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BIOCHEMISTRY AND ENDOCRINE CHANGES AFTER A 6-WEEK MEDITERRANEAN DIET INTERVENTION WITH FASTING FOR WEIGHT LOSS.

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ABSTRACT

Fasting may be effective for reducing weight and improving health. We analysed the effect of a Mediterranean diet intervention, with a 5-day semi-fast, on inflammation, blood lipids and carbohydrate metabolism in overweight type II (BMI>27-30) or obesity (BMI>30-40). Glucose, total cholesterol, triglycerides, HDL-/LDL-cholesterol, albumin, AST-GOT, ALT-SGTP, C-reactive protein, insulin, IGF-1, T3, T4 and TSH were monitored, during a 6-week period, in 42 subjects with overweight type-II/obesity, between the ages of 30-65. Glucose, triglycerides, insulin, AST, IGF-1 and T3 values were not significantly decreased ($p>0.05$) during fasting and non-fasting diets, while albumin and T4 increased in both groups. C-reactive protein decreased significantly when fasting but increased when not. Total cholesterol, HDL, LDL and ALT ($p<0.05$) were significantly decreased after fasting. Significant differences ($p>0.05$) were found for total cholesterol, HDL, LDL and TSH in the Control group. Fasting resulted in greater insulin sensitivity and reduced levels of body fat, IGF-I, insulin, glucose, atherogenic lipids and inflammation.

1. Introduction

In humans, fasting is achieved by ingesting no or minimal amounts of food and caloric beverages for periods that typically range from 12 hours to three weeks (1).

Fasting has been practiced for millennia, but only recently studies have shed light on its role in adaptive cellular responses that reduce oxidative damage and inflammation, optimize energy metabolism and bolster cellular protection (1). Fasting results in ketogenesis, promoting significant changes in metabolic pathways and cellular processes such as stress resistance, lipolysis and autophagy, and can have medical applications that in some cases are as effective as those of approved drugs (2). In lower eukaryotes, chronic fasting extends longevity in part by reprogramming metabolic and stress resistance pathways. In rodents, intermittent or periodic fasting protects against diabetes, cancer, heart disease and neurodegeneration, while in humans it helps reduce obesity, hypertension, asthma and rheumatoid arthritis (1).

Important metabolic and cardiovascular benefits have been reported in humans that deserve further consideration in therapeutic fasting trials, such as decreases in fat mass, low-density lipoprotein (LDL) cholesterol particle size, LDL cholesterol, triglycerides, and C-reactive protein (CRP)(3,4). It has also been hypothesized that intermittent prolonged fasting could positively affect the inflammatory state, by suppressing proinflammatory cytokine expression and decreasing body fat and circulating levels of leukocytes (5).Interestingly, the inflammatory biomarkers such as interleukin-6 (IL-6) and C-reactive protein are found to be significantly depressed by short and long intermittent fasting (6). Furthermore, it is noteworthy to point out that previous studies have shown that intermittent fasting improves insulin sensitivity (7).

In addition, some studies have demonstrated no change in aspartate transaminase (AST) and alanine transaminase (ALT) (8), but conversely, others have reported a decrease in AST and ALT (9). This proves that the impact of fasting on the liver remains to be clarified.

During fasting, severe changes in the regulation of the hypothalamus-pituitary-thyroid axis also occur in order to save energy and limit catabolism.

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In this setting, serum triiodothyronine (T3) and thyroxine (T4) are decreased without an appropriate thyroid-stimulating hormone (TSH) and thyroid-releasing hormone (TRH) response, reflecting central down-regulation of the hypothalamus-pituitary-thyroid axis (10).

On the other hand, the Mediterranean diet is a dietary pattern associated with positive effects on health and well-being. This dietary pattern is known to actively modulate the cell membrane properties (11).

Whether fasting actually causes improvements in metabolic health, cognitive performance, and cardiovascular outcomes over the long term, how much fasting is actually beneficial, and where the threshold of hormesis resides (i.e., a balance between long-term benefit from fasting compared with harm from insufficient caloric intake) remain open questions.

We hypothesised that the implementation of 5-day semi-fasting diet results in a better lipid profile and improvement of biomarkers related to carbohydrates metabolism and inflammation in overweight or obese adults.

