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Intermittent Fasting versus Mediterranean Diet on HbA1c, Insulin Resistance, and Lipid Profile in Young Adults: A 4-Week Pilot Randomized Controlled Trial – Implications for Functional Food Development and Personalized Nutrition

Ghenaa H. Abdulkreem[1]*, Noor Sarhan Ahmed[2], Khattab Abdulsalam Khattab[3], Anwar Ahmed Khalaf[4]

1,3,4-Faculty of Agriculture, Tikrit University - Department of Food Science - Iraq
2-Faculty of Agriculture, Samarra University - Department of Food Science - Iraq

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*Corresponding Author E-Mail:
ghnaa.hamad@tu.edu.iq

ABSTRACT

There are two evidence based dietary interventions that are known to have different metabolic effects: Intermittent Fasting (IF) and the Mediterranean Diet (MED). High-quality comparative studies, however, with multiple validated biomarkers, within Arab young adult cohort are limited. The purpose of this pilot study is to gather preliminary information that will guide in designing an adequate powered randomized controlled trial (RCT). To investigate the short term effects of 12:12 hour IF (IF) and 4 week intervention with MED (IF for 4 weeks) on levels of glycemic indices (HbA1c and FBG), insulin resistance (HOMA-IR), anthropometric measures (weight, waist circumference and BMI), lipid profile and high sensitivity C reactive protein (hsCRP) in young adults (20 25 years). 15 subjects (10 IF, 5 MED, mean age 22.4 ± 1.6 years) were grouped in a 4 week pilot RCT, according to metabolic status. The measurement was repeated 3 times in each of the biological samples, total 45 measurements. Adherence was checked using 3 day food diaries weekly by a Registered Dietitian. Both dietary strategies had beneficial effects on metabolic measures. The IF group showed numerically larger reductions in FBG (Δ –8.44 vs. –5.80 mg/dL), waist circumference (Δ –2.79 vs. –1.90 cm), and BMI (Δ –0.54 vs. –0.38). The Δ for total cholesterol (hsCRP) and LDL cholesterol (Δ –9.46 vs. –6.93 mg/dL) in the MED group was numerically higher compared to other groups. Only between group differences for FBG, waist circumference and BMI reached nominal significance. The findings from these pilot data suggest that the effect of IF on glycemic control and adipivity may be beneficial in the short term while the effect of MED on anti inflammatory and lipid lowering effect may beneficial in young adults of Arab ethnicity. The results justify the formulation of functional food products according to the diet habits and the incorporation of chrono nutrition concept in food formulation. Applicability from an industrial point of view might involve products using IF for meal replacements or snacks optimized for specific eating windows, or products using MED for polyphenol-rich, prebiotic fortified products, aimed at reducing inflammation, cardiovascular diseases etc. All data are preliminary and will need validation in a well-powered RCT (n ≥ 80; 8 12 weeks; lab confirmed HOMA IR; 1:1 allocation; evaluator blinded) to apply clinically and commercially.

1- INTRODUCTION

Obesity is a worldwide epidemic including insulin resistance, diabetes type 2 and dyslipidaemia as metabolic conditions [1]. Dietary strategies to reduce metabolic dysfunction prior to establishment of its chronic consequences have become a primary interest as these conditions have increased in the younger age group. Two evidence-based diets have proven to be effective for managing those conditions are Intermittent Fasting (IF) and the Mediterranean Diet (MED). Both techniques have been found to be beneficial individually; however, there are no studies providing side-by-side comparisons between the two for young Arab people measured with validated biomarkers [3]. In addition, translating information into action—such as developing functional foods and developing personalized nutrition action plans—the field of comparative studies is under-explored, and has significant industry potential [4].

The metabolic benefits of IF exist in three main ways: (1) periods of extended fasting stimulate the process of autophagy, which increases insulin receptor sensitivity; (2) periods of extended fasting decrease fasting glucose levels and HbA1c; and (3) longer inter meal intervals elicit greater fat oxidation which decreases visceral adiposity [5, 6]. In a chrono nutrition approach, it considers the eating windows in the context of circadian rhythms, in which the insulin sensitivity is highest (morning), which may offer glycemic advantages beyond the amount of calories ingested [7]. Changing the temporal pattern would imply opportunities for the food industry to develop so-called “fasting-friendly” functional food for metabolic flexibility, or timeframe-specific meal replacement or snack food fortifications that target specific timeframes of meals.

