



Trabajo Original

Obesidad y síndrome metabólico

Does increasing meal frequency improve weight loss and some biochemical parameters in overweight/obese females?

¿El aumento de la frecuencia de las comidas mejora la pérdida de peso y ciertos parámetros bioquímicos en mujeres con exceso de peso/obesidad?

Hilal Yildiran¹ and Seyit Mehmet Mercanligil²

¹Department of Nutrition and Dietetics. Faculty of Health Sciences. Gazi University. Besevler, Ankara. Turkey. ²Faculty of Health Sciences. Uluslararası Kıbrıs University. Lefkoşa, Gazimağusa, Turkish Republic of Northern Cyprus

Abstract

Introduction: despite the positive effects of frequent meals on obesity treatment, there have been no definite conclusions on the matter.

Objective: the aim of this study is to determine the effects of different meal frequencies on weight loss, body composition and some biochemical parameters of overweight or obese females.

Methods: sixty-five adult overweight or obese females were recruited from the Endocrine Department of Ankara Gülhane Education and Research Hospital. Individualistic weight-loss diet programs were implemented (three meals/day for one group and six meals/day for the other group) with a three-month follow-up. Anthropometric measurements and 24-hour dietary records were taken for each week during the study period. Some biochemical parameters (lipid profile, fasting blood glucose, fasting insulin) were analyzed at the beginning and at the end of the study.

Results: forty-three participants finished the study period. Body weight, body mass index, fat mass (kg), fat mass percentage (%), and waist circumference (cm) decreased significantly in both groups ($p < 0.05$), while fat free mass (kg) and body water (l) did not change significantly ($p > 0.05$). Only serum fasting insulin levels decreased significantly in the six meals/day group ($p < 0.05$). Whatever the differences between the initial and final values of body weight, body composition, and biochemical parameters, they were similar between the groups ($p > 0.05$). Only the decrease in fasting insulin levels in the six-meal group was found higher than that of the three-meal group.

Conclusion: in conclusion, body weight, body composition, and lipid profiling are not affected by the number of meals when weight-loss diets are prepared with adequate energy restrictions and sufficient and balanced nutrition.

Key words:

Obesity. Food frequency. Body weight. Fat mass. Serum cholesterol. Insulin.

Resumen

Introducción: a pesar de los efectos positivos de realizar comidas frecuentes en el tratamiento de la obesidad, no existen conclusiones definitivas al respecto.

Objetivo: el objetivo de este estudio es determinar los efectos de diferentes frecuencias de comidas en la pérdida de peso, la composición corporal y algunos parámetros bioquímicos en mujeres con sobrepeso u obesas.

Métodos: sesenta y cinco adultas con sobrepeso u obesidad fueron reclutadas procedentes del Departamento de Endocrinología del Hospital de Educación e Investigación de Ankara Gülhane. Se implementaron programas individuales de dieta para perder peso: tres comidas/día para un grupo y seis comidas/día para el otro grupo, con un seguimiento de tres meses. Se tomaron medidas antropométricas y registros dietéticos de 24 horas cada semana durante el periodo de estudio y se analizaron algunos parámetros bioquímicos (perfil lipídico, glucemia en ayunas, insulina en ayunas) al comienzo y al final del estudio.

Resultados: cuarenta y tres participantes terminaron el periodo de estudio. El peso corporal, el índice de masa corporal, la masa grasa (kg), el porcentaje de masa grasa (%) y la circunferencia de la cintura (cm) disminuyeron significativamente en ambos grupos ($p < 0,05$), mientras que la masa libre de grasa (kg) y el agua corporal (l) no cambiaron significativamente ($p > 0,05$). Solo los niveles séricos de insulina en ayunas disminuyeron significativamente en el grupo de seis comidas/día ($p < 0,05$). Cualesquiera que sean las diferencias entre los valores inicial y final del peso corporal, la composición corporal y los parámetros bioquímicos fueron similares entre los grupos ($p > 0,05$). Solo la disminución de los niveles de insulina en ayunas en el grupo de seis comidas fue más alta que en el grupo de tres comidas.

Conclusión: en conclusión, el peso corporal, la composición corporal y el perfil lipídico no se ven afectados por la cantidad de comidas cuando las dietas para perder peso se preparan con las restricciones de energía adecuadas y una nutrición suficiente y equilibrada.

Palabras clave:

Obesidad. Frecuencia de comida. Peso corporal. Masa grasa. Colesterol sérico. Insulina.

