Journal of the American College of Nutrition

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Published online: 08 May 2014.

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Original Research

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Key words: lifestyle, diet, morning, Mediterranean, breakfast, fat loss, body composition, 7-day diet diaries, metabolic Holter

Objective: To evaluate the effects of 2 low-calorie diets but with different distributions of calories throughout the day on weight loss and other major obesity-related metabolic parameters.

Methods: We randomly assigned 42 nonsmoking homemakers (age $= 46.3 \pm 2.3$ years, body mass index [BMI] $= 35.7 \pm 0.8$ kg/m^2, mean $\pm$ SD) in 2 groups of 21 subjects (G1 and G2). The participants underwent a 3 month individualized Mediterranean-style diet (55% carbohydrate, 30% fat, 15% protein and fiber > 30 g), calorie (600 kcal daily deficit compared to the total energy expenditure measured by a metabolic Holter). Diets consisted of the same food and complied with cardiovascular disease prevention guidelines but differed in the distribution of calories throughout the day (G1: 70% breakfast, morning snack, lunch and 30% afternoon snack and dinner; G2: 55 breakfast, morning snack, lunch and 45% afternoon snack and dinner). Dual-energy X-ray absorptiometry was used for pre- and postintervention body composition assessment.

Results: Thirty-six subjects completed the study (G1 $= 18$, G2 $= 18$). Both groups had significant improvements in body composition and metabolic parameters but G1 had enhanced results for weight loss (G1: -8.2 ± 3.0 kg; G2: -6.5 ± 3.4 kg; $p = 0.028$), waist circumference reduction (G1: -7 ± 0.6 cm; G2: -5 ± 0.3 cm; $p = 0.033$), and fat mass loss (G1: -6.8 ± 2.1 kg, G2: -4.5 ± 2.9 kg, $p = 0.031$; mean $\pm$ SD). Improvements were detected in both groups for blood pressure and blood and lipid parameters. G1 subjects showed a greater improvement in insulin sensitivity measured by homeostasis model assessment–estimated insulin resistance (G1: -1.37 ± 0.27, G2: -0.74 ± 0.12, $p = 0.017$).

Conclusions: These data suggest that a low-calorie Mediterranean diet with a higher amount of calories in the first part of the day could establish a greater reduction in fat mass and improved insulin sensitivity than a typical daily diet.

BACKGROUND

It is generally accepted that obesity affects health because of its association with numerous metabolic complications such as dyslipidaemia, type 2 diabetes, and cardiovascular diseases [1]. Unfortunately, prevalence of obesity is increasing worldwide. Reasons include urbanization of the world's population, enhanced availability of food supplies, and reduction in physical activity. Because the improvement in health that occurs with weight loss is, in most cases, lost on weight regain [2], it is crucial to learn how to help people to maintain weight loss over the long term.

A frequent aim of weight management programs that may lead to long-term success in weight maintenance is breakfast eating. Breakfast consumption is associated with lower body mass index (BMI) [3,4] due to lower food cravings and prevention of overeating later [5,6] and differences in subjective appetite, micronutrient intakes [7], insulin sensibility, and lower percentages of calories from fat [8] in a number of cross-sectional studies of adults. Thus, the addition of breakfast leading to brain activation of regions previously associated with food motivation and reward with additional alterations following breakfast [9].

Despite these findings, the incidence of skipping breakfast has increased between 1965 and 1991 from 14% to 25% for US

adults [10]. The relationship between skipping breakfast with overweight and obesity has been also established in children and adolescents in European countries [11,12]. Reasons cited for skipping breakfast include lack of time for preparation and consumption as well as a fear of weight gain [13].

Energy homeostasis is probably influenced by biological and circadian rhythms. There are substantial and important changes in ingestive behavior that occur over the course of the day, but little is known regarding how the timing of nutrient ingestion impacts body weight. By analyzing 7-day diet diaries of 867 free-living individuals using epidemiological studies, De Castro suggested that 150% of energy is commonly ingested in the evening meals compared to morning meals [14]. As the calories of the meal increases during the day, the time before eating again decreases because food intake in the morning is particularly satiating, whereas food intake late at night is not satiating and can lead to greater overall energy intake [15,16]. The satiating property of morning food is due to the expression and secretion of hormones that modulate the secretion of satiety- and hunger-related hormones such as peptide YY, glucagon-like peptide-1, cholecystokinin, and ghrelin.