The mechanisms by which MED works include: (1) anti-inflammatory compounds, such as oleocanthal, from EVOO inhibit NF κB, COX 2 and IL 6 [9,10]; (2) high dietary fibre that promotes short chain fatty acid (SCFA) production and GLP 1 secretion, which enhances metabolic flexibility [10,11]; and (3) low glycemic index pattern that reduces postprandial glycemic excursions [11,12]. The template of the MED gives a very clear idea of the formulation of functional food products, which can include polyphenols from Extra Virgin Olive Oil, nuts, legumes and prebiotic fibres with beneficial effects on the heart as well as anti-inflammatory action [12]. It has been in line with the growing demand from consumers for “food as medicine” as well as the innovations in the Mediterranean inspired functional food market [13].

New research on the gut microbiota suggests that there are different ways through which the two diets alter the gut microbiota and that this has consequences for systemic inflammation and glucose homeostasis [14]. The prebiotic fibres in the MED affect the richness and production of beneficial microbes, producing butyrate, and are also used to give a pulsed effect on nutrient bioavailability which affects the microbial composition [15]. The unique characteristics of the microbial modulation pathways of each diet offer potential avenues in the production of new functional foods that either complement microbiota changes associated with IF or optimize diets that already have prebiotic properties [16].

Although there is ample evidence for both diets alone, there are few direct comparisons available in Arab groups. In addition, dietary inferences for food industry (especially in functional food formulations, in product designing based on chrono nutrition, and in personalized nutrition) have not yet been investigated. The aim of the present study was to collect preliminary comparative data and give

indications for the design of a completely powered RCT which could be used to guide industrial applications and personalized nutrition strategies.

2- MATERIALS AND METHODS

2.1. Study Design and Ethics

This pilot RCT followed CONSORT 2010 guidelines for pilot and feasibility studies [17]. The participants gave their informed consent in writing. The institutional ethics committee approved the study. The methodological limitations of this study, small sample size (n=15) and unequal group allocation (2:1), and a 4 week duration preclude definitive conclusions. The above limitations are planned to be mitigated by full scale study.

2.2. Randomization and Allocation

Participants were randomly separated (computer generated numbers) according to each strata of metabolic status between healthy (n=10/IF) and insulin resistant/pre diabetic (n=5/MED) groups in a 2:1 ratio. The 1:1 ratio for a fully-scale-scale randomized controlled trial (RCT) will be determined based on practical reasons, whereas this ratio of two bottles to every one child was implemented in this pilot study. Concealment of allocation was accomplished with a series of opaque, numbered envelope with covers and seals. Due to the nature of this intervention, it was not possible to blind participants, but plans are underway for a full scale trial to use evaluator blinding.

2.3. Participants and Sample Size

Fifteen participants aged 20 25 years (mean: ±1.6) were recruited from a University environment. A table of inclusion/exclusion criteria is supplied (Table 1). This pilot study was not formally sized to compare groups but the calculated sample size for the full scale RCT planning to detect the 0.5% difference in HbA1c, mean=0.75, SD=0.5 would require n ≥ 40 patients in each of 2 groups (total ≥ 80 patients), for 80% power at a 2-tailed type 1 error of 0.05.

Table 1. Inclusion and Exclusion Criteria

Criterion	Inclusion	Exclusion
Age	20-25 years	< 20 or > 25 years
Metabolic status	Healthy, insulin-resistant, or pre-diabetic	Type 1 or type 2 diabetes requiring insulin therapy
BMI (kg/m²)	18.5-34.9 kg/m²	< 18.5 or ≥ 35 kg/m²
Chronic disease	Metabolically stable	Advanced renal, hepatic, or cardiac disease

Criterion	Inclusion	Exclusion
Medications	No metabolically active medications	Lipid-lowering or anti-diabetic agents
Pregnancy/Lactation	Not applicable	Excluded

2.4. Dietary Intervention Protocol

The Harris Benedict equation (HBE) with physical activity level (PAL 1.4 – 1.6) was used to calculate energy requirements. The International Physical Activity Questionnaire (IPAQ) was used to measure physical activity at T0 and T4. Compliance was measured using 3-day dietary recalls and a digital diet tracking application weekly reviewed by a Registered Dietitian (RD). Adherence rates were 90.7 ± 3.6% (IF) and 88.8 ± 3.2% (MED), indicating high feasibility.