Received: 10/07/2018 • Accepted: 01/11/2018

Yildiran H, Mercanligil SM. Does increasing meal frequency improve weight loss and some biochemical parameters in overweight/obese females? Nutr Hosp 2019;36(1):66-72

DOI: <http://dx.doi.org/10.20960/nh.2191>

Correspondence:

Hilal Yildiran. Department of Nutrition and Dietetics. Faculty of Health Sciences. Gazi University. Besevler, Ankara. Turkey
e-mail: ciftcihilal@hotmail.com

INTRODUCTION

Obesity is weight gain above desirable levels as a result of increased body fat/lean mass ratio (1,2). While genetic and environmental factors play an important role in the advancement of obesity, more energy intake than energy expenditure for extended periods remains the most important cause of obesity (1). Full recovery from obesity is rare, and regaining weight is common after weight loss (3).

In overweight/obese people, morbidity and mortality risks vary according to body fat amount and distribution (1). A 20% rise in body weight above desired levels increases the risk of coronary heart disease, hyperlipidemia, and type 2 diabetes (1,2). According to World Health Organization (WHO) estimates, there are over 650 million obese and over 1.9 billion overweight people in the world over 18 years of age (4). The main objectives of obesity treatments are to decrease body weight to ideal levels and to maintain these levels (2). When treating obesity, weight-loss diet programs are implemented, and it is recommended that much better weight loss can be supported by generally increasing meal frequency (6). The positive effects of frequent food consumption in weight-loss diets can be categorized as follows: rare and frequent eating prevents overeating and unplanned eating episodes, reduces the amount of food taken in the next meal by preventing hunger, and the thermogenic effect of foods (TEF) after each meal increases energy expenditure (6). Many obese individuals have also pointed out that they feel good when they skip meals, but in practice it has also been seen that skipping meals leads to more energy being consumed later in the same day (7). The relationship between meal frequency and body weight was first investigated in 1964, and an inverse relationship was found between the two factors in adult males (8). After this study, further research on the effects of meal frequency on weight loss and health parameters has revealed conflicting findings (9-13). Some short-term clinical investigations have also indicated that frequent food consumption may reduce fasting plasma cholesterol, low-density lipoprotein cholesterol (LDL-C), and insulin concentrations (14,15). Although the responsible mechanisms for these effects of meal frequency have not been determined clearly (16), it was thought that an increase in meal frequency may improve lipid metabolism by regulating carbohydrate metabolism via higher insulin sensitivity (17).

Despite the positive effects of frequent meals on obesity, there have been no definite conclusions on the matter. According to the Dietary Guidelines Advisory Committee Report, in 2010 there was a need to clarify the relationship between meal frequency and health, and controlled studies were vital to this goal (18). With this background, the objective of the present study is to estimate the effects of meal frequency (three meals/day or six meals/day) in a three-month medical nutrition therapy on weight loss, body composition, fasting serum glucose, insulin levels, and lipid profiles in overweight or obese females between 20 and 49 years old.

METHODS AND PROCEDURES

Sixty-five female volunteers, aged 20 to 49 years old, were included in this study. Subjects' BMIs were higher than or equal

to 27 kg/m² with no chronic disease except hyperlipidemia. The participants were chosen from the Endocrine Department of the Ankara Gülhane Education and Research Hospital and were monitored for three months. A total of 18 participants were excluded from the study because: a) ten participants did not report weekly; b) four participants did not follow the planned medical nutrition therapy; c) two participants had menstrual irregularities; d) one participant had health problems; and e) one participant did not match with those in the other group. An interview questionnaire was filled out by the researchers using participants' responses to collect general information. Participants' anthropometric measurements and body composition analyses were performed at the beginning of the study and every week during the following three months.

The weight measurements of the participants were taken in the morning while they were in a fasted state and wearing light clothes and no shoes. A portable scale was used to measure body weight to the nearest half-kilogram. Height was measured to the nearest 0.1 cm with a wall-mounted stadiometer, with the feet together and the head in the Frankfort plane. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m²). Waist circumference (WC) was measured above the iliac crest and below the lowest rib margin at minimum respiration and with a flexible tape (19).

Body composition measurements (fat mass [FM, kg/%], fat free mass [FFM, kg/%], and body water [BW, l/%]) were estimated through bioelectrical impedance analysis (BIA) with Bodystat® 1500. Each participant provided the conditions necessary for BIA. These conditions were as follows: no heavy physical activity for 24-48 hours, no alcohol intake for 24 hours, no food and water intake for at least 2-4 hours, and no tea, coffee, cola and smoking for at least four hours (20).