The primary aim of this study was to analyse the effect of a Mediterranean diet intervention, with a 5-day semi-fast, on biomarkers related to overweight type II (body mass index, BMI >27-30) or obesity (BMI >30-35), by comparing the fasting group with a non-fasting group before and after intervention. The secondary objectives were to understand the impact of a semi-fast on inflammation, blood lipids and carbohydrate metabolism.

2. Methods

Study Population

A randomized, controlled, prospective clinical trial was conducted. A sample of 50 participants, of both sexes and between the ages of 30 and 65, was selected from the Community of Madrid (Spain) in 2017. Inclusion criteria were subjects between 30 and 65 years-old, of both sexes, with no severe diseases (chronic, autoimmune or neurological diseases, eating disorder, cancer, hepatitis, chronic obstructive pulmonary disease or intellectual disabilities) and no bariatric surgery, with overweight type II (BMI >27-30) or obesity (BMI >30-40), who agreed to participate voluntarily by following the diet. Participants who did not meet inclusion criteria (n=2), who did not complete all questionnaires (n=3), did not follow the diet as stated (n=3), or were under medication (n=0), all belonging to the semi-fast group, were excluded. Hence a total of 42 participants (76.2% women and 23.8% men) were finally included.

Declarations of Helsinki principles were followed, and the rights of all participants were respected. They all signed an informed consent to participate in the study. This research project was evaluated by the Ethical Committee of Clinical Research of *Hospital Universitario Severo Ochoa*, Madrid (Spain).

The study population included 42 subjects (n = 25 in group 1 [G1] and n = 17 in group 2 [G2]), who were distributed by randomization. G1 was the study group (semi-fast) and G2 was the control group (standard hypocaloric Mediterranean diet).

The sample size was calculated by comparison of means: $n = 2 * (1.645 + 1.282)^2 * 2.29^2 / 2^2 = 22.46$ subjects per group. The dropout rate was estimated at 10%, so 50 subjects were recruited in total (25 subjects per group). A 95% confidence interval and a statistical power of 90% were used.

A deviation of ±2.29 kg for a habitual weight loss in the Spanish obese population in a slimming program with a hypocaloric Mediterranean diet (500 kcal restriction), with a mean loss of 2-4 kg/month, were taken into account. A 3 kg loss was estimated in the Mediterranean diet group and 5 kg in the semi-fast group, assuming a difference of 2 additional kg in the study group.

Study factors

An anthropometric study of the subjects participating in the study was conducted. Their eating habits were analysed for the adjustment of dietary guidelines at baseline (results not shown), using the PREDIMED questionnaire (12) and NHANES Food Frequency Questionnaire (13). Data on biochemical markers were also collected through a blood test.

a. Anthropometric Study

The anthropometric study was carried out by a single trained researcher, ensuring the homogeneity and standardisation of criteria and the of the methodology. The anthropometric measurements included: height, weight, BMI, fat mass, visceral fat, muscle mass and waist circumference. Height was measured with the subjects standing barefoot, according to the WHO (14) protocol, with a SECA mobile stadiometer with a 1 mm accuracy. Weight, body fat mass, visceral fat and muscle mass were measured with a digital bioimpedance analyser TANITA model BP-601, ranging from 0.1 to 150 kg. Quetelet index, based on weight and height, was used to calculate BMI (15). BMI >27-30 was considered overweight type II and BMI >30-40 was considered obese. Waist circumference was measured around the midpoint between the lowest rib and the iliac crest, with a non-extensible tape measure (range 0-150 cm).

b. Dietary assessment

G1 (semi-fast group) followed the standard hypocaloric Mediterranean Diet for 6 weeks including 5 days of semi-fast. G2 (control group) followed the same standard hypocaloric Mediterranean Diet with no fasting period.

Basal and total metabolic expenditure were calculated for every subject, and their diet was adjusted to a caloric restriction of 500 kcal, deducted from the estimated individual resting energy expenditure. Diets were established by trained dieticians. There guidelines were adjusted during two previous weeks, so that all participants acquired similar habits from baseline.