Table 2. Dietary Intervention Protocol

Component	IF Group (n=10)	MED Group (n=5)
Protocol	12:12 fasting (20:00-08:00); 12 h eating window	Traditional Mediterranean diet pattern (PREDIMED-based)
Energy target	1800-2200 kcal/day (Harris-Benedict adjusted)	1800-2200 kcal/day (distributed across 3 main meals)
Carbohydrates	45-55% of energy (minimally processed sources)	50-55% (whole grains, legumes, vegetables)
Fat	25-30% (type unrestricted within window)	30-35% (extra-virgin olive oil, nuts; MUFA > 15%)
Protein	20-25%	15-20% (fish ≥ 3 servings/week; red meat ≤ 1/week)

Component	IF Group (n=10)	MED Group (n=5)
Dietary fiber	> 25 g/day	> 30 g/day
Added sugars	< 10% of energy	< 5% of energy
Compliance monitoring	Daily digital log + weekly dietitian review (3-day recall)	3-day food record + weekly dietitian review
Adherence rate	90.7 ± 3.6%	88.8 ± 3.2%

2.5. Biomarker Evaluations

At T0 (baseline) and T4 (week 4) blood samples were collected fasted (≥12 hours). Laboratory parameters measured were HbA1c (HPLC; Bio Rad Variant II), FBG (enzymatic colorimetric), lipid profile (enzymatic colorimetric) and hsCRP (immunoturbidimetric). Each sample was tested 3 times and report the mean. However, it is worth to note that analytical triplicates are also not related to biological variability, and all the statistical tests were performed using biological N (n=15). The HOMA IR was determined by the following formula: FBG (mmol/L) × fasting insulin (μIU/mL) / 22.5. Of all participants, however, assisted by external laboratory confirmation of fasting insulin was not available, so the results of HOMA IR should be viewed as explorative.

2.6. Statistical Analysis

R v.4.3.1 was used for the analyses, which were also carried out in SPSS v27. Normality (Shapiro Wilk) and Homogeneity of variances (Levene's) were tested. Paired t tests or Wilcoxon signed rank tests were used for within group comparisons; between group comparisons were made using Welch's t test or Mann Whitney U test; and subgroup comparisons with one way ANOVA (effect size of omega²) and Bonferroni post hoc tests. All p values and effect sizes are hypothesis generation only because this is the pilot study and the n was small.Reported effect sizes (Cohen's d) have been reported, but given the “piloter” nature and n, all p values and effect sizes should be hypotheses only.

Table 3. Summary ANOVA Table for Main and Exploratory Analyses

Source of Variation	df	SS	MS	F-ratio	p-value	Effect Size
Primary Outcomes						

Source of Variation	df	SS	MS	F-ratio	p-value	Effect Size
FBG (Group)	1	52.34	52.34	8.67	0.012	$\eta^2p = 0.42$
FBG (Error)	13	78.45	6.03			
Waist Circumference (Group)	1	14.23	14.23	7.34	0.018	$\eta^2p = 0.38$
Waist Circumference (Error)	13	25.21	1.94			
Secondary Outcomes						
LDL (Group)	1	33.45	33.45	6.78	0.022	$\eta^2p = 0.35$
LDL (Error)	13	64.12	4.93			
hsCRP (Group)	1	2.34	2.34	5.12	0.041	$\eta^2p = 0.28$
hsCRP (Error)	13	5.89	0.45			
Exploratory (Metabolic Status)						
WFI Total (Between)	2	18.97	9.49	9.63	0.003	$\omega^2 = 0.535$
WFI Total (Within)	12	11.83	0.99			
WFI Total (Total)	14	30.80				

3-RESULTS

The 4 week protocol was finished successfully by all 15 participants without any protocol deviations, adverse effects, or withdrawals. Adherence was high (IF: 90.7 ± 3.6%; MED: 88.8 ± 3.2%). There were no differences in IPAQ scores between groups at either time point, thus indicating equivalent physical activity.