Speechly and Buffenstein [17] showed that an isocaloric preload meal over the course of the morning as opposed to a single breakfast leads to a significant 26% lower energy intake at a following ad libitum lunch and that splitting breakfast over the morning could help to better regulate cravings and show advantageous effects in weight loss regimens.

We hypothesized that the time of day of food intake is related to total intake. Higher morning ingestion would tend to reduce overall ingestion, whereas ingestion later in the day would tend to increase intake over the entire day. Total daily calories in the morning (morning snack and lunch) may be an effective strategy to reduce intake in the second part (afternoon snack, evening meal, and evening snack), the period when the satiating power of food is lower. If this is true, then a dietary regimen that encourages the ingestion of relatively large amounts of food in the morning and restricts intake during the evening might reduce overall intake and serve as a treatment or preventative measure for obesity.

The purpose of this study was to evaluate metabolic and body composition changes made by a lifestyle intervention in a randomized homogeneous sample of 2 groups of homemakers with equal daily caloric reduction but different caloric distributions in the 2 parts of the day.

SUBJECTS AND METHODS

Subjects

Among individuals attending the outpatient service of the Obesity Centre of the University Hospital "Policlinico Tor Vergata," 42 overweight and obese female homemakers were included in the present study. All subjects were adult white Eu-

ropeans and provided written informed consent to participate. The investigation was conducted in accordance with the Declaration of Helsinki. Exclusion criteria were as follows: age <18 or >65 years; pregnant or nursing; any lifestyle treatment in the year before; alcoholism; diabetes mellitus; chronic kidney disease; glucocorticoid, estrogen, or anticonvulsant therapies; and history of cardiovascular, neoplastic, or other systemic diseases (both chronic and acute). Patients whose alimentary diaries reported daily caloric values < 110% of the Basal Metabolic Rate (BMR) were also excluded.

Study Procedures and Diagnostic Criteria

All participants underwent a comprehensive medical evaluation including clinical history, physical examination, and anthropometric parameters with body composition assessment, blood pressure measurement, and blood sampling. Weight and height were measured after the subjects fasted overnight and wearing only underwear. BMI was calculated as weight (kg) divided by height (m^2). Blood pressure was measured in the seated position with a standard mercury sphygmomanometer on the left arm after at least 10 min of rest. For all parameters the mean values were determined from 2 independent measurements. The total energy expenditure was measured by a multisensory armband (SenseWear Pro2 Armband, Bodymedia Inc., Pittsburgh, PA, USA) worn on the back of the upper right arm that recorded data for 36 hours in a free-living context.

Lifestyle Intervention

All participants were placed on a 3-month hypocaloric nutritionally balanced diet tailored to the individual. The diet was a Mediterranean-style diet. The main features of this diet are as follows: eating primarily plant-based foods, such as fruits and vegetables, whole grains, legumes, and nuts; replacing butter with healthy fats such as olive oil; using herbs and spices instead of salt to flavor foods; limiting red meat to no more than a few times a month; and eating fish and poultry at least twice a week. Other features are energy intake of around 600 kcal less than the total energy expenditure and a macronutrient composition of about 16% proteins, 25% fat, and 59% carbohydrates. Nutritional intakes were divided in 3 main meals and 2 snacks. Nutritional therapies differed in the distribution of calories throughout the day. In group 1 (G1) daily food intake was divided as follows: 70% for breakfast, morning snack, and lunch and 30% for afternoon snack and dinner. In the control group (G2), 55% of total calories was consumed in the first part of the day and 45% was consumed later. Patients were required to complete a 3-day diet diary in the beginning of the study and then weekly throughout the follow-up. Diaries included one weekend day. To avoid underreporting all subjects whose reported intake was <110% of their estimated basal metabolic rate were excluded [18]. Twice a month patients met dieticians for a nutritional rehabilitation program that aimed to improve and promote changes in eating

habits and consisted of individual sessions (dietary assessment, evaluation of nutrient intake and adequacy, nutritional status, anthropometric, eating patterns, readiness to adopt change). A telephone consultation service was also provided for patient support to families and the health care professionals caring for them. On a weekly basis, generally not overlapping with clinical appointments or teaching time, phone counseling with a dietician was scheduled. The goals of the phone call evaluations were to assess the correct daily application of the nutritional plan and determine whether an ambulatory evaluation was necessary. Participants performed a 50-minute low-intensity aerobic exercise 3 days per week on nonconsecutive days. A 7-day diary assessed adherence to the exercise program.

