) which activates a series of nutrient sensing pathways (28). Increased BHB concentrations lowers oxidative stress and inflammation, and increases the AMP/ATP ratio, activating AMP-activated protein kinase (AMPK) in muscle cells (29,30).

TRE and Weight Loss. Preliminary studies reported that TRE (with ad libitum intake) produces weight loss (1–4 % with ad libitum intake and ≥ 5 % when combined with CR) in adults with overweight and obesity (7). There is evidence that weight loss can improve inflammation and lower oxidative stress (5). Therefore, weight loss induced by TRE is another pathway, which plays a role in the metabolic benefits of TRE.

The effects of TRE on inflammation and oxidative in different population has been explored by previous studies (18,20,31,32). However, to date, no study has specifically examined the effects of TRE, with ad libitum intake, on pro-inflammatory markers and oxidative stress in older adults. Therefore, the primary aim of this secondary analysis was to evaluate the effects of TRE on pro-inflammatory markers including TNF-alpha, IL-1beta, IL-6, hs-CRP, and the oxidative stress marker 8-isoprostane in older adults with overweight. We hypothesized that short-term TRE can initiate reductions in pro-inflammatory markers and oxidative stress in older adults with overweight.

Methods

Study Design

This secondary analysis aimed to assess the effects of time-restricted eating (TRE) on inflammatory markers (IL-1β, IL-6, TNF-α) and oxidative stress (8-isoisoprostane) in 10 older adults from a pilot single-arm study that has already been published (33). The study was designed to assess the feasibility and safety of TRE in overweight, sedentary older adults over a four-week period. Participants were included if they were aged ≥ 65 years, self-reported difficulty walking ¼ mile or climbing a flight of stairs, were sedentary (<30 minutes of structured exercise per week), had a walking speed <1 m/sec on the 4 m walk test, and had a body mass index between 25–40 kg/m² (inclusive).
The exclusion criteria included fasting >12 hours per day, actively trying to lose weight by participating in a formal weight loss program or significantly restricting calorie intake, weight loss > 5 lbs in the month prior to the intervention, and having medical history or conditions such as a resting heart rate >120 beats per minute, systolic blood pressure >180 mmHg or diastolic blood pressure >100 mmHg, unstable angina, heart attack or stroke in the 3 months prior to the intervention, insulin-dependent diabetes mellitus, active treatment for cancer in the past year, rheumatoid arthritis, Parkinson's disease, or currently being on dialysis or requiring continuous use of supplemental oxygen to manage a chronic pulmonary condition or heart failure. Additionally, taking medications that precluded fasting for 16 hours (e.g., must be taken with food at least 12 hours apart) was also an exclusion criterion.

Intervention

Study participants were asked to fast for a target of 16 hours per day for a period of 4 weeks while they were allowed to eat ad libitum during the 8-hour eating window. The first week involved a ramp-up to a full 16-hour fasting period (Days 1-3 fast for 12-14 hours per day, Days 4-6 fast for 14-16 hours per day, Days 7-28 fast for 16 hours per day). Participants were allowed to consume calorie-free beverages, tea, black coffee, and sugar-free gum, and were encouraged to drink plenty of water throughout the intervention period. Participants were asked to record the time of

first and final food/drink consumption each day. All testing sessions were performed at Institute on Aging Clinical Translational Research Building, University of Florida.

The fasting times were self-selected by participants and were allowed to vary daily. The first week involved a gradual increase in fasting duration: Days 1–3 included a 12–14 hour fast, Days 4–6 included a 14–16 hour fast, and Days 7–28 involved a full 16-hour fasting period. Participants were encouraged to stay hydrated during fasting times. No dietary restrictions regarding the amount or types of food consumed during the 8-hour eating window were imposed, allowing participants to select a timeframe that best fit their lifestyle.

To enhance adherence, participants were provided with detailed instructions on how to follow the TRE protocol during the baseline visit. These instructions included a pictorial flyer for future reference that depicted permissible and non-permissible items during the fasting windows. "Go Foods" (e.g., water, diet soda, unsweetened teas, sugar-free gum, black coffee) were indicated in green, while "No Foods" (e.g., coffee creamer, sweet teas, alcohol, snacks, calorie-containing drinks) were indicated in red. Participants also received an eating time log to record the times when they first consumed calories and when they stopped each day.