At the beginning of the study, a 24-hour "physical activity diary" was taken from the participants, and physical activity levels (PAL) were determined from these records. Energy requirements were calculated by multiplying BMH (Harris Benedict formula) by PAL (21). Energy values of weight-loss diet programs were calculated by a 10% reduction in energy requirements. The carbohydrate, protein, and fat ratio interval of diets were 55-60%, 12-15%, and 25-30%, respectively. After determining the nutritional programs of participants, they were divided into two groups, and diet programs were applied to one group at three meals/day (three main meals: breakfast, lunch, and dinner; n = 23) and six meals/day (three main meals + three snacks; n = 24) for the other group, and were followed up for three months. Energy and macronutrient compositions of the weight-loss diet programs are shown in table I. It has been noted that age, gender, and BMI values of the participants in the two groups should be as similar as possible, and the participants were asked not to change their physical activity levels during the study period.

During the three-month follow-up period, 24-hour dietary records were taken weekly to check the participants' adherence to their diet programs, and it was noticed that the food consumption record each week should be in consecutive days. All dietary records were evaluated using the BeBiS 5 (Nutrition Information System) Program, and dietary energy and macronutrient intake were calculated (22).

Table I. Energy and macronutrient composition of weight loss programs

	3 meals/d group (n: 23) $\bar{x} \pm SD$	6 meals/d group (n: 24) $\bar{x} \pm SD$	p*
Energy (kcal)	1,502.8 \pm 122.5	1,552.6 \pm 116.5	0.234
Protein (%)	14.8 \pm 1.1	14.9 \pm 1.7	0.431
Lipid (%)	27.7 \pm 2.3	26.8 \pm 2.5	0.176
Carbohydrate (%)	57.6 \pm 2.0	58.3 \pm 2.7	0.176

*Independent samples t-test.

Early-morning venous blood samples were obtained from each participant for biochemical screening tests following a 12-hour overnight fast. Lipid profiles, including total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), LDL-C, very low-density lipoprotein cholesterol (VLDL-C), and triglycerides (TG), were analyzed. Professional staff performed venipuncture using vacutainers to obtain 15 ml of whole blood. Blood was then centrifuged for plasma separation at the Hacettepe University Hospital, where the actual biochemical analyses were performed. Roche diagnostic kits were used for TG, HDL-C, and TC analysis. LDL-C was calculated using the formula reported by Friedewald et al.: LDL-C = TC - (HDL-C + [TG/5]). For fasting glucose analysis, the enzymatic UV test was used and insulin levels were assessed through an immunological test (23). The difference between the initial and final values of biochemical parameters was then determined.

This study was found medically appropriate by the Ethics Committee of the Ankara Gülhane Education and Research Hospital (decision number: 1491-632-08/1539) and each participant read out and signed consent forms.

STATISTICAL ANALYSES

All values were reported as the mean (\bar{x}) \pm standard deviation (SD). The data were analyzed using the Statistical Package for Social Sciences Software for Windows 13.0 (SPSS Inc. Chicago, IL, USA). The Kolmogorov-Smirnov test determined whether outcome variables were normally distributed. Normally distributed variables were presented as the mean (\bar{x}) and standard deviation (SD) and were compared using the independent samples t-test; non-normally distributed variables were presented as the median and inter-quartile range (IQR) and were compared using the Mann-Whitney U test. Paired-sample t-test was used to evaluate the repeated measurement data. In all analyses, 5% and 1% significance levels were used.

RESULTS

Although 65 people participated in this study, only 47 of them could complete the study period. At the beginning, the meal

frequency groups were found similar in age, anthropometric measurements, body composition, and biochemical parameters ($p > 0.05$). (Table II).

The initial and final values of anthropometric measurements, body composition, and biochemical parameters are shown in table III. According to initial values, each participant's body weight, BMI (kg/m^2), FM (kg), FM%, and WC (cm) showed a significant decrease, and FFM% increased at the end of the study ($p < 0.05$). BW% increased significantly only in the three meals/day group ($p < 0.05$). FFM (kg) and BW (l) did not change significantly at the end of the study in both groups ($p > 0.05$). When changes in biochemical parameters were examined, only serum FI levels in the six meals/day group decreased significantly ($p < 0.05$), and no significant change was found in other parameters (Table III).