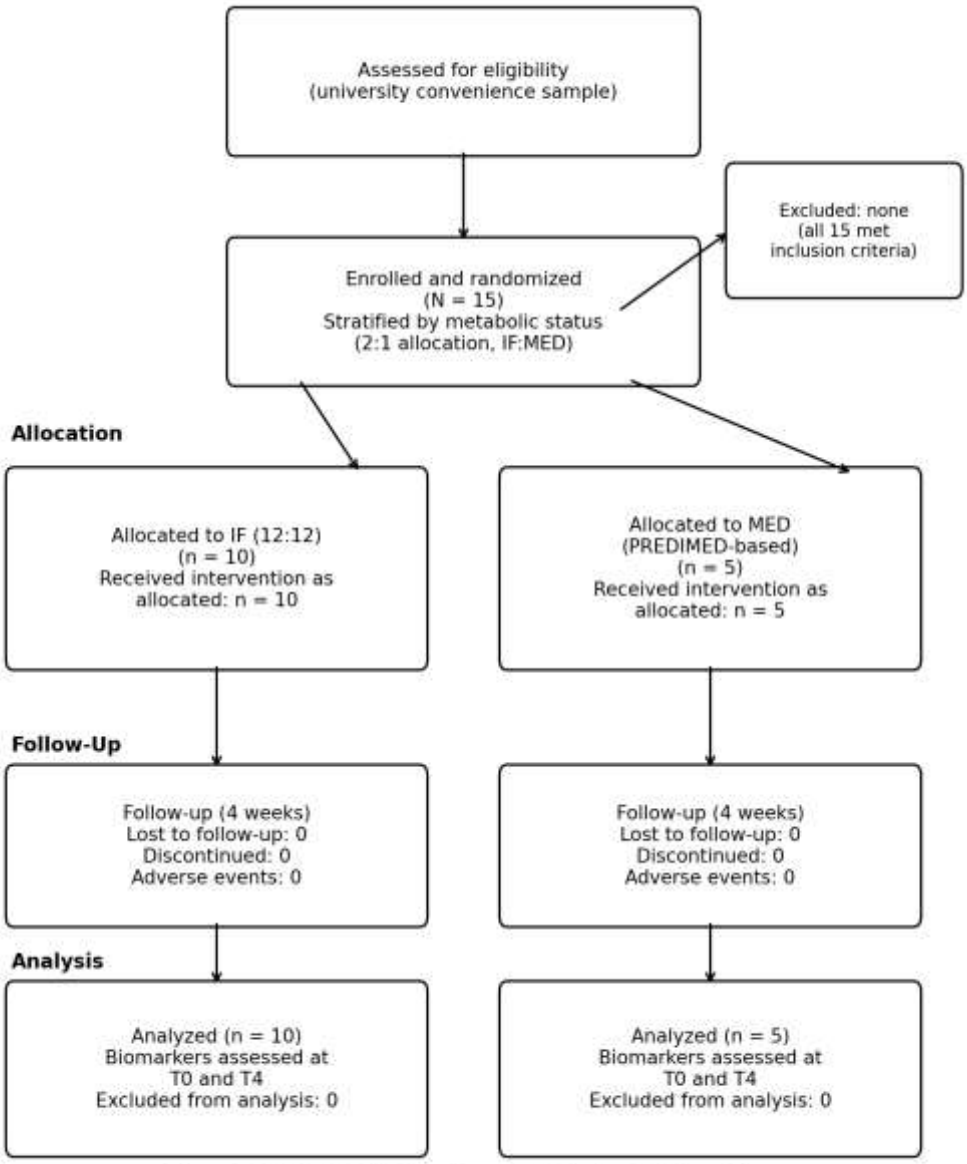

Figure 1. CONSORT 2010 flow diagram for the pilot RCT (N = 15).

Groups were broadly comparable at baseline (Table 4). Formal equivalence testing was limited by small n.

