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ORIGINAL RESEARCH

The effect of low-calorie diet on cardiometabolic risk factors in normal and overweight patients with type 2 Diabetes: a randomized controlled trial

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Abstract

Objective: Weight loss is a basic strategy for the management of Type 2 diabetes mellitus (T2DM). Some studies have suggested that calorie restriction has beneficial effects in the management of diabetes symptoms. The aim of the present research was to evaluate the effects of a low-calorie diet (LCD) on cardio-metabolic risk factors, body composition, and liver enzymes in normal and overweight patients with T2DM.

Materials and Methods: 120 eligible patients (49 female and 71 male) were randomly assigned to one of the four following groups: low caloric (LCD with -500 kcal lower than need) and isocaloric (control) diets for 12 weeks for normal and overweight T2DM patients. Glycemic and lipid profiles, liver enzymes and body composition were assessed before and after the intervention.

Results: 104 patients completed the study. LCD groups presented significant decrease in anthropometric measurements compared to control groups ($P<0.05$). Glycemic markers, triglyceride, and alkaline phosphatase (ALP) were significantly decreased only in normal-weight LCD group compared to control group. Homeostatic model of insulin resistance, LDL-C, and ALP were significantly decreased only in overweight LCD group ($P<0.05$). However, insulin sensitivity was significantly increased in LCD groups compared to control groups ($P<0.05$).

Conclusion: It was found that LCD improved cardio-metabolic risk factors, liver enzymes and body composition in normal and overweight T2DM patients.

Keywords: Type 2 diabetes, Low calorie diet, Body composition, Lipid profile, Glycemic indexes, Liver enzymes

Introduction

Type 2 diabetes (T2DM) is one of the most common chronic diseases and World Health Organization predicts that by 2025, 300 million people will suffer from this disease[1]. Since 1980, there has been a dramatic increase in the prevalence of T2DM in middle- and low-income countries. The number of adults with T2DM is estimated to increase from 463.0 million to 700.2 million between 2019 and 2045[2, 3]. This disease has many short-term and long-term complications and leads to micro and macrovascular disorders[4]. On the other hand, this disease imposes great costs to health systems in different countries and significantly affects the life quality of patients. The total annual cost of managing this disease is expected to increase accordingly from 760.3 to 845.0 billion USD in the above period[5]. Over the past few decades, a number of factors have increased the prevalence of T2DM in the world, including higher prevalence of obesity, sedentary and stressful lifestyle, and consumption of high-calorie foods[6].

The fundamental pathophysiologic pathways leading to the formation of diabetes are now well recognized. To forecast the etiology and pathophysiology of T2DM development and reversal, twin cycle theory was developed in 2008 and evaluated in several trials[7-9]. Various studies have shown that an unhealthy diet containing high amounts of calorie, sugars, and saturated fatty acids (SFA) and low fiber is one of the important factors in T2DM etiology [10].

Although T2DM is related to excess weight, the rate of this disease among people with normal weight has increased by ~10–20% over the past decade[11, 12]. Generally, non-obese T2DM patients have higher abdominal obesity and total fat mass and lower muscle mass compared to normal control group with similar body mass index (BMI)[13]. Therefore, although obesity is an important risk factor for T2DM, body composition including fat and lean mass also plays an important role in predisposing people to T2DM[14].

Recent research works on obese patients after gastric bypass surgery have shown that metabolic irregularities of T2DM could be corrected and diabetic drugs could be stopped shortly after surgery[15]. Caloric restriction in the early postoperative phase has been linked to the correction of plasma glucose level within the first week after surgery[16]. These findings have increased the interest of researchers to investigate the effects of weight loss in the management of diabetes symptoms[17]. However, the results of studies on the severity of weight loss and its effect on biomarkers related to diabetes are contradictory. Some studies have shown that the rate of diabetes remission was higher among patients who had lost more weight[18]. Also, it has been reported that the effect of weight loss among people with severe obesity was different compared to patients with overweight and mild obesity[9].

Therefore, the aim of this research was to investigate the effect of low-calorie diet (decrease of 500 kcal/day) on metabolic status in two overweight and normal weight groups of T2DM patients.