To further ensure adherence, the trial interventionist contacted each participant weekly. These calls aimed to reinforce adherence to the protocol, monitor for adverse events, and provide support and guidance for any challenges encountered. Daily activities, changes to routine, and any health changes were collected via calls. The eating time log was reviewed during each call, and participants received support and guidance for any intervention-related challenges.

Measures:

Fasting blood samples were collected for inflammatory cytokines, including IL-1β, IL-6, and TNF-α, which were assessed using the Magnetic Luminex Assay Human Premixed Multi-Analyte Kit (R&D Systems, Minneapolis, MN, USA). Human Quantikine ELISA (R&D Systems) was performed to measure hs-CRP levels. The oxidative stress marker 8-isoprostane was measured by 8-iso-Prostaglandin (CELL BIOLABS, San Diego, CA, USA) at Screening/Baseline and the Week 4 visit.

Statistical Analysis

Data were checked for missing values and outliers. Any missing data were excluded from the analysis to ensure accuracy in the results. Initial descriptive statistics, including mean and standard deviations (SD), were calculated for each inflammatory and oxidative stress marker at baseline and at week four. Primary analyses were performed using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA). Statistical analysis included Paired Sample t-Tests to compare baseline and week four values for each marker. Statistical significance was set at $p<0.05$. Changes and percent changes in mean and SD from baseline to week four were calculated for each outcome to assess the intervention's effect. Effect sizes were calculated using Cohen's d to measure the magnitude of the changes in inflammatory and oxidative stress markers, given the small sample size and the study being a pilot and not powered to detect the changes.

Results

Ten sedentary, overweight older adults (the mean (SD) age 77.1 ± 6.1 years; 6 women and 4 men) were included in the current secondary analysis. Participants had mild to moderate functional decline and reported a high mean adherence rate of 84% through self-reported food diaries. Participants reported a gradual increase in their fasting duration from 14:41(hr:mins) mean daily fasting for 16:07 (hr:mins) at week 4. The changes in anthropometric outcomes has already been published (33) (Table 1).

Changes in Inflammatory and Oxidative Stress Markers

Table 2 summarizes the changes in inflammatory and oxidative stress markers including TNF-α, IL-1β, IL-6, hs-CRP, and 8-Isoprostane from baseline to the end of 4-weeks of time-restricted eating. There were no significant differences in the outcomes of interest compared to baseline ($p > 0.05$).

TNF-α

TNF- α levels decreased from a baseline mean (SD) of 43.2 (11.2) pg/ml to 39.7 (10.0) pg/ml at the end of the intervention. However, this change was not statistically significant ($p = 0.101$). The mean change (SD) reduction was

-3.5 (5.7) pg/ml, which corresponds to an 8% decrease. The effect size (Cohen's d) was 0.33, which indicates a small effect.

IL-1β (pg/ml)

IL-1β levels showed a slight reduction from a baseline mean (SD) of 1.4 (0.8) pg/ml to 1.3 (0.6) pg/ml at the end of the intervention, with a mean (SD) reduction of -0.1 (0.7) pg/ml, representing to an 11% decrease. The effect size (Cohen's d) was 0.23, which represents a small effect size. There was not statistically significant difference between post-intervention and baseline ($p = 0.499$).

IL-6

IL-6 levels remained almost unchanged, with a mean (SD) of 10.8 (7.2) pg/ml at both baseline and post-intervention. The mean (SD) change was -0.001 (2.4) pg/ml, representing 0% change. The effect size (Cohen's d) was 0.03, indicating a negligible effect, with no statistical significance ($p = 0.998$).

hs-CRP

hs-CRP levels increased from a baseline mean (SD) of 3300.5 (4089.5) ng/L to 3625.1 (3429.5) ng/L at the end of the intervention, demonstrating a mean (SD) increase of 324.6 (1766.1) ng/L, showing a 10% increase. However, this change was not statistically significant ($p = 0.596$). The effect size (Cohen's d) of 0.01 indicates a negligible effect.

8-isoprostane

The change in 8-isoprostane levels showed a slight reduction from a baseline mean (SD) of 39117.6 (12787.5) pg/ml to 38694.7 (11631.4) pg/ml at the end of the intervention, with a mean (SD) reduction of -422.9 (12206.3) pg/ml, indicating a 1% decrease. The change was not statistically significant ($p = 0.919$). The effect size (Cohen's d) of 0.07 indicates a negligible effect (Table 1).