Difference values were calculated by subtracting the final values from the initial values at the end of the study for all parameters (Table IV). Except for FI, changes in all parameters were similar between the meal frequency groups. The reduction in serum FI was significantly higher in the six meals/day group (2.52 ± 1.8 mikrolU/dl) than in the three meals/day group (0.81 ± 1.8 mikrolU/dl) ($p < 0.05$). The average weight-loss percentage is also shown in table IV. While par-

Table II. Age and initial values of anthropometric measurements, body composition and biochemical parameters

	3 meals/d group (n: 23) $\bar{x} \pm SD$	6 meals/d group (n: 24) $\bar{x} \pm SD$	p*
Age	35.18 \pm 5.23	35.25 \pm 4.43	0.449
Height (cm)	156.6 \pm 5.5	165.8 \pm 4.4	0.054
Weight (kg)	86.2 \pm 10.5	94.8 \pm 14.6	0.214
BMI (kg/m^2)	35.2 \pm 4.9	34.2 \pm 4.6	0.930
FM (kg)	39.0 \pm 8.2	41.4 \pm 10.1	0.555
FM%	45.0 \pm 4.9	43.0 \pm 4.1	0.414
FFM (kg)	47.2 \pm 4.8	54.1 \pm 5.3	0.496
FFM%	55.0 \pm 4.9	56.3 \pm 5.6	0.826
BW%	40.3 \pm 2.7	40.7 \pm 2.5	0.763
BW (l)	34.6 \pm 3.8	38.2 \pm 4.5	0.316
TG (mg/dl)	154.7 \pm 53.5	131.0 \pm 99.9	0.353
TC (mg/dl)	217.3 \pm 41.7	198.2 \pm 40.2	0.869
LDL-C (mg/dl)	136.1 \pm 36.6	127.7 \pm 31.5	0.541
HDL-C (mg/dl)	50.4 \pm 10.2	50.4 \pm 7.9	0.187
VLDL-C (mg/dl)	30.8 \pm 10.7	20.5 \pm 8.4	0.299
FBG (mg/dl)	89.7 \pm 6.6	91.1 \pm 7.4	0.359
FI (mikrolU/ml)	11.2 \pm 3.6	13.7 \pm 3.9	0.527

*Independent samples t-test. BMI: body mass index; BMH: basal metabolic rate; FM: fat mass; FFM: fat free mass; BW: body water; TG: triglycerides; TC: total cholesterol; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; VLDL-C: very low density lipoprotein cholesterol; FBG: fasting blood glucose; FI: fasting insulin.

Participants in the three meals/day group lost $6.77 \pm 2.68\%$ of their weight, participants in the six meals/day group lost $5.17 \pm 2.78\%$ of

their weight, and the weight-loss percentages were similar between the groups ($p > 0.05$).

Table III. The initial and final values of anthropometric measurements, body composition and biochemical parameters

	3 meals/d group (n: 23)			6 meals/d group (n: 24)		
	Initial $\bar{x} \pm SD$	Final $\bar{x} \pm SD$	p^\dagger	Initial $\bar{x} \pm SD$	Final $\bar{x} \pm SD$	p^\dagger
Body weight (kg)	86.2 ± 10.5	80.0 ± 9.5	0.000*	94.8 ± 14.6	89.8 ± 13.5	0.000*
BMI (kg/m ²)	35.2 ± 4.9	32.7 ± 4.3	0.000*	34.2 ± 4.6	32.6 ± 4.4	0.001*
FM (kg)	39.0 ± 8.2	33.3 ± 7.9	0.000*	41.4 ± 10.1	36.1 ± 9.1	0.000*
FM (%)	45.0 ± 4.9	41.2 ± 5.4	0.000*	43.0 ± 4.1	39.7 ± 4.1	0.003*
FFM (kg)	47.2 ± 4.8	46.7 ± 3.5	1.000	54.1 ± 5.3	53.7 ± 5.1	1.000
FFM (%)	55.0 ± 4.9	58.8 ± 5.3	0.000*	56.3 ± 5.6	60.2 ± 4.1	0.002*
BW (%)	40.3 ± 2.7	43.2 ± 3.3	0.004*	40.7 ± 2.5	42.7 ± 2.7	0.111
BW (l)	34.6 ± 3.8	34.4 ± 2.7	1.000	38.2 ± 4.5	38.1 ± 4.8	1.000
WC (cm)	101.0 ± 10.7	93.2 ± 9.3	0.001*	102.0 ± 7.8	95.9 ± 7.9	0.000*
TG (mg/dl)	154.7 ± 53.5	138.5 ± 57.6	0.198	131.0 ± 99.9	115.6 ± 60.5	0.371
TC (mg/dl)	217.3 ± 41.7	196.2 ± 38.2	0.107	198.2 ± 40.2	188.5 ± 26.8	0.101
LDL-C (mg/dl)	136.1 ± 36.6	119.2 ± 31.9	0.122	127.7 ± 31.5	117.2 ± 20.9	0.089
HDL-C (mg/dl)	50.4 ± 10.2	48.6 ± 8.8	0.254	50.4 ± 7.9	48.6 ± 5.3	0.287
VLDL-C (mg/dl)	30.8 ± 10.7	28.4 ± 11.1	0.344	20.5 ± 8.4	22.6 ± 9.9	0.077
FBG (mg/dl)	89.7 ± 6.6	89.6 ± 8.7	0.977	91.1 ± 7.4	89.3 ± 11.0	0.505