Body Composition Assessment

Body composition was assessed with dual-energy X-ray absorptiometry Briefly, individuals were scanned using a Delphi W scanner (HOLOGIC Co., Bedford, MA) and images were analyzed using Hologic Discovery software (version 12.2, Discovery Software Ltd., Bellingham, WA, USA). Measurements of total body fat, total fat-free mass, and percentage of fat were acquired and analyzed as previously described [19].

Laboratory Data

Blood samples were taken in the morning (between 7:00 AM and 9:00 AM). Measurements of glucose, total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, creatinine, calcium, phosphorus, and fibrinogen concentrations were assessed by standard immune-enzymatic methods. Insulin levels were measured by immunoradiometric assay.

Statistical Analysis

Statistical analysis was performed using SPSS 18.0 software (SPSS Inc., Chicago, IL). Mean $\pm$ SD or median (interquartile range) was used for normally distributed or skewed continuous variables, respectively. All quantitative variables were tested for normality of distribution using the Kolmogorov-Smirnov test. Triglycerides and fasting insulin were logarithmically transformed before being used in the subsequent parametric procedures. Baseline differences in continuous variables between subjects in groups G1 and G2 were assessed with Student's t test for unpaired data. Changes from baseline between groups were tested for significance using a general linear model for continuous variables with respective baseline values included as a covariate.

For all analyses a p value < 0.05 based on 2-sided test was considered statistically significant.

RESULTS

Baseline Data

The final sample was composed of 36 subjects (G1 = 18, G2 = 18). Two patients dropped out due to family reasons.

Table 1. Anthropometric and Main Metabolic Parameters of the Study Subjects, Expressed as Mean $\pm$ SD or Median (interquartile range) as Appropriate

	Group 1 (n = 18)	Group 2 (n = 18)	p Value*
Age (years)	39 ± 17	43 ± 16	0.23
BMI (kg/m^2)	35.8 ± 5.2	35.1 ± 4.5	0.46
Waist (cm)	100 ± 3	100 ± 5	0.37
Total body fat (kg)	40.7 ± 3.5	37.8 ± 3.8	0.18
sBP (mmHg)	132 ± 16	124 ± 11	<0.05
dBP (mmHg)	83 ± 9	77 ± 13	<0.05
Total cholesterol (mg/dl)	198 ± 37	203 ± 41	0.32
HDL cholesterol (mg/dl)	48 ± 12	49 ± 14	0.36
Triglycerides (mg/dl)	117 (101–143)	122 (98–161)	0.24
Fasting glucose (mmol/l)	5.35 ± 0.66	5.12 ± 0.82	0.21
Fasting insulin (μU/ml)	16.2 (12.4–19.1)	17.3 (11.9–19.5)	0.45
Homeostasis model assessment–estimated insulin resistance	4.12 ± 2.62	4.15 ± 2.5	0.47

BMI = body mass index, sBP = systolic blood pressure, dBP = diastolic blood pressure, HDL = high-density lipoprotein.*Differences between groups assessed with Student's t test.

Three subjects were excluded because their reported intakes were <110% of their estimated basal metabolic rate. One patient was excluded due to failure to complete the 7-day diary for assessing observance to the training plan.

The mean age and BMI for group 1 was 39 $\pm$ 17 years and 35.8 $\pm$ 5.2 kg/m^2, respectively. The mean age and BMI for the group 2 were 43 $\pm$ 16 years and 35.1 $\pm$ 4.5 kg/m^2, respectively.