Discussion

Main Findings

The purpose of this secondary analysis was to investigate the effects of a short-term (4 weeks) TRE intervention on inflammatory and oxidative stress markers in older adults with overweight. The main finding of the current study was that overall the results suggest modest changes in inflammatory markers following the short-term intervention. This finding imply that TRE may induce anti-inflammatory benefits in adequately powered and longer-duration studies among older populations.

To our knowledge, this is the first trial investigating the effects of TRE with ad libitum intake on inflammation and oxidative stress in older adults. Previous short-term studies have tested the effects of TRE on these outcomes among different populations (18, 31-32,34). Martens et al. examined eucaloric TRE (16:8), without weight loss, in 22 healthy non-obese middle-age and older adults in a randomized controlled trial (RCT). After 6 weeks, there were no significant differences between TRE and normal diet control for IL-6 and CRP levels (34). Similarly, others have found no significant changes in IL-6 and CRP/ hs-CRP levels with short-term TRE interventions (5 to 8 weeks) (18,31). Consistent with this, the present study found no change in these outcomes after 4 weeks. In contrast, early 16:8 TRE (eating window between 8a.m-4 p.m.) resulted in significant reductions in hs-CRP levels (42%) in overweight and obese women with polycystic ovary syndrome over 5 weeks (35).

TNF-α significantly decreased only with early (6 a.m.–3 p.m.) but not mid-day TRE (11 a.m.–8 p.m.) in previous short-term RCTs (20). In the current study, TNF-α levels demonstrated a small decrease with a Cohen's d effect size of small to medium, and IL-1β also exhibited minimal changes with TRE, with a small effect size. However, a one-year long RCT reported a significant reduction in IL-1β with late TRE (3 meals: 1 p.m., 4 p.m., and 8 p.m.) compared to a control in healthy resistance-trained males (32).

To our knowledge, the present study is the only study that examined oxidative stress following 16:8 TRE. Oxidative stress marker 8-isoprostane has slightly decreased after intervention with a very small effect size. In contrast, longer daily fasting durations of 18 and 20h has shown to lower 8-isoprostane significantly in prediabetic men or obese adults

after 5 and 8 weeks, respectively (18,31). Notably, 18:6 early TRE significantly improved oxidative stress marker 8-isoprostane even in the absence of weight loss (18), whereas a 2.6 kg weight loss occurred in the present study following four weeks. This suggests that the 16 h of fasting may not have been sufficient to activate the metabolic switch, thereby lowering oxidative stress and inflammation. However, given the small sample size and short duration of the study, this finding should be interpreted with caution. Future well-powered studies with longer durations are needed to elucidate the effect of 16:8 TRE on inflammation and oxidative stress in older populations.

Circadian-Driven Benefits: The Effects of Meal-timing on Inflammation and Oxidative Stress

TRE has the potential to alleviate various metabolic conditions through the reduction of inflammation, exemplifying a refined approach to health and longevity. In animal models, TRE has been shown to reduce the levels of nuclear factor-kB (NF-kB), and IL-17 in adipocytes. It also reduces the mRNA expression of inflammatory factors, including TNF-α, IL-10, and IL-1 (36). As a result, TRE lowers the number of macrophages within adipose tissue, thereby preventing the increase in the CD8+/CD4+ ratio and effectively mitigating adipose tissue-induced inflammation. Notably, TRE enhances the release of adiponectin within adipose tissue, which significantly contributes to ameliorating insulin resistance and the modulation of AMP-activated protein kinase (AMPK), enhancing signaling molecules, including endothelial nitric oxide synthase, NAD-dependent sirtuin proteins, PPARs, CREB, and the activity of deacetylated PPAR-gamma, PGC1-alpha (36-38).

TRE has shown to stimulate AMPK even without weight loss (36). The activation of AMPK leads improved metabolic health by lowering cholesterol and triglyceride levels, muscle glucose uptake, and the rates of glycolysis and lipolysis; and improving the cellular redox environment. Activating AMPK can upregulate SIRT1 by increasing PGC1-α expression, a master regulator of mitochondria leading to enhanced oxidative phosphorylation, mitochondrial biogenesis, and mitochondrial autophagy (36). There is evidence suggesting that altering meal timing can significantly increase AMPK, PPAR-alpha, and CREB activity in the liver (21).