Materials and Methods

This study was performed as a parallel randomized controlled trial. Patients were recruited from Diabetes Clinic of Urmia Imam Khomeini Educational Hospital in Urmia, Iran, between October 2021 and May 2022. T2DM was defined as the fasting plasma glucose (FPG) level ≥126 mg/dl (7 mmol/L), 2-hr glucose level after an oral glucose tolerance test (OGTT) ≥200 mg/dl (11.11 mmol/L) or use of glucose-lowering medication(s) (only metformin and glibenclamide). Inclusion criteria at the time of screening were age range of 18-65 years, BMI range of 18.5 to 29.9 kg/m^2, no evidence of the complications of diabetes, glycated hemoglobin level (HbA1C) ≥6.5% (48 mmol/mol), DM duration <10 years, and good compliance to study protocol. All patients were at pre hypertension stage and did not take any blood pressure-lowering drugs. Patients with alcohol consumption and smoking, pregnancy and lactation, previous treatment with a thiazolidinedione or a glucagon-like peptide-1 receptor agonist within the past 3 months, previous use of insulin, concurrent use of steroid, involvement in a weight loss program, application of weight loss medications, use of herbal and chemical supplements, mal-absorption diseases, untreated hypothyroidism, change in physical activity level, and consumption of fat-lowering and diuretic medications were excluded from the study. Sample size was calculated on the basis of a study by Lee *et al*.[19] to detect a significant change in serum HbA1c

concentration between groups using a two-sided test with α=0.05 and power=0.8. Sample size was calculated to be 30 participants in each group using the following equation:
$n = (Z_{1-\alpha/2} + Z_{1-\beta})^2 (S_1^2 + S_2^2)/(\mu_1-\mu_2)^2$ and n` = n√k-1 (k is the number of group).
Study design was described in details using CONSORT recommendation, and study protocol was approved by the Ethics Committee of Urmia University of Medical Sciences (Code no. IR.UMSU.REC.1397.034) and was registered in Iranian Registry of Clinical Trials (IRCT20170214032571N10). Written informed consent was obtained from all participants.
A total of 120 eligible patients (60 normal weight subjects with BMI 18.5-22.9 and 60 overweight subjects with BMI 23-29.9) were randomly assigned to one of the four following groups: low-calorie diet group with normal weight (*n*=30), low-calorie diet group with overweight (*n*=30), control group with normal weight (*n*=30), control group with overweight (*n*=30). A BMI cutoff of above 23 kg/m^2 was chosen to represent being overweight according to WHO criteria for Asians[20].
Low-calorie diet for participants in normal and overweight intervention groups was prescribed for 12 weeks. The diet contained 15% protein, 55% carbohydrate and 30% fat.Calorie requirements for intervention groups were calculated and limited, so that 500 kcal was reduced from total calculated energy for normal and overweight patients in intervention groups. Study protocol was consisted of three periods, a 2-week run-in period, a 12-week caloric restriction period, and a 4-week transition period. In run-in period, participants were tried on an LCD (500 calories less than required) for 10 days over a period of 2 weeks (weeks −2 to 0) to assess for compliance. Those who passed 90% rate of compliance were invited to continue to caloric restriction period, during which participants received LCD for 12 weeks (weeks 0–12). Participants in the control groups received no intervention and consumed their habitual diet. All patients were advised to follow their usual physical activity during the study. All patients were assessed adherence in terms of attendance at monthly visits. Total energy expenditure was calculated using the equations set by Food and Nutrition Board in Institue of Medicine[21].
Demographic information and medical history were obtained at baseline. All patients underwent body composition measurements and laboratory tests at the baseline and week 12. Heights were measured without shoes using a stadiometer (Biospace, model BSM370, Korea) with 0.1 cm accuracy. Body weights were measured using a scale with 0.1 kg accuracy without shoes and with minimum clothing. Body composition included fat mass (FM), skeletal muscle mass (SMM) and visceral fat area (VFA) which were assessed through Bioelectrical Impedance Analysis (InBody770, Korea).
Blood pressure was measured using a mercury sphygmomanometer and stethoscope after subjects had rested for a minimum of 10 minutes [17]. Blood samples were collected after an overnight 12-h fast and were kept at -80°C until analyzing. Fasting blood sugar (FBS), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides were measured using BT 4500 auto-analyzer (Italy) with reagent kits (Dialab, Wr.Neudorf, Austria).
Fasting insulin was measured by electro-chemiluminescence Cobase e411 using Roche® kits. Homeostasis model assessment for insulin resistance (HOMA-IR) and quantitative insulin-sensitivity check index (QUICKI) were calculated using the equations previously reported in literature [18,19].
Dietary intakes were assessed using 3-day and 24-h dietary recall and were analyzed using Nutritionist IV software. Physical activity level was evaluated using an International Physical Activity Questionnaire (IPAQ). For blood pressure measurements at baseline and at the end of 12th week, patients were first asked to rest for 15 minutes, then a trained nurse measured their blood pressure twice in seated position, with a 10-minute interval, by using a standard mercury sphygmomanometer and