Table 1 provides a comparison of the baseline characteristics of the 2 groups. No significant differences were detected in almost all of the baseline criteria, except for lower blood pressure levels in group G2 than in group G1 (systolic blood pressure 124 $\pm$ 11 vs 132 $\pm$ 16 mmHg; diastolic blood pressure 77 $\pm$ 13 vs 83 $\pm$ 9 mmHg; $p < 0.05$ for both).

Analysis of the diet diaries showed a different distribution of energy throughout the day between groups as expected. Calorie intake was 35% in the morning and 30% in the evening for G1 and 20% and 45% respectively for G2 (Fig. 1). Lunch calories were similar between groups (35% of total daily energy). Macronutrient calories in both groups were distributed as shown in Fig. 2. Carbohydrates provided most of the total caloric intake with low-fat snacks between meals, in order to limit blood sugar levels and help boost energy throughout the day. Table 3 shows energy expenditure and main diet features of both groups.

Anthropometrics and Body Composition

People in group G1 experienced the most weight loss. Subjects in groups G1 and G2 lost on average 8.2 $\pm$ 3.0 kg and 6.5 $\pm$ 3.4 kg ($p = 0.028$), respectively (Table 2 and Fig. 3). Mean BMI change was 3.1 $\pm$ 0.2 kg/m^2 (G1) and 1.8 $\pm$ 0.4 (G2; $p = 0.046$; see Table 2). The reduction in waist circumference (Table 2 and Fig. 4) was also higher in group G1 (G1, −7 $\pm$ 0.6 cm; G2, −5 $\pm$ 0.3 cm, $p = 0.033$). Subjects in group G1
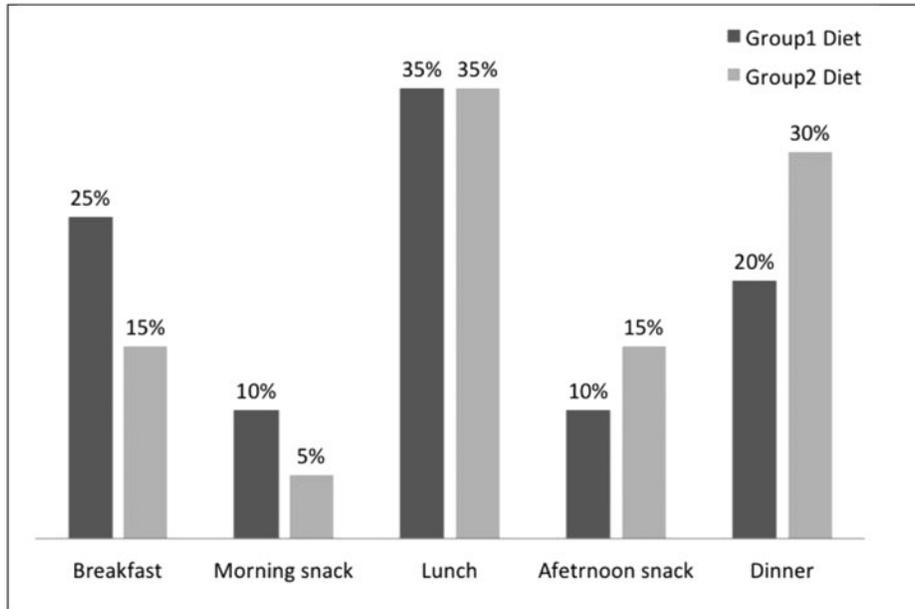
Fig. 1. Groups difference in distribution of calories throughout the day (%).

lost also a higher proportion of body fat mass (-6.8 ± 2.1 vs -4.5 ± 2.9 kg, $p = 0.031$; see Table 2). Lifestyle treatment effects on body composition are shown in Table 4.

Cardiometabolic Risk Factors

Improvements were detected in blood lipid parameters (total cholesterol, HDL, low-density lipoprotein) and glucose metabolism-related parameters in both groups. Major differences were observed in G1 for blood pressure and lipid param-

eters. G1 had a significant reduction in diastolic blood pressure (-6 ± 2 vs $+4 \pm 1$ mmHg, $p = 0.004$) and an improvement in HDL levels (4 ± 0.5 vs 1 ± 0.2 mg/dl, $p = 0.034$; see Table 2).