The general Mediterranean diet guidelines (16) that dieticians provided to participants included the following recommendations: a) use of olive oil for cooking and dressing dishes; b) consumption of ≥2 daily servings of vegetables (at least one of them raw, such as salad), not including side dishes; c) ≥2-3 daily servings of fresh fruits (including natural juices); d) ≥3 weekly servings of legumes; e) ≥3 weekly servings of fish or seafood (at least one them fatty fish); f) ≥1 weekly serving of nuts or seeds; g) select white meats (poultry without skin or rabbit) instead of red meats or processed meats (burgers, sausages); and h) cooking regularly (at least twice a week) with tomato, garlic and onion, and dressing vegetables, pasta, rice and other dishes with a sauce made by slowly simmering minced tomato, garlic and onion with abundant olive oil. Recommendations were also given to eliminate or limit the consumption of cream, butter, margarine, cold cut meat, pâté, duck, carbonated and/or sugary beverages, pastries, industrial bakery products (such as cakes, donuts, or cookies), industrial desserts (puddings, custard), French fries and/or potato chips, and out-of-home pre-cooked cakes and sweets.

The aim of the control diet was to reduce all types of fat, with particular emphasis on the consumption of lean meats, low-fat dairy products, cereals, potatoes, pasta, rice, fruits and vegetables. In the control diet, advice on vegetables, red meat and processed meats, high-fat dairy products, and sweets concurred with the recommendations of the Mediterranean diet, but the use of olive oil for cooking and dressing and the consumption of nuts, fatty meats, sausages, and fatty fish were discouraged.

Semi-fast (so-called because the number of calories ingested is greater than a fast and less than a caloric restriction) consisted of 5 days with two previous days of adaptation and two days of exit from the semi-fast.

The 5-day semi-fast consisted of a liquid diet based on organic fruits and vegetables delivered by *BioSabor S.A.* with the corresponding instructions. The main recommendations were to ingest only the food supplied made puree and juice, add spices for flavour, moderate salt and olive oil.

The one-day menu consisted of: a) 700 ml of juice to be distributed throughout breakfast, mid-morning and afternoon snack. Water could be added to obtain a greater quantity; b) 400 ml of puree (add water for flavour) distributed between lunch and dinner; c) 500 ml of organic gazpacho gluten-free Biosabor to be distributed between lunch and dinner. Infusions could be taken without sugar and sweeteners.

The ingredients, which included zucchini, leek, aubergine, carrot, pineapple, papaya, orange and packaged gazpacho, were adapted to each participant's preference. All ingredients used were organic ensuring more nutritious and chemical-free food.

The two previous days of adaptation included: a) 1 whole-yogurt with chopped fruit and oat for breakfast, b) 300 ml of multifruit juice for mid-morning, c) salad with white fish and gazpacho for lunch, d) 300 ml of multifruit juice and 5 nuts for afternoon snack and e) meat or fish with boiled vegetables for dinner.

The two days of exit from the semi-fast included: a) 1 yogurt and 4 plums or any other fruit for breakfast, b) multifruit juice and yogurt or cooked ham for mid-morning, c) steamed vegetables with white fish and gazpacho for lunch, d) multifruit juice or chopped fruit for afternoon snack, and e) 1 avocado with chopped tomato dressed with olive oil, garlic, salt and lemon, and a French omelette for dinner (day 1); steamed vegetables with white fish (day 2).

c. Biochemical markers

Venous blood was drawn into EDTA vacutainer tubes by medical staff, after a 12-hour fasting period by the subjects, following the standard protocol (17). The biochemical parameters determined were: glucose, total cholesterol, triglycerides, HDL- cholesterol, LDL- cholesterol, albumin, AST-GOT, ALT-SGTP, CRP, insulin, somatomedin C (insulin like growth factor 1, IGF-1), T3, T4 and TSH.

d. Visit schedule

All participants signed informed consent, filled out food habit questionnaires, had blood drawn and anthropometric measures taken at the first visit. Participants were supervised at 3 weeks, where a diet control and adherence to treatment survey was conducted. In the case of G1, participants were indicated how to follow the semi-fast diet between week 3 and 4. The final visit, after 6 weeks of intervention, was used to take the final anthropometric measures and a final blood analysis.