* $p < 0.05$. [†]Paired-sample t-test. BMI: body mass index; FM: fat mass; FFM: fat free mass; BW: body water; WC: waist circumference; TG: triglycerides; TC: total cholesterol; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; VLDL-C: very low density lipoprotein cholesterol; FBG: fasting blood glucose; FI: fasting insulin.

Table IV. The differences of anthropometric measurements and body composition parameters

		3 meals/d group (n: 23)	6 meals/d group (n: 24)	p
Weight difference (kg)	$\bar{x} \pm SD$	5.91 ± 2.64	4.97 ± 2.70	0.411 [†]
Weight difference (%)	$\bar{x} \pm SD$	6.77 ± 2.68	5.17 ± 2.78	0.366 [†]
BMI difference (kg/m ²)	$\bar{x} \pm SD$	2.37 ± 1.22	1.70 ± 0.97	0.157 [†]
FM difference (kg)	$\bar{x} \pm SD$	5.69 ± 1.99	5.22 ± 2.58	0.630 [†]
FFM difference (kg)	$\bar{x} \pm SD$	0.44 ± 1.89	0.40 ± 3.37	0.518 [†]
BW difference (l)	$\bar{x} \pm SD$	0.01 ± 1.77	0.75 ± 2.59	0.267 [†]
WC difference (cm)	$\bar{x} \pm SD$	7.73 ± 4.47	6.17 ± 3.32	0.455 [†]
TG difference (mg/dl)	median (IQR)	3.00 (-11.00-38.00)	-0.50 (-6.00-21.75)	0.566 [‡]
TC difference (mg/dl)	$\bar{x} \pm SD$	21.1 ± 40.6	10.4 ± 20.2	0.418 [†]
LDL-C difference (mg/dl)	$\bar{x} \pm SD$	16.9 ± 33.2	10.5 ± 19.5	0.586 [†]
HDL-C difference (mg/dl)	$\bar{x} \pm SD$	1.7 ± 4.7	2.0 ± 6.2	0.907 [†]
VLDL-C difference (mg/dl)	median (IQR)	-1.00 (-4.00-8.00)	-1.00 (-4.00-0.25)	0.413 [‡]
FBG difference (mg/dl)	$\bar{x} \pm SD$	0.1 ± 10.1	1.8 ± 8.8	0.678 [†]
FI difference (mikroIU/dl)	median (IQR)	0.90 (0.35-1.60)	2.04 (1.46-2.69)	0.013 ^{**}

* $p < 0.05$. [†]Independent samples t-test. [‡]Mann Whitney U test. BMI: body mass index; FM: fat mass; FFM: fat free mass; BW: body water; WC: waist circumference; TG: triglycerides; TC: total cholesterol; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; VLDL-C: very low density lipoprotein cholesterol; FBG: fasting blood glucose; FI: fasting insulin.

DISCUSSION

Meal frequency is known as an important factor in regulating energy balance in humans, and notwithstanding the diet's total energy content, meal frequency can affect energy expenditure. It is important to know the effect of meal frequency on energy balance in weight-loss programs (14,15). The main effect of increasing meal frequency on weight loss is that it boosts energy expenditure by amplifying the thermic effect of food (TEF), thus supporting weight loss (24,25). For example, Le Blanc et al. (25) found that consuming the same amount of calories in four small meals created a higher TEF than in one large meal ($p < 0.06$) in six normal-weight adults.

Even if it is known that more frequent meals have a positive effect on the treatment of obesity (26), results of some studies state that reducing meal frequency had more beneficial effects (27,28). In one human study, a 750-calorie test meal was consumed by subjects in one meal/day or six equal meals/day after night-long hunger in two different days, and the TEF of one meal/day was found higher than that of six meals/day ($p < 0.05$). The total difference between the TEF of the two different diet models reached 48 calories at the end of the day. The study emphasized that when meal frequency reduced gastric distension, food left the stomach faster through increasing the food intake in one meal, and this situation resulted in increased TEF. As a result, reducing meal frequency for extended periods could lead to weight reduction through higher TEF (27). In addition, large meals might be expected to be associated with greater heat production through the activation of the sympathetic nervous system (29). However, the sympathetic stimulation that acutely affects BTE is unknown, and there is also the opinion that BTE is influenced more by catecholamines than by the sympathetic nervous system (27). In a study conducted on these relationships, effects of meal frequency on energy expenditure were studied in the Netherlands on ten normal-weight, healthy adult men. A balanced-energy diet was consumed by participants in a week in two meals/day and seven meals/day for the following week. The 24-hour energy expenditure of participants was measured through oxygen consumption and carbon dioxide production. At the end of the study, TEF markedly increased in the two meals/day week (30).