thereafter, the mean of 2 measurements was considered as the participant's blood pressure.
Statistical analysis was conducted using SPSS statistical software version 20.0 (Chicago, IL, USA). Normality of quantitative variables was checked using Kolmogorov–Smirnov test. Continuous variables were compared among the four groups by one-way ANOVA with Tukey's post hoc test for normal distribution or Kruskal-Wallis test for non-normal distribution. Within-group comparison was performed using paired *t*-test or Wilcoxon test. Chi-square test or Fisher's exact test was applied for categorical variables. $P < 0.05$ was considered as statistically significance level.

Results

A total of 120 patients with T2DM were recruited from October 2020 to May 2021. From 120 patients participated in the study, only 104 finished study period. The reasons for attrition in each group are shown in Figure 1.

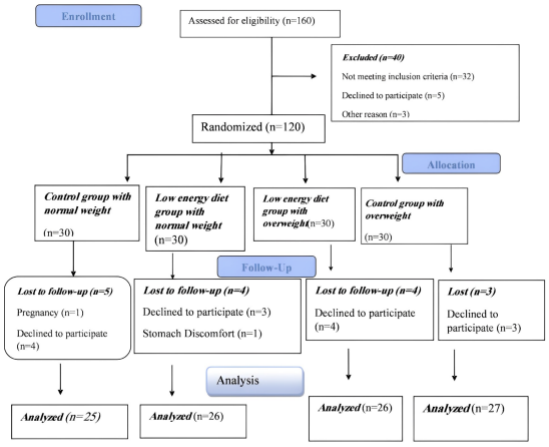

Figure 1: Flowchart of the study protocol

The baseline characteristics of participants are summarized in Tables 1 and 2. There were not any significant differences between groups in term of age (P=0.825), gender (P=0.596), and physical activity before (P=0.173) and after (P=0.168) the intervention.
All participants in intervention groups consumed more energy than the prescribed amount.
During the study, there was not any statistically significant difference in the physical activity level of participants at the beginning (P=0.173) and end of trial in the four groups (P=0.168) (Table1). No adverse events were reported during the study. As given in Table 2, in the comparison of 4 groups, there was a significant difference in the weights of participants(P<0.001). However, when diet group with overweight was compared with overweight control group (P=0.543), and when normal weight diet group was compared with normal weight control group (P=0.110), no significant differences were observed. Also, no significant difference was found between the two overweight groups and normal weight groups in terms of BMI (P=0.853), FM (P=0.802), SMM (P=0.691) and VFA (P=0.297). Moreover, we did not find any significant differences in two-by-two analysis in term of other baseline variables including TG (P=0.369), cholesterol (P=0.666), LDL-C (P=0.637), HDL-C (P=0.496), ALT (P=0.203), AST (P=0.650), ALP (P=0.952), FBS (P=0.776), HbA1c (P=0.343), SBP (P=0.215), DBP (P=0.223) and dietary intake of macronutrients (P>0.05).

Table 1. General characteristics in interventions and control groups

Variables		Diet group with overweight (n=26)	Control group with overweight (n=27)	Diet group with normal weight (n=25)	Control group with normal weight (n=26)	P. value
Age (years) mean		54.69±9.14	56.74±6.9	55.0±9.7	56.65±7.91	0.825*
Gender, n (%)	Male	15 (57.7)	14 (51.9)	16 (64.0)	18 (69.2)	0.596¶
	Female	11 (42.3)	13 (48.1)	9 (36.0)	8 (30.8)	
Education, n (%)	≤Diploma	20 (76.9)	19 (70.4)	18 (72.0)	17 (65.4)	0.835¶
	>Diploma	6 (23.1)	8 (29.6)	7 (28.0)	9 (34.6)	
Income (million rials/month) n (%)	<10	6 (23.1)	5 (18.5)	9 (36.0)	8 (30.8)	0.810¶¶
	10-30	7 (57.7)	16 (59.3)	12 (48.0)	7 (557.7)	
	>30	5 (19.2)	6 (22.2)	4 (16.0)	3 (11.5)	
Physical activity before intervention n (%)	Low	10 (38.5)	10 (37.0)	16 (64.0)	10 (38.5)	0.173¶¶
	Moderate	14 (53.8)	14 (51.9)	5 (20.0)	11 (42.3)	
	Severe	2 (7.7)	3 (37.0)	4 (16.0)	5 (19.2)	
Physical activity after intervention n (%)	Low	11 (42.3)	11 (40.7)	17 (68.0)	10 (38.5)	0.168¶¶
	Moderate	13 (50.0)	13 (48.1)	5 (12.0)	10 (38.5)	
	Severe	2 (7.7)	3 (11.1)	3 (12.0)	6 (23.1)	