Statistical analysis

Analysis of the data collected was processed with SPSS® software (version 20). A descriptive analysis for the baseline data was conducted with demographic, lifestyle and anthropometric data. For the analysis of the impact of the diet on the selected biomarkers over time, a mixed ANOVA was performed. A statistical significance of 0.05 was considered. All variables were checked for the assumptions of normality, homoscedasticity, significance of outliers and covariance before running the test. Sphericity was assumed, as it only applies when 3 or more groups are compared, and this study only examined two treatments.

3. Results

We evaluated 42 participants between the ages of 30 and65 (mean 46.95 ± 7.83 years), 32 (76.2%) females and 10 (23.8%) males. BMI showed that 71.4% (n=30) were obese and 28.6% (n=12) were overweight type II. The baseline characteristics of participants are summarized in Table 1. There were no statistically significant differences between groups at the beginning of the study.

		Semi-fastgroup (61.4 %)		No semi-fastgroup (38.6 %)		p-value
		Mean	SD[a]	Mean	SD[a]	
Age (years)		46.32	8.03	47.88	7.67	0.532
Height (m)		1.67	0.09	1.66	0.08	0.674
Weight (kg)		92.21	13.82	97.99	18.05	0.247
BMI (kg/m^2)		32.83	3.73	35.92	5.32	0.035
Body fat mass (%)		40.76	6.61	44.51	6.40	0.075
Visceral fat (%)		11.96	3.77	13.12	4.00	0.346
Muscle (kg)		51.81	10.02	51.47	11.16	0.919
Waist circumference (cm)		106.24	11.89	110.49	14.17	0.299
Exercise (h/week)		4.54	3.33	4.13	3.39	0.785
Sex (%)	Men	28.0		17.6		0.452
	Women	72.0		82.4		
Physical exercise (%)	No	20.0		47.1		0.065
	Yes	80.0		52.9		

Table 1. **Baseline characteristics of the participants according to study group.** ***([a] SD, standard deviation)***

Table 2 summarizes the biochemical parameters measured at the beginning and at the end of the study.

Plasma glucose values decreased to 80.0 ± 14.32 mg/dL for all subjects after the fast intervention (p>0.05), and to 83.06 ± 6.87 mg/dL in subjects of the non-fast intervention (p>0.05). Baseline plasma insulin levels also changed in both conditions but were significantly reduced to 10.21 ± 5.03 (μU/mL) in the non-fast condition (p>0.05) (Table 2).

Total cholesterol, triglycerides, LDL and HDL were decreased after fasting. The same situation was observed in the non-fasting group. There was a more pronounced decrease in triglycerides in this latter group (mean difference 18.6 ± 44.97 mg/dL vs. -4.00 ± 30.63 mg/dL).

Statistically significant differences were found in total cholesterol (p<0.001), LDL (p<0.001) and HDL (p<0.001) after fasting and in total cholesterol (p<0.001), LDL (p=0.01) and HDL (p=0.001) after ingesting the hypocaloric Mediterranean diet (Table 2).

Fasting serum levels of liver function tests, including AST, ALT and albumin were measured. Mean AST decreased in G1 and increased in the second group (p=0.568 and p=0.744, respectively) compared to baseline levels. Mean ALT decreased significantly (p=0.008) only in the first group. Serum albumin increased and IGF-1 decreased in both groups, after fasting (p=0.447, p=0.485, respectively) and non-fasting (p=0.328, p=0.175, respectively), but not significantly. C-reactive protein (CRP) decreased statistically significantly in G1 (p=0.007).

Serum T3 levels decreased after fasting in both semi-fast and hypocaloric Mediterranean Diet intervention groups, while serum T4 levels increased in both groups. TSH expression and release from the pituitary gland is negatively regulated by thyroid hormone. Fasting decreased TSH levels in G1 and G2, being statistically significant in G2 (p=0.005), despite low serum thyroid hormone concentrations (Table 2).