TRE improves gut microbiota stability by regulating circadian rhythms, lowering low-grade inflammation, which is linked to T2D, CVD and other chronic conditions (39). There is evidence suggesting the impact of gut microbiota

including Lactobacillus, Bifidobacterium, Roseburia, and Akkermansia to attenuate proinflammatory cytokines, including IL-1β, IL-6, IL-8, IL-17, and TNF-α (39). Consistent with this, an RCT by Xie et al. reported significant improvements in TNF-α and IL-8 levels, gut microbial diversity, and insulin sensitivity only with early-TRE from 6 a.m. to 3 p.m., but not with mid-day TRE from 11 a.m. to 8 p.m., in 82 healthy young adults without obesity over a 5-week period (20).

Fasting-Driven Benefits of TRE

The periods of nutrient deprivation (fasting ≥12) triggers a cascade of cellular and biochemical changes leading to a shift in metabolism towards fat oxidation in response to the depletion of glycogen stores and production of ketone body such as β-hydroxybutyrate (BHB). Elevated BHB activates a series of nutrient sensing pathways leading to many of the benefits of prolonged fasting (28).

The increased circulation of BHB lowers oxidative stress, and increase the AMP/ATP ratio, activating AMPK in muscle cells. AMPK promotes mitochondrial biogenesis and enhances glucose uptake and fatty acid oxidation. It also stimulates insulin sensitivity by promoting glucose transporter type 4 (GLUT4) translocation to the cell membrane, thereby enhancing glucose uptake and reducing insulin levels and signaling pathways resulting in decreased PI3K/AKT pathway activity. Furthermore, AMPK activation suppresses mechanistic Target Of Rapamycin Complex 1 (mTORC1) activity, a nutrient sensor key to regulating protein synthesis and autophagy. Suppressed mTORC1 activity, as a result of nutrient deprivation during fasting, results in inhibition of pro-inflammatory cytokines and activates autophagy, including mitophagy mediated by Unc-51-like kinase 1 (ULK1). Additionally, downregulation of mTOR, and reduction in AKT activity during TRE enhances insulin sensitivity by reducing cellular stress and enhancing autophagy through FOXO3 activation (29).

Several studies have showcased longer daily fasting hours in TRE interventions can induce improvements in markers of health and aging such as inflammation, oxidative stress, and glucoregulatory markers regardless of timing of fast ing initiation or weight loss (18,31,32). Of note, Cienfuegos et al. reported improvements in insulin, insulin sensitivity,

and oxidative stress with longer fasting hours (18 and 20h; eating from 1-7pm vs. 3-7pm) in adults with obesity over 8 weeks. These improvements in markers of aging can be attributed to other metabolic pathways such as increasing circulating BHB, and therefore enhanced autophagy, induced by longer fasting hours (typically ≥18 hours).

Collectively, findings from RCTs indicate the benefits of TRE for improving insulin sensitivity, glucose hemostasis, triglycerides, blood pressure, oxidative stress, and inflammation. Nonetheless, future research should determine whether extended fasting hours are the main driving factor for the weight-loss-independent benefits of TRE.

Implications and Future Directions

Despite non-significant p-values, our study found notable effect sizes for several inflammatory markers. Specifically, TNF-α demonstrated a small to medium effect size, suggesting a trend towards lowered inflammation with TRE. Likewise, IL-1β showed a small effect size, indicating modest improvements in inflammatory markers. Oxidative stress marker 8-isoprostane exhibited very small effect sizes, suggesting minimal impact of 16:8 TRE within the short study's timeframe.

The current pilot study implemented self-selected eating windows, and the mean stating eating time occurred 61 minutes later at week 4 compared to week 1 (mean start time 9:32a.m. at week 1 vs. 10:33 a.m. at week 4) (40). This is in line with the findings of previous studies in other populations suggesting the importance of meal timing in lowering inflammation (18,20). While a 5 week-early TRE intervention (6 a.m. to 3 p.m.) improved pro-inflammatory cytokines (TNF-α and IL-8) compared to a control in young adults without obesity, mid-day TRE (11 a.m. to 8 p.m.) failed to improve those markers (20).