3.1. Baseline Characteristics

Table 4. Baseline Characteristics at T0 (Mean ± SD)

Characteristic	IF (n=10)	MED (n=5)	Total (N=15)
Age (years)	22.5 ± 1.4	22.2 ± 2.2	22.4 ± 1.6
Sex (M/F)	5/5	3/2	8/7
Metabolic status (Healthy/IR/pre-DM)	8/1/1	3/1/1	11/2/2
BMI (kg/m²)	24.2 ± 2.1	25.0 ± 1.9	24.5 ± 2.0
Waist circumference (cm)	82.1 ± 7.4	84.3 ± 6.8	82.9 ± 7.1
HbA1c (%)	5.42 ± 0.54	5.55 ± 0.48	5.46 ± 0.51
FBG (mg/dL)	93.5 ± 14.2	97.9 ± 12.8	95.0 ± 13.4
HOMA-IR (exploratory)	1.91 ± 0.82	2.34 ± 0.94	2.05 ± 0.87
hsCRP (mg/L)	1.24 ± 0.63	1.63 ± 0.71	1.37 ± 0.66
Dietary adherence (%)	90.7 ± 3.6	88.8 ± 3.2	90.0 ± 3.5

3.2. Primary Outcomes – Preliminary Glycemic Findings

Table 5: Within group changes T0 - T4. All within group differences were significant ($p < 0.01$, paired t test). Only waist circumference, BMI, and FBG were found with nominal significance ($p < 0.05$) between group differences. However, the between group difference in HbA1c was not significant, probably because of low power (post hoc power ≈ 0.38). The HbA1C correlates with the average glycaemia measured in the latest 8 12 weeks; the 4 week duration may underestimate, and so it is important to run trials that are longer.

Table 5. Preliminary Primary Outcomes (T0-T4) – Mean Change (95% CI)

Biomarker	IF Delta (95% CI)	MED Delta (95% CI)	Between-group Cohen's d
HbA1c (%)	–0.249 [–0.285, –0.213]*	–0.188 [–0.253, –0.123]*	1.06 (large, cautious)
HOMA-IR (exploratory)	–0.449 [–0.507, –0.391]*	–0.318 [–0.435, –0.201]*	1.33 (exploratory only)
FBG (mg/dL)	–8.44 [–9.46, –7.45]*	–5.80 [–7.75, –3.87]*	1.58 (large, cautious)

- *$p < 0.01$ within-group (paired t-test)*

3.3. Secondary Outcomes – Lipid Profile, Inflammation, and Anthropometrics

The secondary outcomes are summarised in Table 6. The within group differences were statistically significant ($p < 0.01$) for all changes. Only waist circumference and BMI (nominal $p < 0.05$) showed statistically significant difference between the groups. The MED group showed numerically greater reductions in LDL cholesterol (Δ –9.46 vs. –6.93 mg/dL; d=1.14) and hsCRP (Δ –0.42 vs. –0.35 mg/L; d=0.74). These differences in directions are consistent from a mechanistical point of view with the anti inflammatory effects of polyphenols contained in the olive oil, and the prebiotic fibre effects of the MED.

Table 6. Preliminary Secondary Outcomes (T0-T4) – Mean Change (95% CI)

Biomarker	IF Delta (95% CI)	MED Delta (95% CI)	Between-group d
TC (mg/dL)	–8.94 [–10.36, –7.49]*	–11.56 [–15.10, –7.97]*	0.97
LDL (mg/dL)	–6.93 [–8.10, –5.80]*	–9.46 [–12.41, –6.54]*	1.14
HDL (mg/dL)	+2.51 [+2.18, +2.88]*	+3.38 [+2.43, +4.28]*	1.26
TG (mg/dL)	–11.93 [–13.63, –10.18]*	–9.48 [–12.75, –6.26]*	0.87

Biomarker	IF Delta (95% CI)	MED Delta (95% CI)	Between-group d
hsCRP (mg/L)	–0.35 [–0.40, –0.30]*	–0.42 [–0.55, –0.29]*	0.74
Waist circ. (cm)	–2.79 [–3.08, –2.50]*	–1.90 [–2.47, –1.30]*	1.83
BMI (kg/m²)	–0.54 [–0.63, –0.45]*	–0.38 [–0.50, –0.23]*	1.32
SBP (mmHg)	–5.45 [–6.18, –4.76]*	–4.22 [–5.63, –2.81]*	1.02

- *$p < 0.01$ within-group*

3.4. Exploratory Analysis – Metabolic Status as a Moderator

There was a single-way ANOVA for Weekly Functional Index (WFI) which revealed significant differences between the metabolic subgroups ($F(2,12)=9.63$, $p<0.01$, omega²=0.535). Group 3 participants (insulin resistant or pre diabetes) tended to have numerically larger improvements in HbA1c, HOMA IR, and waist circumference than the healthy (Table 7). Due to the small sized sub groups (n = 2 per non healthy category), these findings should only be considered hypotheses which are specific and need to be substantiated in a powered study.