	Semi-fastgroup						No semi-fastgroup						P value
	Baseline		Final		Difference		Baseline		Final		Difference		
	M[a]	SD[b]	M	SD	M	SD	M	SD	M	SD	M	SD	
Glucose (mg/dL)	82.28	11.16	80.00	14.32	2.92	8.40	85.31	6.78	83.06	6.87	2.93	6.51	0.431
Total Cholesterol (mg/dL)	221.24	37.18	187.24	31.82	27.29	28.10	234.81	32.71	215.63	33.82	27.53	33.46	0.10
Triglycerides (mg/dL)	110.24	48.54	102.64	32.13	-4.00	30.63	125.63	59.47	121.06	44.92	18.60	44.97	0.134
HDL (mg/dL)	56.08	14.92	47.68	12.69	8.17	7.41	55.31	10.50	49.31	9.21	7.20	5.25	0.659
LDL (mg/dL)	143.08	32.72	118.33	25.88	20.91	25.16	154.31	27.96	142.06	26.76	16.47	30.73	0.008
Albumin (g/L)	38.98	10.82	39.22	11.01	-0.51	1.54	42.47	1.96	43.09	1.92	-0.05	2.27	0.173
AST_GOT (U/L)	27.44	8.78	26.48	8.03	0.83	7.12	24.50	6.71	24.56	6.76	0.53	8.50	0.434
ALT_SGTP (U/L)	31.68	16.73	25.44	10.63	4.17	6.81	24.50	9.29	23.31	11.26	3.87	12.57	0.545
CRP (mg/L)	3.32	3.30	2.32	2.78	1.12	2.39	6.26	6.31	6.65	7.65	-0.46	3.44	0.015
Insulin (µU/mL)	12.49	6.30	10.66	10.27	2.67	9.51	13.20	5.40	10.21	5.03	2.20	6.23	0.871
Somatomedin C (IGF-1) (ng/mL)	115.10	29.62	111.68	25.41	7.30	25.30	118.76	32.87	106.53	18.59	3.29	21.76	0.494
T3 (ng/mL)	1.05	0.22	1.01	0.18	0.00	0.21	1.08	0.11	1.04	0.17	0.07	0.09	0.554
T4 (µg/mL)	7.41	1.09	7.74	1.59	-0.22	0.74	7.26	0.92	7.42	0.90	-0.12	0.92	0.478
TSH (µUI/mL)	1.60	0.71	1.49	0.75	0.24	0.84	2.47	1.47	1.81	0.94	0.43	0.55	0.245

Table 2. Differences between groups in baseline and final biochemical markers values. ***([a]M, mean; [b] SD, standard deviation)***

When it comes to the impact of the intervention in the analysed biomarkers, only RCP, F (1,35)=5.106. p=0.030 and TSH F (1,35) =5.655, p=0,023, showed differences between the means of the fasting and no-fasting group (Table 3).

The fasting group showed a lower TSH value before the trial (1.602 µUI/mL vs. 2.583 µUI/mL for the non-fasting group). After the intervention, both groups exhibited a decrease in TSH values. The non-fasting group experienced the biggest difference, as seen in Figure 1.

Overall, both groups improved the biomarkers with both diets, even though no statistically significant differences were observed between groups. However, significant differences were observed in both groups over time, demonstrating safety and efficacy of both interventions.

Measure	Group	Mean	SD	Mean difference (Baseline-Final)	P value
Glucose	Fasting group	79.932	1.808	-4.535	0.119
	No-fasting group	84.467	2.189		
Insulin	Fasting group	11.290	1.182	-0.872	0.642
	No-fasting group	12.162	1.432		
Cholesterol	Fasting group	205.795	6.760	-20.471	0.062
	No-fasting group	226.267	8.187		
HDL	Fasting group	50.591	2.297	-0.342	0.925
	No-fasting group	50.933	2.782		
LDL	Fasting group	133.295	5.579	-16.238	0.072
	No-fasting group	149.533	6.756		
TG	Fasting group	109.455	8.712	-19.312	0.167
	No-fasting group	128.767	10.551		
Albumin	Fasting group	42.275	0.368	-0.602	0.305
	No-fasting group	42.877	0.445		
AST	Fasting group	27.795	1.473	3.062	0.194
	No-fasting group	24.733	1.784		
ALT	Fasting group	28.682	2.641	3.982	0.344
	No-fasting group	24.700	3.198		
CRP	Fasting group	3.050	1.045	-3.710	0.030
	No-fasting group	6.760	1.266		
Somatomedin C	Fasting group	114.341	5.235	2.611	0.753
	No-fasting group	111.730	6.340		
T3	Fasting group	1.034	0.034	-0.035	0.523
	No-fasting group	1.069	0.041		
T4	Fasting group	7.620	0.240	0.308	0.420
	No-fasting group	7.312	0.291		
TSH	Fasting group	1.520	0.192	-0.717	0.023
	No-fasting group	2.237	0.233		

Table 3. Mean and mean difference of biochemical markers values for fasting and non-fasting group.