Glucose Metabolism-Related Parameters

After 3 months of lifestyle intervention, fasting glucose levels and fasting insulin were lower than baseline levels in both groups, with no significant differences between them. In contrast, insulin resistance significantly improved in group G1

Fig. 2. Macronutrients between meals and snacks in both groups.

Table 2. Treatment Effect on Anthropometrics and Cardiometabolic Risk Factors, Expressed as Mean $\pm$ SD or Median (interquartile range) as Appropriate

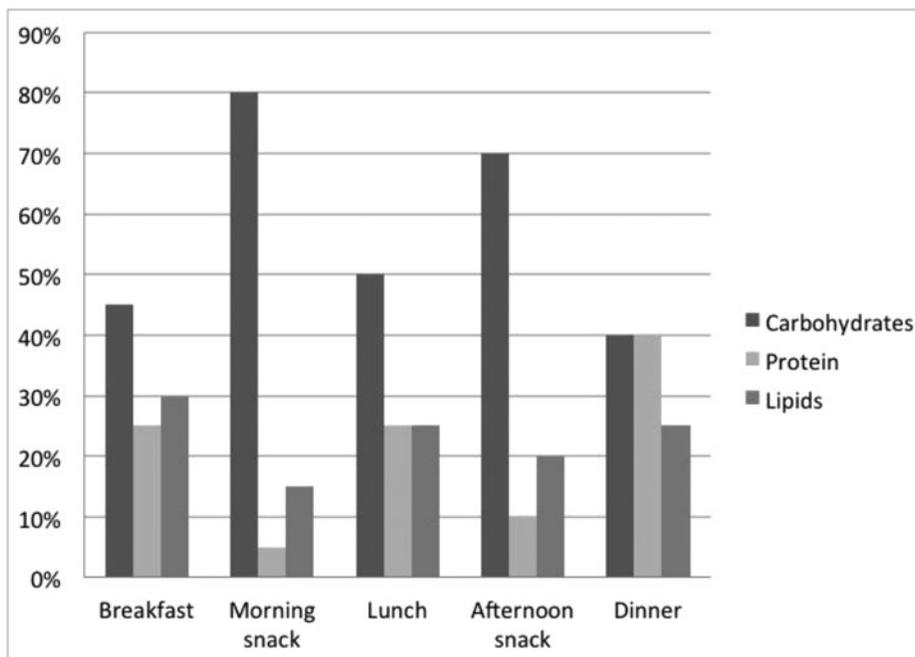|	G1 ($n = 18$)			G2 ($n = 18$)			
	Baseline	3 Months	Δ	Baseline	3 Months	Δ	*p* Value*
Anthropometrics and body composition							
Body weight (kg)	94.2 $\pm$ 2.1	86.3 $\pm$ 1.9	-8.2 ± 3.0	88.2 $\pm$ 1.3	81.7 $\pm$ 0.9	-6.5 ± 3.4	0.028
BMI (kg/m^2)	35.8 $\pm$ 5.2	32.7 $\pm$ 2.3	-3.1 ± 0.2	35.1 $\pm$ 4.5	33.3 $\pm$ 3.9	-1.8 ± 0.4	0.036
Waist (cm)	100 $\pm$ 3	93 $\pm$ 4	-7 ± 0.6	100 $\pm$ 5	95 $\pm$ 5	-5 ± 0.3	0.033
Body fat mass (kg)	40.7 $\pm$ 3.5	33.9 $\pm$ 3.9	-6.8 ± 2.1	37.8 $\pm$ 3.8	33.6 $\pm$ 3.7	-4.5 ± 2.9	0.031