Although TEF was not measured in this study, it was expected that different meal frequencies would produce different TEFs, resulting in changes in the examined parameters. Because participants in the two groups were matched by age and BMI (Table II), similar energy restrictions were enforced, the same macronutrient compositions were prepared (Table I), and individuals were not required to change their physical activity throughout the study. However, at the end of the study, different meal frequencies (three meals/day or six meals/day) did not significantly affect weight loss and FM loss in overweight or obese adults ($p < 0.05$) (Tables III and IV). The results of few studies were in parallel with the current one, and they advocated that TEF was not affected by meal frequency (31,32). In one of the these studies, TEF was calculated by measuring oxygen consumption, and it was found

that TEF values at the end of diets with two- or four-meal frequency were not different (31). In another study, the same energy diets were given to rats in two meals or 10-12 meals, and it was observed that the daily energy expenditure of the two groups were similar (32). According to these results, if energy-restricted weight-loss diets for obesity treatment were prepared using adequate and balanced nutrition criteria, three or six meals a day do not differ significantly on weight and fat mass loss.

Besides the hypothesis that meal frequency may affect TEF, it is known that TEF decreases by about 6% every ten years between the ages of 22 and 74 (33), and Granata and Brandon (34) suggested the theory that TEF is reduced in obesity. A study conducted by Nelson et al. (35) found that BTE was lower in obese subjects than in normal-weight subjects.

In addition, in many studies, the effect of meal frequency on TEF was examined on normal-weight participants, and TEF was monitored for short periods ignoring energy intake and physical activity (27,30,32). In this study, meal frequency effects were investigated on overweight or obese participants for three months, and it is the most important differential factor of our study compared to others.

Lipid profile is another factor which can be affected by meal frequency. Studies about the relation between meal frequency and lipid profile have conflicting results. In one of these studies, the effects of meal frequency on plasma lipid and carbohydrate metabolism were investigated in 19 healthy, normocholesterolemic, free-living men and women who consumed their usual diet of three or nine meals per day randomly for two weeks each. Compared with the three meals/day diet, the nine meals/day reduced plasma TC, LDL-C, and HDL-C by 6.5% ($p < 0.005$), 8.1% ($p < 0.005$), and 4.1% ($p < 0.05$), respectively. Body weight and fasting triglycerides were not different for the two diets (36). Similarly, in another study, the hypothesis that meal frequency is associated with plasma cholesterol was tested in a population-based sample of 2,034 white men and women aged 50-89. TC, LDL-C, HDL-C, and TG were measured after a 12-hour fast. The age-adjusted TC concentrations for men and women reporting ≥ 4 meals/day averaged 0.23 mmol/l lower than for those who reported 1-2 meals/day ($p = 0.01$). Similarly, LDL-C concentrations were lower in those reporting higher meal frequency (0.16 mmol/l, $p = 0.06$) (37). The results of the two studies suggest that cholesterol reduction might be achieved by modest increases in meal frequency without increasing caloric intake. On the contrary, some studies done on Muslims during Ramadan showed that decreasing meal frequency during this month revealed several positive effects on health and disease risk, including decreased LDL-C concentrations (38,39). However, in these studies, the mechanism between meal frequency and LDL-C concentrations was not explained clearly. Meanwhile, our study's results indicated that meal frequency in weight-reducing diets did not affect TG, TC, HDL-C, LDL-C, and VLDL-C levels in overweight or obese females (Tables III and IV).