Data are presented as n (%). *Analyzed by one-way ANOVA between four groups, ¶P values for Chi-square test. ¶¶P values for Fisher's Exact test.

Table 2. Baseline anthropometric profile and biochemical and nutritional variables in interventions and control groups

Variables	Diet group with overweight (n=26)	Control group with overweight (n=27)	Diet group with normal weight (n=25)	Control group with normal weight (n=26)	P. Value	P Diet versus Control (overweight)	P Diet versus Control (normal weight)
Anthropometric Parameters							
Weight (kg)	77.13 ± 9.08	75.71 ± 6.95	69.47 ± 9.17	65.66 ± 8.45	<0.001*	0.543*	0.110*
BMI (kg/m²)	27.95 ± 1.54	28.14 ± 1.19	23.84 ± 1.21	23.76 ± 1.72	<0.001*	0.644*	0.853*
FM (kg)	25.25 ± 4.67	25.44 ± 4.47	18.63 ± 4.16	16.63 ± 3.87	<0.001*	0.872*	0.802*
SMM (kg)	28.71 ± 6.46	27.62 ± 5.14	28.06 ± 5.82	26.84 ± 5.35	0.691*	-	-
VFA (cm²)	123.07 ± 33.31	126.21 ± 30.61	88.30 ± 25.54	80.05 ± 21.19	<0.001*	0.685*	0.297*
Biochemical Parameters							
FBS (mg/dl)	117.34 ± 22.56	116.92 ± 36.43	115.13 ± 18.45	121.40 ± 26.67	0.776**	-	-
HbA1c (%)	7.5 ± 1.13	7.5 ± 1.38	7.1 ± 1.14	7.7 ± 0.87	0.343*	-	-
Insulin (µu/ml)	5.83 ± 1.97	6.16 ± 2.09	5.16 ± 2.50	4.24 ± 2.00	0.005**	0.590**	0.130**
HOMA-IR	29.10 ± 10.95	29.76 ± 12.29	26.90 ± 12.64	22.30 ± 9.83	0.107*	-	-
QUICKI	0.35 ± 0.02	0.35 ± 0.02	0.36 ± 0.02	0.37 ± 0.03	0.013**	0.607**	0.159**
Triglycerides (mg/dl)	106.62 ± 45.8	105.92 ± 35.05	124.75 ± 51.37	106.0 ± 44.8	0.369*	-	-
Cholesterol (mg/dl)	150.34 ± 31.55	142.18 ± 26.8	141.87 ± 32.0	140.92 ± 32.41	0.666*	-	-
LDL-C (mg/dl)	81.50 ± 23.38	77.00 ± 18.80	74.36 ± 19.31	74.70 ± 25.89	0.637*	-	-
HDL-C (mg/dl)	43.16 ± 6.87	44.42 ± 8.57	40.91 ± 10.18	43.26 ± 6.53	0.496*	-	-
ALT (IU/l)	20.95 ± 5.58	21.00 ± 5.37	17.86±5.31	18.13±6.20	0.203**	-	-
AST (IU/l)	20.92 ± 3.79	20.08 ± 4.68	19.54±4.40	19.61±4.05	0.650**	-	-
ALP (IU/l)	141.30 ± 34.21	145.51 ± 32.30	140.17 ± 28.19	143.44 ± 35.07	0.952**	-	-
Blood Pressure							
SBP (mmHg)	13.03 ±1.67	12.6 ± 1.43	12.45 ± 1.19	12.1 ± 1.97	0.215*	-	-
DBP (mmHg)	7.66 ±1.10	7.46 ± 1.36	7.48 ± 0.78	7.12 ± 0.83	0.223**	-	-
Dietary Intakes							
Energy intake (kcal)	2330.89 ±36.36	2314.16 ± 388.47	2291.38 ± 340.98	2267.67 ± 349.59	0.930*	-	-
Dietary carbohydrate (g/day)	334.40 ±69.28	327.33 ± 78.50	315.21 ± 57.96	312.72 ± 62.6	0.623*	-	-
Dietary fat (g/day)	82.05 ±17.82	79.73 ± 25.31	82.57 ± 22.61	84.43 ± 20.65	0.890*	-	-
Dietary protein (g/day)	77.48 ±14.41	77.23 ± 16.67	75.42 ± 15.45	76.94 ± 21.32	0.975*	-	-