Cardiometabolic risk factors							
Cholesterol (mg/dl)							
Total	198 $\pm$ 37	191 $\pm$ 32	-7 ± 2	203 $\pm$ 41	195 $\pm$ 34	-8 ± 3	ns
LDL	127 $\pm$ 21	122 $\pm$ 17	-5 ± 2	128 $\pm$ 24	127 $\pm$ 21	-1.3 ± 0.1	ns
HDL	48 $\pm$ 12	52 $\pm$ 9	4 ± 0.5	49 $\pm$ 14	50 $\pm$ 12	1 ± 0.2	0.034
Triglycerides (mg/dl)	117 (101–143)	90 (74–103)	-27 ± 5	122 (98–161)	107 (88–134)	-15 ± 3	ns
sBP (mmHg)	132 $\pm$ 16	124 $\pm$ 9	-9 ± 2	124 $\pm$ 11	125 $\pm$ 13	1 ± 0.3	ns
dBP (mmHg)	83 $\pm$ 9	77 $\pm$ 6	-6 ± 2	77 $\pm$ 13	81 $\pm$ 10	4 ± 1	0.004
Fasting glucose (mmol/l)	5.35 $\pm$ 0.66	5.21 $\pm$ 0.32	-0.14 ± 0.06	5.12 $\pm$ 0.82	5.16 $\pm$ 0.57	0.04 ± 0.02	ns
Fasting insulin (μU/ml)	16.2 (12.4–19.1)	9.5 (6.3–11.5)	-6.7 ± 1.4	17.3 (11.9–19.5)	15.5 (11.3–18.7)	-1.8 ± 0.02	ns
Homeostasis model assessment– estimated insulin resistance	4.12 $\pm$ 2.62	2.75 $\pm$ 1.34	-1.37 ± 0.27	4.15 $\pm$ 2.51	3.41 $\pm$ 1.95	-0.74 ± 0.12	0.017
hs CRP (mg/l)	5.6 $\pm$ 2.1	5.4 $\pm$ 1.8	-0.2 ± 0.1	4.3 $\pm$ 1.6	4.4 $\pm$ 2.1	0.1 ± 0.01	ns