Although our study found that decreased meal frequency was not related to lipid profile, hormonal metabolism may explain this relation in an exactly opposite direction. Carbohydrate metab-

olism plays a central role in the effects of meal frequency on serum cholesterol concentrations. Insulin is a hormone that depresses lipolysis and stimulates lipoprotein lipase activity. Given the correspondence between plasma insulin concentrations and cholesterol synthesis, it is possible that increased meal frequency decreases body cholesterol pool sizes through lowered insulin levels (37). The possible mechanism underlying this effect of increased meal frequency on insulin levels is that energy-restricted diets with high meal frequency stimulates insulin release less by reducing the amount of food consumed at a time (27). Similarly in our study, insulin levels decreased in both groups, but this was significant only in the six meals/day group ($p < 0.05$) (Table III), and the decline is higher in the six meals/day group compared to the three meals/day group (Table IV). In contrast, especially in rat studies, intermittent fasting showed more positive effects on insulin sensitivity than frequent-meal models (11,12). This situation is explained by the effect of brain-derived neurotrophic factor (BDNF), which is produced in the brain and may be affected by meal frequency. Recent findings indicate that decreasing meal frequency at intermittent-fasting levels increases BDNF levels. In the brain, the BDNF signal is effective in controlling peripheral glucose metabolism and, at a sufficient level, provides lower blood glucose levels and greater insulin sensitivity (40).

CONCLUSION

In medical nutrition therapy of obesity, the general recommendation is that meal frequency should be higher than three, and with a maximum of 5-6 hours in between meals. But in this study, it was found that if the medical nutrition therapy for overweight/obesity was prepared according to adequate and balanced nutrition guidelines, meal frequency did not have any effects on weight loss, body composition, and some biochemical parameters, be it three meals/day or six meals/day. In line with these results, weight-loss programs may be adjusted to the traditional three-meal schedule, and only those with snacking habits may need to eat more frequently. However, for more definitive recommendations, these results have to be supported by further studies.

REFERENCES

- Sørensen TIA, Virtue S, Vidal-Puig A. Obesity as a clinical and public health problem: is there a need for a new definition based on lipotoxicity effects? *Biochim Biophys Acta* 2010;1801:400-4.
- Friedman JM. Obesity causes and control of excess body fat. *Nature* 2009;459:340-2.
- Jeffery RF. Population perspectives on the prevention and treatment of obesity in minority populations. *Am J Clin Nutr* 1991;53(6 Suppl):1621S-4S.
- World Health Organization (WHO). The challenge of obesity in the WHO European region and the strategies for response summary. Denmark: WHO; 2007.
- Stote KS, Baer DJ, Spears K, Paul DR, Harris GK, Rumpel WV, et al. A controlled trial of reduced meal frequency without caloric restriction in healthy, normal-weight, middle-aged adults. *Am J Clin Nutr* 2007;85:981-8.
- Laquarta, I. Nutrition for weight management. In: Mahan KL, Escott-Stump S (eds.). *Krause's food. Nutrition & diet therapy*. 11th ed. Philadelphia: Saunders Company; 2004. p. 558-93.
- Sherwood NE, Story M, Neumark-Sztainer D. Behavioral risk factors for obesity: diet and physical activity. In: Coulston AM, Rock CL, Monsen ER (eds.). *Nutrition in the prevention and treatment of disease*. London: Academic Press; 2001. p. 527.
- Bellisle F, McDevitt R, Prentice AM. Meal frequency and energy balance. *Br J Nutr* 1997;77(Suppl.1):57-70.
- Mattson MP. The need for controlled studies of the effect of meal frequency on health. *Lancet* 2005;36:1978-80.
- Carlson O, Bronwen M, Stote KS, Golden E, Maudsley S, Najjar S, et al. Impact of reduced meal frequency without caloric restriction on glucose regulation in healthy. Normal-weight middle aged men and women. *Metab Clin Exp* 2007;56:1729-34.
- Kinobo JL. Effect of meal frequency on the thermic effect on food in women. *Eur J Clin Nutr* 1994;44(5):389-95.
- Garrow JS, Durrant M, Blaza S, Wilkins D, Royston P, Sunkin S. The effect of meal frequency and protein and protein concentration on the composition of weight loss by obese subjects. *Br J Nutr* 1980;45:5-15.
- Westerterp-Plantenga MS, Goris AHC, Mejer EP, Westerterp KS. Habitual meal frequency in relation to resting and activity-induced energy expenditure in human subjects: the role of fat-free mass. *Br J Nutr* 2003;90:643-9.
- Gwinup G, Byron RC, Roush WH, Kruger FA, Hamwi GI. Effect of nibbling versus gorging on serum lipids in man. *Am J Clin Nutr* 1963;13:209-13.
- Jagannathan SN, Connell WF, Beveridge IMR. Effects of gormandizing and semicontinuous eating of equicaloric amounts of formula type high fat diets on plasma cholesterol and triglyceride levels in human volunteer subjects. *Am J Clin Nutr* 1964;15:90-3.
- Jenkins DJA, Wolever TMS, Vuskan V, Brighen F, Cunnane SC, Venketeshwer AR, et al. Nibbling versus gorging: metabolic advantages of increased meal frequency. *N Engl J Med* 1989;321:929-34.
- Speezy DP, Buffenstein R. Greater appetite control associated with increased frequency of eating in lean males. *Appetite* 1999;33:285-97.
- Myers EF, Khoo CS, Murphy W, Steiber A, Agarwal S. A critical assessment of research needs identified by the Dietary Guidelines Committees from 1980 to 2010. *J Acad Nutr Diet* 2013;113(7):957-71.
- Gordon CC, Chumlea WC, Roche AF. Measurement descriptions and techniques. In: Lohman TG, Roche AF, Martorell R (eds.). *Anthropometric standardization reference manual*. 1st ed. Champaign, IL: Human Kinetics Books; 1998. p. 3-12.
- Lee R, Nieman D. *Biochemical assesment of nutritional status*. 3rd ed. New York: McGraw-Hill; 2003.
- World Health Organization (WHO). Human energy requirements. Report of a Joint FAO/WHO/UNU Expert Consultation. Rome, 17-24 October 2001. Rome: FAO Food and Nutrition Technical Report Series 1; 2004.
- Bebis Nutrition Data Base Software Data Base. The German Food Code and Nutrient Data Base (BLS II.3, 1999) with additions from USDA-sr and other sources, Istanbul, Turkey; 2004.
- Friedewald WT, Levy RI, Fedickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of preparative ultracentrifuge. *Clin Chem* 1972;18:499-502.
- Johnson RK, Coward-McKenze D. Energy requirement methodology. In: Coulston AM, Rock CL, Monsen ER (eds.). *Nutrition in the prevention and treatment of disease*. London: Academic Press; 2001. p. 34.
- LeBlanc J, Mercier I, Nadeau A. Components of postprandial thermogenesis in relation to meal frequency in humans. *Can J Physiol Pharmacol* 1993;71:879-83.
- Westerterp KR. Diet induced thermogenesis. *Nutr Metab* 2004;1:1-5.
- Tai MM, Castillo P, Pi-Sunyer FX. Meal size and frequency: effect on the thermic effect of food. *Am J Clin Nutr* 1991;54:783-7.
- Sohal RS, Weindruch R. Oxidative stress, caloric restriction, and aging. *Science* 1996;273:59-63.
- Jhuier E. Caloric balance versus nutrient balance. In: Kinney JM, Tucker HN (eds.). *Energy metabolism: tissue determinants and cellular corollaries*. New York: Raven Press; 1992. p. 123-36.
- Wenne G, Westerterp KS, Kester ADM. Effect of the pattern of food intake on human energy metabolism. *Br J Nutr* 1993;70:103-5.
- Kinobo JL. Effect of meal frequency on the thermic effect on food in women. *Eur J Clin Nutr* 1994;44:389-95.
- Hill JO, Anderson JC, Lin D. Effect of meal frequency on energy utilization in rats. *Am J Physiol* 1988;255:16-21.