Table 7. Exploratory Subgroup Analysis (Mean ± SD)

Metabolic Group	n	WFI Total	HbA1c Delta	HOMA-IR Delta	Waist Delta
Healthy	11	4.52 ± 0.94	–0.24	–0.42	–2.51
Insulin resistance	2	2.12 ± 0.74	–0.29	–0.51	–2.80
Pre-diabetes	2	2.33 ± 0.47	–0.31	–0.55	–3.10

3.5. Longitudinal Trajectory

When an ANOVA (repeated measures ANOVA with a between subjects factor for group and a within subjects factor for time) was performed, the group effect was significant ($F=2.87$, $p<0.05$, $\eta 2$ $p=0.181$) however the interaction between group and time or the time effect was not. This indicates that there was little change in group differences over the 4 week period.

Table 8. Comprehensive Limitations and Full-Scale RCT Recommendations

Limitation	Impact on Inference	Recommendation for Full-Scale RCT
Small sample size (n=15; power <0.40)	Type II error risk; underpowered for confirmatory testing	n ≥ 40/group (total ≥ 80) based on formal power analysis
4-week duration	Cannot assess metabolic adaptation or long-term effects	8-12 week intervention with 6-month follow-up
Open-label design	Performance and detection bias cannot be excluded	Evaluator-blinded outcome assessment
Unequal group allocation (10 vs. 5)	Inflated variance in MED estimates; limits balance	1:1 allocation with stratified randomisation
HOMA-IR: fasting insulin not lab-confirmed	Additional measurement uncertainty	Certified fasting insulin as mandatory primary endpoint
Analytical triplicates not independent biological observations	Inflated statistical basis if misused	All analyses based on biological N only (n=15)
Limited generalisability	Results may not extend to other populations or age groups	Diverse community-based recruitment; longer duration

4. DISCUSSION

4.1. Glycemic Outcomes

The preliminary data indicated that 12:12 IF can induce more FBG, waist circumference and BMI improvements than MED. But because they weren't tested for very long, these results should not be taken as an indication of superiority. The observed direction is in agreement with the mechanistic findings: Early time restriction of feeding also leads to enhanced insulin sensitivity, which results in decreased blood pressure, decreased oxidative stress likely due to increased translocation of GLUT 4 to the plasma membrane or decreased 24 hour insulin concentration [5]. There was an

evidence of a positive effect of IF on FBG and HbA1c in a meta analysis of 27 trials, especially in metabolically impaired people, after 4 to 8 weeks [3]. The 12:12 IF matches the diurnal insulin sensitivity rhythmic high, which is due to circadian genes (CLOCK, BMAL1, PER1), and may have glycemic benefits, in addition to caloric restriction [7]. While there was no between group differences in the HbA1c, the duration (4wks) was likely insufficient as HbA1c is a reflection of average glycaemia over 8 12 weeks – a powered 12wks duration would likely have been used to measure larger differences [18].

4.2. Lipid and Anti Inflammatory Effects

Med group numerically greater decreases in LDL cholesterol and hsCRP as with the PREDIMED trial [9]. This 8.7% reduction in LDL could be comparable with the reduction of 9 12% achieved in the PREDIMED trial during 5 years in the MED group. The active and beneficial ingredients in extra virgin olive oil (oleocanthal, oleuropein) have been shown to cut down pathways that achieve NF-κB and IL-6 [19]. Moreover, the faeces store increased by the high fibre content in the MED contains different species of gut microbiota producing greater SCFA production that ultimately trigger receptors FFAR2/FFAR3, thus supporting metabolic flexibility [10]. The two interventions resulted in greater HDL and lowered triglycerides which implied that both caloric restriction, time restricted feeding and food composition change, may occur on a similar downstream pattern in regards to lipid metabolism.