BMI = body mass index, LDL = low-density lipoprotein, HDL = high-density lipoprotein, sBP = systolic blood pressure, dBP = diastolic blood pressure, hs CRP = high-sensitivity C-reactive protein.*Differences between groups, adjusted for respective baseline values (general linear model).

only, as shown by the group difference in homeostasis model assessment–estimated insulin resistance at the end of the study ($p = 0.017$; see Table 2).

DISCUSSION

Stories about food and nutrition are in the news on an almost daily basis, but information can sometimes be confusing and contradictory. Clear messages should be proposed in order to reach the greatest number of people. One clear communication from physicians could be "If you want to lose weight, eat more in the morning than in the evening."

In previous studies [14] it was established that the higher the proportion of total food intake ingested in the morning the lower the daily intake, whereas the higher the proportion of total intake ingested in the late evening the higher the daily intake. These results suggest that intake during the morning is associated with lower intake throughout the day, whereas intake late at night is
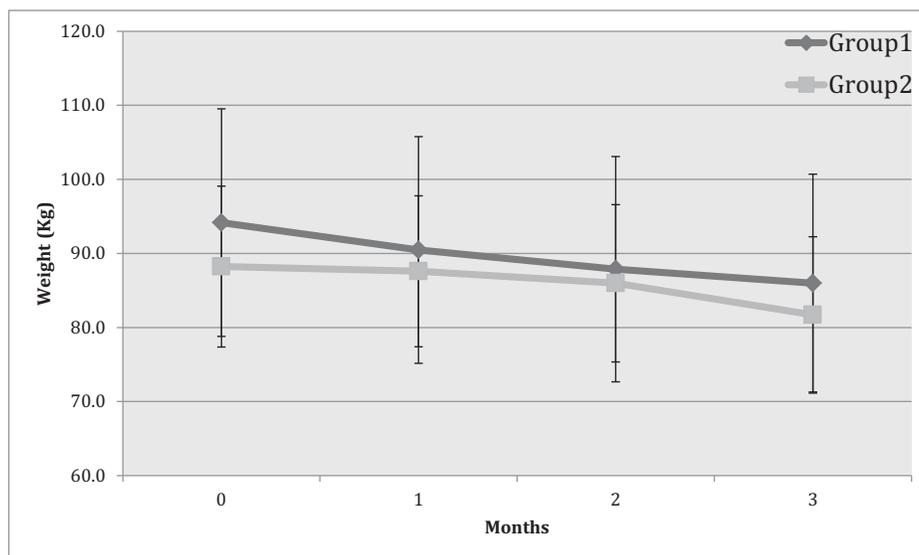
Fig. 3. Groups difference in weight loss (p = 0.028).
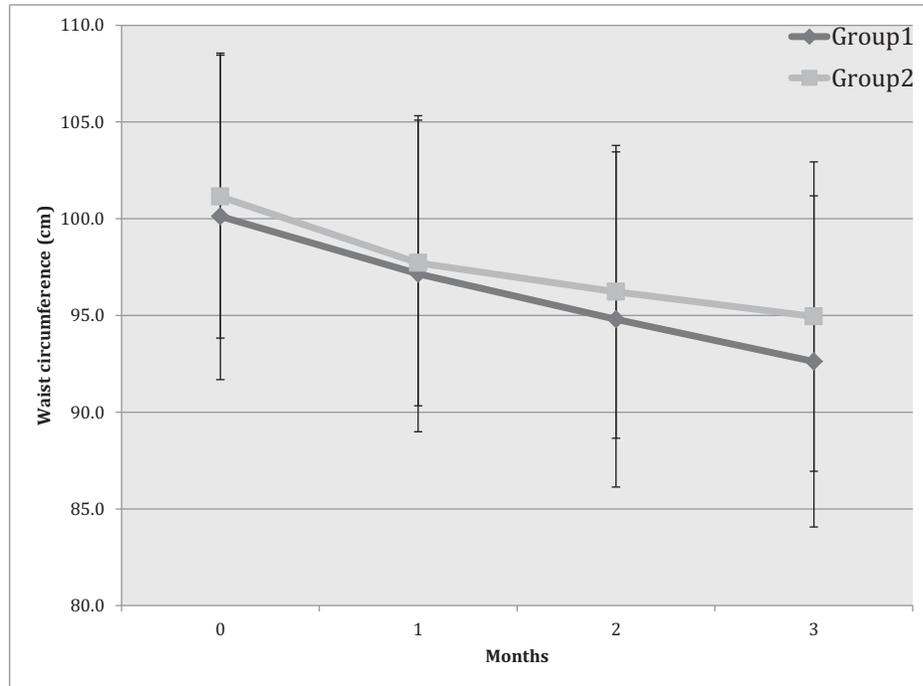
Fig. 4. Waist circumference reduction.

associated with higher levels of overall daily intake. The present study supports this theory in a 3-month lifestyle intervention.

Our study revealed that a lifestyle intervention with more energy intake in the first part of the day had a higher impact on weight and fat reduction. Many people overlook nutrition in the morning due to work or a conviction that this is a method to lose weight. Total daily calories and macronutrients are often considered but time of ingestion is not.

The satiating power of morning meals can be explained by evolutionary theory, hormone theory, and metabolic theory. Earlier in human evolutionary history, the loss of light in the evening significantly limited activity. In modern times, however, the extensive use of artificial lighting has allowed people to remain active as well as eat late into the night. Thus, obesity in recent evolutionary years could be explained due to eating in the evening when satiation is weak. Foods containing complex

Table 3. Energy Expenditure Measured by Metabolic Armband and Main Diet Features of Both Groups (expressed as mean $\pm$ SD)