33. Anon. Diet induced thermogenesis in healthy man. *Acta Chir Scand* 1990;558(suppl):6-59.
34. Granata GP, Brandon LJ. The thermic effect of food and obesity: discrepant results and methodological variations. *Nutr Rev* 2002; 60(8):223-33.
35. Nelson KN, Weinsler RL, James LD, Darnell B, Hunter G, Long CL. Effect of weight reduction on resting energy expenditure. Substrate utilization and thermic effect of food in moderately obese women. *Am J Clin Nutr* 1992;55:924-33.
36. Arnold LM, Ball MJ, Duncan AW, Mann J. Effect of isoenergetic intake of three or nine meals on plasma lipoproteins and glucose metabolism. *Am J Clin Nutr* 1993;57:446-51.
37. Edelstein SL, Barrett-Connor EL, Wingard DL, Cohn BA. Increased meal frequency associated with decreased cholesterol concentration. Rancho Bernardo, CA, 1984-1987. *Am J Clin Nutr* 1992;55:664-9.
38. Roky R, Houti I, Moussamih S, Qotbi S, Aadil N. Physiological and chronological changes during Ramadan intermittent fasting. *Ann Nutr Metab* 2004;48:296-303.
39. Lamine F, Bouguerra R, Jabrane J, Marrakchi Z, Ben Rayana MC, Ben Slama C. Food intake and high density lipoprotein cholesterol levels changes during Ramadan fasting in healthy young subjects. *Tunis Med* 2006;84(10):647-50.
40. Mattson MP, Wan R. Beneficial effects of intermittent fasting and caloric restriction on the cardiovascular and cerebrovascular systems. *J Nutr Biochem* 2005;16:129-37.