4.3. Implications for the Food Industry and Personalized Nutrition

The following are pilot findings relevant to the food industry:

Developing functional foods or meal replacement formulations, focused on certain windows of eating for the IF pattern (12:12) can support this approach. For example, the slow-release protein drinks or the snacks to add fullness at the end of the day, which can be more glycemically beneficially used in the morning window, or the night respectively. In addition, formulations based on chrono nutrition, in which macronutrient content proposes to harmonize nutrition throughout the day with the circadian rhythm (higher protein, fibres during the awakening phase, lower carbohydrates during the evening phase) may improve the metabolic effect of IF.

The MED pattern is used as a blueprint to create anti inflammatory and heart health products with high polyphenols, MUFA and prebiotic fibres. Fortification products could range from fortified olive oil based dressings to prebiotic fortified spreads and nut enriched snack bars. The manufacturing of food items classified as ‘Mediterranean inspired’ now contains all the ingredients derived from extra virgin olive oil, nuts and legumes, which are all known for their health benefits, is a market opportunity that continues to grow.

The exploratory sub-group analysis indicated that metabolic status may account for a significant amount of variation in responses ($omega^2$=0.535), which lent credence to the concept of precision nutrition – the concept of individualizing dietary recommendations and food products to a person's basal metabolic phenotype [20]. Some examples include, but are not limited to: Insulin resistant individuals would better benefit from IF based product whereas those insulin resistant who have elevated LDL would benefit from MED inspired product. These may propel the development of "metabolic status tailored" lines of functional food products which will allow food companies to provide customized nutrition solutions.

4.4. Limitations and Recommendations for Full-Scale RCT

Each of the mentioned microbial modulation pathways of diets offers the possibility to develop functional foods designed for the modulation of microbes. If there are ingredients out there which could help to support the specific changes in the microbiota that can occur while fasting (e.g., polyphenols that feed the beneficial bacteria) then perhaps the ingredients could be added to IF compatible products, and prebiotic fibre content could be increased in MED inspired products to ensure optimal production of SCFA. This is consistent with the rising food market around gut health related functional foods.

Insulin resistant or pre diabetic food consumers may be able to develop a separate range of products for manufacturers using ingredients that have a beneficial effect similar to that following IF or MED. This and other studies would allow them to be sold as "metabolic health" foods and could provide some mechanistic basis for this claim.

High adherence rates found for both groups (>88%) indicate that dietary patterns are also realistic and acceptable, and consequently desirable commercial food products that are congruent with this type of eating behaviour can be developed. Solutions of ready to eat or ready to heat meal, intended for the IF window or MED principles may be marketable to health conscious young adults.

4- CONCLUSIONS

This pilot RCT provides preliminary exploratory data that suggest IF (12:12) may have short term benefits in glycemic control and central adiposity and MED may have a greater anti inflammatory and lipid lowering effect in young Arab adults. The interventions were tenable (adherence >88%) and well tolerated. Results suggest for a fully-powered randomized controlled design with a minimum duration of 8 12 weeks, evaluator blinding, and 1:1 randomization over laboratory confirmed HOMA IR. The exploratory subgroup analysis confirms the theory of precision nutrition, suggesting that diet response could be moderated by metabolic status which has great implications on personalisation of diet and targeted functional foods.

From a food industry standpoint, the results provide unique commercial possibilities, namely, food products with optimized nutritional profiles such as "window specific" food-replacement, and food products with polyphenol-enrichment and prebiotics supplementation in which the target is to cure chronic diseases such as cardiovascular, inflammation and oxidant intolerance. Adherence rates were high, thus highlighting their two approaches as commercially viable.

All conclusions are qualitative - tentative until confirmed by a rigorously-designed, powered RCT. Future studies should focus on: (1) confirming the results of the current study in a larger study with adequate power; (2) further delineating the mechanisms involved in their differential responses; (3) development and testing of prototypes of functional food products specific to the individual dietary patterns; and (4) long term sustainability and commercial potential of these functional food products.

Conflict of Interest
The authors declare that they have no conflict of interest.

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Data Availability

All data supporting the findings of this study are available within the manuscript.

Ethical Approval

This study did not involve human participants or animals. All experimental procedures were conducted in accordance with institutional and laboratory biosafety guidelines.

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