	G1 ($n = 18$)			G2 ($n = 18$)			
	Baseline	3 Months	Δ	Baseline	3 Months	Δ	*p* Value*
Energy expenditure and intake							
Total energy expenditure	2918 $\pm$ 75	2845 $\pm$ 61	−18	2895 $\pm$ 97	2799 $\pm$ 88	−96	ns
Diet calories (kcal)	2008 $\pm$ 67	1992 $\pm$ 89	−12	1993 $\pm$ 87	1967 $\pm$ 95	−26	ns
Macronutrient intake (percentage energy from)							
Fat	24.5 $\pm$ 6.7	24.3 $\pm$ 5.9	−0.2	24.4 $\pm$ 6.5	24.6 $\pm$ 7.8	0.2	ns
Carbohydrates	58.7 $\pm$ 7.4	58.5 $\pm$ 6.5	−0.2	58.9 $\pm$ 8.2	58.4 $\pm$ 8.1	−0.5	ns
Sugars	12.9 $\pm$ 3.2	12.5 $\pm$ 3.1	−0.4	12.6 $\pm$ 3.6	12.3 $\pm$ 3.9	−0.3	ns
Protein	16.8 $\pm$ 4.1	17.2 $\pm$ 4.7	+0.3	16.5 $\pm$ 4.3	16.8 $\pm$ 4.9	0.6	ns
Alcohol	0	0	0	0	0	0	ns
Macronutrient intake (g)							
Fat	54.7 $\pm$ 5.6	54.2 $\pm$ 5.1	−0.5	54 $\pm$ 6.4	54.5 $\pm$ 7	0.5	ns
Carbohydrates	294.7 $\pm$ 32.4	297.2 $\pm$ 31	2.5	293.5 $\pm$ 31	291 $\pm$ 29.7	−2.5	ns
Sugars	64.8 $\pm$ 13.4	65 $\pm$ 13.2	0.2	62.8 $\pm$ 13.6	61.3 $\pm$ 14.1	−1.5	ns
Fiber	27.5 $\pm$ 6.5	28.2 $\pm$ 6.7	0.7	27.7 $\pm$ 6.8	28.5 $\pm$ 7.2	0.8	ns
Protein	84.3 $\pm$ 10.1	86.3 $\pm$ 12	2	82.2 $\pm$ 9.6	83.7 $\pm$ 10.3	1.5	ns
Alcohol	0	0	0	0	0	0	ns

p value < 0.05 was considered significant.

Table 4. Lifestyle Treatment Effects on Body Composition (expressed as mean ± SD)

	Baseline	3 Months	Δ	*p* Value
Body weight (kg)				
Intervention group	94.2 ± 2.1	86.3 ± 1.9	−8.2	
Control group	88.2 ± 1.3	81.7 ± 0.9	−6.5	0.028
Fat mass (%)				
Intervention group	43.2 ± 4.2	39.3 ± 5.1	−3.9	
Control group	42.8 ± 5.2	41.1 ± 5.3	−1.7	0.031
Lean body mass (%)				
Intervention group	54.2 ± 2.8	57 ± 4.2	+3.8	
Control group	54.4 ± 5.2	56.1 ± 5	+2.7	ns
Water (%)				
Intervention group	40.6 ± 3.7	40.8 ± 1.4	0.2	
Control group	42.6 ± 3.1	42.5 ± 1.2	−0.1	ns

carbohydrates, like whole grains commonly consumed at breakfast, could affect [20] the secretion or activity of gastrointestinal hormones such as cholecystokinin, or other incretins, including gastric inhibitory peptide and glucagon-like peptide-1, which may influence insulin secretion [20] and postprandial satiety [21] and have a role in absorption of glucose that is not dependent on insulin [22]. Promoting a high morning calorie intake may also prevent weight regain by reducing diet-induced compensatory changes in hunger, cravings, and ghrelin suppression. In a recent study a high-carbohydrate and high-protein breakfast reported less hunger and fewer cravings [23]. Analysis of patients' lipid profiles showed further benefits in association with the high-calorie breakfast: total cholesterol, low-density lipoprotein, and triglycerides all dropped significantly more among patients assigned to the high-calorie breakfast. Other studies [24] provide evidence for the importance of timing as a modulator of the adipocyte–hypothalamic axis and its impact on body weight. Short-term changes have an immediate effect on food intake rhythmicity and, over time, the changes in rhythmic food intake lead to an increase in body weight.

Higher calorie ingestion in the first part of the day determines changes in metabolism by increased satiation [25], improved insulin sensitivity [26], and reduced total daily energy intake [27]. It has been demonstrated that a time-restricted feeding regimen mice is a nonpharmacological strategy against obesity and associated diseases [28] and protects against obesity, hyperinsulinemia, and hepatic steatosis. Improved CREB (cAMPresponse element binding protein transcription factor), mTOR (mammalian target of rapamycin), and AMPK (AMP-activated protein kinase) pathway function and oscillations of the circadian clock altered liver metabolism and improved nutrient use and energy expenditure.

CONCLUSIONS

Results of a 3-month lifestyle intervention including a low-calorie Mediterranean diet with a greater caloric intake in the first part of the day could establish a greater reduction in fat mass and improved insulin sensitivity than a typical daily diet.

ACKNOWLEDGMENT

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