Data are presented as mean ± SD or percent of valid cases. *Analyzed by one-way ANOVA

between 4 groups, **Analyzed by Kruskal-Wallis between 4 groups, BMI=Body mass index; FM=Fat mass; SMM=Skeletal muscle mass; VFA=Visceral fat area; WC=Waist circle; FBS=Fasting blood sugar; HbA1c: glycated hemoglobin A; LDL-C=Low-density lipoprotein cholesterol; HDL-C=High-density lipoprotein cholesterol; ALT= alanine aminotransferase; AST= aspartate aminotransferase; ALP= Alkaline Phosphatase; HOMA-IR=Homeostasis model assessment of insulin resistance; QUICKI= The quantitative insulin sensitivity check index; SBP=Systolic blood pressure; DBP=Diastolic blood pressure; SD=Standard deviation.

As shown in Tables 3 and 4, calorie restriction caused significant weight loss in overweight (P<0.001) and normal weight (P=0.024) diet groups; moreover, as reported in Table 5, it could be understood that intervention with calorie restriction for 12 weeks led to a more significant wight loss in both low calorie diet groups than control groups (P<0.001).

Similarly, 12-week intervention significantly reduced BMI, FM, SMM, VFA and waist circle (WC) in overweight and normal weight diet groups than control groups (P<0.05). Low-calorie diet for 12 weeks caused significant reduction in BMI, FM, VFA, WC in diet groups compared with control groups (P<0.05).

Table 3. Comparison the baseline and after intervention characteristics within overweight groups

Variable	Diet group with overweight (n=26)			Control group with overweight (n=27)		
	Baseline	Week 12	*P*	Baseline	Week 12	*P*
Anthropometric Parameters						
Weight (kg)	77.13±9.08	75.09±9.34	<0.001*	75.71±6.95	76.41 ±7.31	0.024*
BMI (kg/m²)	27.95±1.54	27.20±1.60	<0.001*	28.14±1.19	28.39 ±1.26	0.024*
FM (kg)	25.25±4.67	23.88±4.34	<0.001*	25.44±4.47	26.62 ±4.80	<0.001*
SMM (kg)	28.71±6.46	28.31±6.39	0.011*	27.62±5.14	27.41 ±5.04	0.171*
VFA(cm²)	123.07±33.31	116.63±31.73	<0.001*	126.21±30.61	133.70 ±33.69	<0.001*
WC	95.47±6.64	93.99±6.42	<0.001*	94.89±4.05	96.99±5.09	<0.001*
Biochemical Parameters						
FBS (mg/dl)	117.34±22.56	104.68±14.68	0.003*	114.35±34.55	117.80 ±34.33	0.490*
HbA1c (%)	7.5±1.13	7.0±1.00	0.053*	7.5±1.38	7.7 ±1.67	0.417*
Insulin (µu/ml)	5.68±1.87	5.11±2.11	0.110**	6.16±2.09	6.29 ±1.95	0.753**
HOMA-IR	28.54±10.93	23.75±11.25	0.174*	29.68±12.85	30.52±13.06	0.762*
QUICKI	0.35±0.02	0.36±0.02	0.012*	0.35±0.02	0.35±0.02	0.493*
Triglycerides (mg/dl)	106.62±45.80	93.83±37.60	0.089*	101.16±30.37	108.20 ±32.34	0.286*
Cholesterol (mg/dl)	150.34±31.55	149.65±27.74	0.895*	142.18±26.80	151.37 ±33.41	0.072*
LDL-C (mg/dl)	81.50±23.38	79.99±20.87	0.695*	77.00±18.80	83.85±24.27	0.076*
HDL-C (mg/dl)	43.16±6.87	43.20±8.05	0.968*	43.64±7.74	44.48±8.14	0.595*
ALT (IU/L)	20.95±5.58	19.00±5.14	0.137*	21.00±5.37	20.36±4.85	0.607*
AST (IU/L)	20.92±3.79	18.76±3.19	0.034**	20.08±4.68	19.24±3.65	0.352**
ALP (IU/L)	141.30±34.21	137.92±31.04	0.507*	145.51±32.30	155.14±37.09	0.045*
Blood Pressure						
SBP (mmHg)	13.03±1.67	12.56±1.95	0.046*	12.60±1.43	12.63±1.83	0.901*
DBP(mmHg)	7.66±1.10	7.56±0.73	0.690*	7.46±1.36	7.65±0.89	0.427*
Dietary Intake						
Energy intake (kcal)	2330.89±369.36	2139.54±427.89	<0.001*	2314.16±388.47	2378.66±424.60	0.025*
Dietary carbohydrate (g/day)	334.40±69.28	313.00±70.62	0.004*	327.33±78.50	351.17±67.30	0.001*
Dietary fat (g/day)	82.05±17.82	76.85±22.93	0.152*	79.73±25.31	77.43±24.55	0.428*
Dietary protein (g/day)	77.48±14.41	67.84±15.22	0.001*	77.23±16.67	74.43±16.14	0.209*