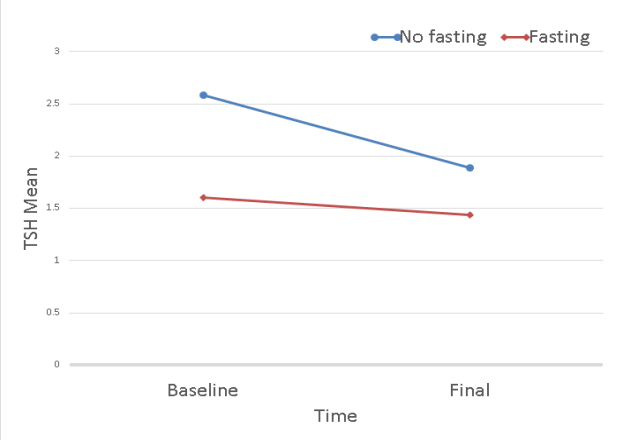

Table 4. TSH value observed before and after the intervention in both groups (Fasting in red and No fasting in blue).

4. Discussion

In most mammals, the liver serves as the main reservoir of glucose, which is stored in the form of glycogen. In humans, depending upon their level of physical activity, 12 to 24 hours of fasting typically results in a 20% or greater decrease in serum glucose and depletion of the hepatic glycogen, accompanied by a switch to a metabolic mode in which non-hepatic glucose, fat-derived ketone bodies and free fatty acids are used as energy sources (18).

In our sample, no such significant decreases were achieved (around 3%) in blood glucose levels, but they followed the trend of evidence.

It is important to determine how the frequency of specific changes such as the lowering of IGF-1 and glucose affect cellular protection, diseases and longevity. Changes in the levels of IGF-1, glucose and insulin are among the major effects of fasting. Fasting for 3 or more days causes a 30% or more decrease in circulating insulin and glucose, as well as a rapid decline in the levels of IGF-1, the major growth factor in mammals, which together with insulin is associated with accelerated aging and cancer (19). In humans, five days of fasting causes an over 60% decrease in IGF-1and a 5-fold or higher increase in one of the principal IGF-1-inhibiting proteins: IGFBP1 (20). This effect of fasting on IGF-1, also visible in our study, is mostly due to protein restriction, and particularly to the restriction of essential amino acids, but is also supported by calorie restriction, since the decrease in insulin levels during fasting promotes reduction in IGF-1 (20). Although no human data are available on the effect of intermittent fasting or periodic fasting in cancer prevention, their effect on reducing IGF-1, insulin and glucose levels, and increasing IGFBP1 and ketone body levels could generate a protective environment that reduces DNA damage and carcinogenesis, while at the same time creating hostile conditions for tumour and pre-cancerous cells (21).

The primary finding of the Kul et al. meta-analysis (22) was that after fasting, low-density lipoprotein (standardized mean difference, SMD = -1.67, 95% confidence interval, CI = -2.48 to -0.86) was decreased in both sex groups and also in the entire group compared to levels prior to fasting. In addition, in the female subgroup, total cholesterol (SMD = 0.05, 95% CI = -0.51 to 0.60), and triglyceride levels (SMD = 0.03, 95% CI = -0.31, 0.36) remained unchanged, while HDL levels (SMD = 0.86, 95% CI = 0.11 to 1.61, p = 0.03) were increased. In males, fasting resulted in a substantial reduction in total cholesterol (SMD = -0.44, 95% CI = -0.77 to -0.11), LDL levels (SMD = -2.22, 95% CI = -3.47 to -0.96) and in triglyceride levels (SMD = -0.35, 95% CI = -0.67 to -0.02). The increase in HDL levels observed in this study did not coincide with those in our study, where HDL was decreased.