Regarding fasting-driven benefits, longer fasting durations (18-20 hours) have been shown to reduce oxidative stress markers in obese individuals who underwent nearly 3% weight loss over 8 weeks (9). In contrast, the current study observed similar weight loss (≈3% of initial body weight) while implemented shorter fasting periods (16 hours), which may not have been sufficient to fully capture the effects within this study's shorter timeframe. Future well-powered

studies with longer duration could provide further clarity on the optimal timing and duration of fasting within TRE for maximizing health benefits in older adults. Additionally, the effects of TRE interventions on hallmarks of aging including autophagy, and epigenetic alteration, cellular senescence and inflammation should be investigated in future trials.

Strengths and Limitations

The strength of our work is that it is the first trial to explore the effects of TRE with ad libitum intake on inflammation and oxidative stress in older populations. Several limitations should be considered in interpreting these findings. The pilot nature of the study limits the interpretation of results, as the study was underpowered to detect effects, thereby increasing the likelihood of both type I and type II errors. Furthermore, the short duration of the study may have limited the magnitude of observed effects, especially for outcomes showing minimal changes. The present study employed self-selected eating/fasting windows. While this enhances adherence to the intervention, it may also limit the ability to assess the effects of meal timing (early, mid-day, late) on the outcomes of interest. Additionally, the study relied on self-reported eating times. Although common in dietary interventions, self-reporting can introduce potential limitations due to recall bias and social desirability bias. The use of technology such as digital tools (e.g., smartphone apps designed for dietary tracking) could improve the validity of findings.

Conclusion

In conclusion, this secondary analysis provides preliminary evidence of TRE's effects on inflammatory and oxidative stress markers in older adults with overweight. While TNF-α and IL1β levels showed small reductions, suggesting potential benefits of the intervention, further research with larger sample sizes and longer durations is warranted to explore the effects of this dietary approach in older populations.

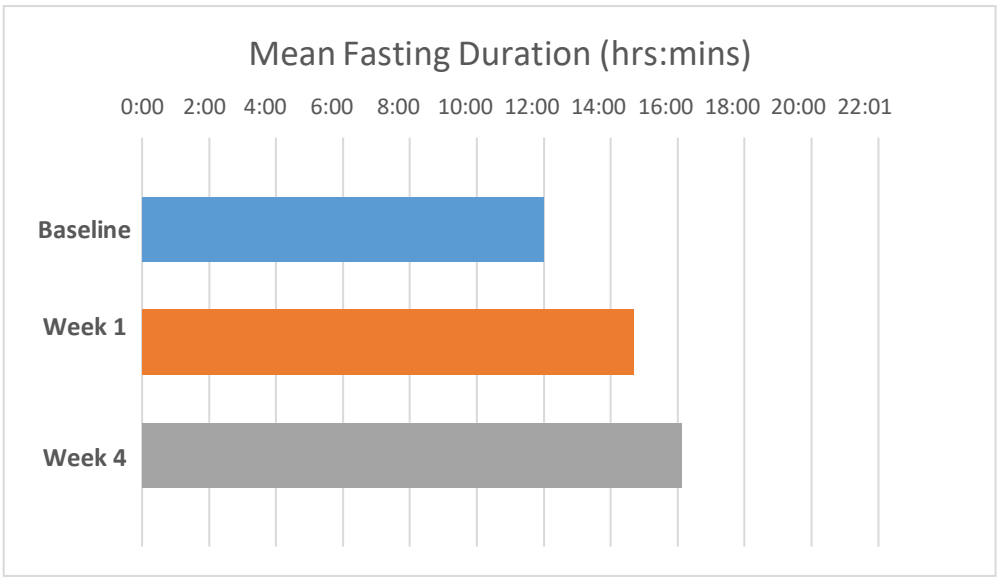

Figure 5-1. The mean fasting duration from baseline to week 4.

Table 5-1.

Baseline and Follow-Up Measures on Anthropometric outcomes.

Study Measures	**Baseline Mean *(SD)***	**TRE Mean *(SD)***	**Cohen's d (Effect Size)**	***p*-Value**
Body Weight	96.96 (16.2)	94.81 (16.9)	0.13	**0.009**
Body Mass Index (BMI)	34.1 (3.3)	33.2 (3.2)	0.29	**0.013**
Waist Circumference (cm)	109.43 (12.9)	109.23 (12.3)	0.02	0.602

Table 5-2.

Changes in inflammatory and oxidative stress markers from baseline to the end of 4-weeks of time-restricted eating

Study Measures	**Baseline *M (SD)***	**TRE *M (SD)***	**Δ Mean (SD)**	**Percent change**	**Cohen's d (Effect Size)**	***p*-Value**
TNF-alpha (pg/ml)	43.2 (11.2)	39.7 (10.0)	−3.5 (5.7)	−8%	0.33	0.101
IL-1β (pg/ml)	1.4 (0.8)	1.3 (0.6)	−0.1 (0.7)	−11%	0.23	0.499
IL-6 (pg/ml)	10.8 (7.2)	10.8 (7.2)	−0.001 (2.4)	0%	0.03	0.998
hs-CRP (ng/L)	3300.5 (4089.5)	3625.1 (3429.5)	324.6 (1766.1)	10%	0.01	0.596
8-isoprostane (pg/ml)	39117.6 (12787.5)	38694.7(11631.4)	−422.9 (12206.3)	−1%	0.07	0.919

TNF-alpha: Tumor Necrosis Factor alpha; IL-1β: Interleukin-1 beta; IL-6: Interleukin-6; hs-CRP: high-sensitivity C-reactive protein

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Chapter 6- Conclusions

This dissertation aimed to investigate the effects of various dietary interventions, particularly time-restricted eating (TRE) and intermittent fasting (IF), on inflammation, oxidative stress, and metabolic health. The findings provide valuable insights into how these dietary interventions may influence biomarkers related to chronic diseases and aging.

Chapter 1 established a theoretical foundation by reviewing the relationship between TRE and inflammation, emphasizing the potential effects of TRE on pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β). The review highlighted the need for further exploration into how TRE may lower oxidative stress, particularly in the context of Alzheimer's disease. This chapter underscored the significance of understanding the biological mechanisms through which TRE may induce its neuroprotective effects.

Chapter 2 explored the broader concept of IF by comparing TRE with other types of IF regimens, including alternate-day fasting, the 5:2 diet, and Ramadan fasting. The chapter highlighted the potential of IF to serve as a therapeutic strategy for reducing chronic inflammation, a common hallmark of chronic diseases and aging.

Chapter 3 provided a systematic review comparing the c effects of isocaloric IF and daily caloric restriction (DCR) on weight loss and metabolic markers of chronic disease in adults with overweight and obesity. The findings revealed that, while both approaches resulted in similar outcomes in terms of weight loss and metabolic risk factors, IF showed particular promise in improving insulin sensitivity and reducing body fat. However, the heterogeneities in outcomes across studies suggested that more controlled and long-term studies are needed to fully understand the differential effects of IF compared to DCR.

In Chapter 4, another systematic review focused specifically on TRE and its potential benefits beyond those attributed to calorie restriction alone. The review revealed that only prolonged fasting, especially when combined with moderate

calorie restriction, led to significant improvements in insulin resistance (HOMA-IR). This chapter provided novel insights into how meal timing and duration of fasting periods might play a crucial role in enhancing metabolic health, independent of calorie reduction.

Chapter 5 presented original research through a secondary analysis of a pilot study on the effects of TRE on inflammation and oxidative stress in older adults with overweight. Although the study found no significant changes in pro-inflammatory and oxidative stress markers after a short-term TRE intervention, there were small reductions in TNF-α and IL-1β levels, suggesting potential anti-inflammatory effects. These findings are consistent with previous studies in other populations, indicating that longer and larger trials are needed to fully assess the impact of TRE on inflammation and oxidative stress in aging populations.

The collective findings from this dissertation provide promising evidence that IF, particularly TRE, may have beneficial effects on inflammation, oxidative stress, and metabolic health. However, further research is needed to explore the long-term effects of TRE on these outcomes to fully understand its potential benefits.

In conclusion, this dissertation contributes to the growing body of literature on the health benefits of IF, particularly TRE. The findings provide groundwork for future studies that will further elucidate the impact of meal timing and prolonged fasting in the prevention and treatment of chronic diseases, as well as in promoting healthy aging.