Alternate-day fasting trials for periods lasting from3 to 12 weeks appear to be effective for reducing total cholesterol (≈10%-21%), and triglycerides (≈14%-42%) in normal-weight, overweight, and obese humans. This result could also be verified in the results of our study. Whole-day fasting trials lasting from 12 to 24 weeks also favourably improve blood lipids (≈5%-20% reduction in total cholesterol and ≈17%-50% reduction in triglycerides) (23).

Abdelaal et al. (24)compared liver function test serum values before and after Ramadan in 216 cirrhotic patients and reported a decrease in ALT and AST. Although they studied cirrhotic patients, their results were similar to those in the present study. In the Nasiri et al. study (8) serum albumin only increased significantly one month after Ramadan ($p<0.05$). Once more, results were in accordance to those in our study, where serum albumin increased after fasting and non-fasting, although not significantly ($p>0.05$). Mild changes in liver function tests may be related to changes in cytokines and alteration in circadian rhythms of hormones during fasting days (25).

The pituitary gland secretes TSH, which stimulates the release of T4 and T3 (26). It has been suggested that the prime effect of fasting might be a reduction of thyroidal secretion of T4 and T3, resulting from reduced stimulation by TSH (27). This evidence is shown in several studies, such as the one by Boelen et al. (27) were a fasting period of 24 hours in mice decreased serum T4 and T3 concentrations, or in the Vries et al. (2014) (10) study were fasting also decreased serum T3 and T4 concentrations. In the Vries et al. comparative study (28), they characterized hepatic thyroid hormone metabolism in two models for caloric restriction: 36h of complete fasting and 21 days of 50% food restriction. Both fasting and food restriction decreased serum T4 concentration, while after 36-h fasting serum T3 also decreased. Fasting decreased hepatic T3 but not T4 concentrations, while food restriction decreased both hepatic T3 and T4 concentrations. However, somehow, we obtained some contradictory results regarding T4 concentration, as it increased instead of decreasing as the evidence suggests.

The observed decrease in serum thyroid hormone concentrations results to some extent from diminished thyroidal secretion of thyroid hormones. The overall result of these complex hypothalamus-pituitary-thyroid axis changes in various tissues during fasting is downregulation of the hypothalamus-pituitary-thyroid axis, which is assumed to represent an energy-saving mechanism, instrumental in times of food shortage (27).

An unexpected increase in insulin in the non-fast condition or in T4 and albumin after fasting in both semi-fast and hypocaloric Mediterranean Diet intervention groups was observed. This, in turn, implies that it is hard to interpret the estimation results. Usually fasting state is associated to a sudden improvement of metabolic parameters.

Widely considered to be the best tests of clinical efficacy and safety, the randomized, placebo-, or standard-controlled clinical trial has a rigorous design that is engineered to balance both observed and unmeasured confounders between the intervention and control arms, which makes any resulting difference observed between the 2 trial arms a causal result of the intervention. Whereas this may be the optimal approach for studying the effects of fasting, no such randomized clinical trial has been performed (29). In addition, studies of fasting regimens have not been performed in children, the very elderly and underweight individuals, and it is possible that IF and PF would be harmful to these populations. Fasting periods lasting longer than 24 hours and particularly those lasting 3 or more days should be done under the supervision of a physician and preferably in a clinic (1).

5. Conclusions

Studies have documented well-established and replicable effects of fasting on health indicators including greater insulin sensitivity, and reduced levels of blood pressure, body fat, IGF-I, insulin, glucose, atherogenic lipids and inflammation, which have also been proven in this study.

Based on the existing evidence from animal and human studies described, we conclude that there is great potential for lifestyles that incorporate periodic fasting during adult life to promote optimal health and reduce the risk of many chronic diseases, particularly for those who are overweight and sedentary.

Intermittent fasting and periodic fasting-based approaches towards treating the current epidemics of overweight, diabetes and related diseases should be pursued in human research studies and medical treatment plans.

The lack of the evaluation of IGF-1 binding proteins posed a limitation in this study, taking into account the relevance of IGF binding proteins to modulate IGF activity and bioavailability in different settings, including caloric restriction. In addition, the use of the bioimpedance using a TANITA device does not represent a gold standard for the definition of fat mass.

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