*P-values for Paired *t*-test within groups comparison, **P-values for Wilcoxon test within groups comparison. BMI=Body mass index; FM=Fat mass; SMM=Skeletal muscle mass; VFA=Visceral fat area; WC=Waist circle; FBS=Fasting blood sugar; HbA1c: glycated hemoglobin A; LDL-C=Low-density lipoprotein cholesterol; HDL-C=High-density lipoprotein cholesterol; ALT= alanine aminotransferase; AST= aspartate aminotransferase; ALP= Alkaline Phosphatase; HOMA-IR=Homeostasis model assessment of insulin resistance; QUICKI= The quantitative insulin sensitivity check index; SBP=Systolic blood pressure; DBP=Diastolic blood pressure; SD=Standard deviation.

Table 4. Comparison the baseline and after intervention characteristics within normal weight groups

Variables	Diet group with normal weight (n=25)			Control group with normal weight (n=26)		
	Baseline	Week 12	*P*	Baseline	Week 12	*P*
Anthropometric Parameters						
Weight (kg)	69.47±9.17	68.28±9.48	0.001*	65.66±8.45	66.06±8.53	0.275*
BMI (kg/m²)	23.84±1.21	23.44±1.26	0.002*	23.76±1.72	23.91±1.71	0.278*
FM (kg)	18.63±4.16	17.92±4.00	0.007*	16.63±3.87	17.60±4.27	0.003*
SMM (kg)	28.06±5.82	27.68±5.96	0.009*	26.84±5.35	26.57±5.22	0.022*
VFA (cm²)	88.30±25.54	86.70±25.62	0.266*	80.05±21.19	84.74±23.44	0.003*
WC	86.92±6.55	86.24±6.37	0.128*	83.75±5.70	85.27±6.76	0.002*
FBS (mg/dl)	115.13±18.45	108.76±16.62	0.026*	121.40±26.67	122.84±28.51	0.808*
HbA1c (%)	7.1±1.14	6.5±1.03	0.001*	7.7±0.87	7.4±1.22	0.222*
Insulin (µu/ml)	5.03±2.46	4.30±1.89	0.161**	4.24±2.00	4.89±2.18	0.057**
HOMA-IR	26.52±12.80	21.24±9.97	0.044*	22.30±9.83	26.73±12.89	0.082*
QUICKI	0.36±0.02	0.37±0.03	0.053*	0.37±0.03	0.36±0.02	0.107*
Triglycerides (mg/dL)	124.75±51.37	105.08±43.36	0.009*	104.48±45.03	94.80±44.01	0.132*
Cholesterol (mg/dl)	141.87±32.00	138.12±27.72	0.528*	140.60±33.04	148.64±33.92	0.116*
LDL-C (mg/dl)	74.36±19.31	69.30±17.90	0.157*	74.70±25.89	82.70±27.49	0.057*
HDL-C (mg/dl)	40.91±10.18	41.20±8.68	0.750*	42.96±6.47	45.20±8.09	0.020*
ALT (IU/l)	17.86±5.31	17.47±4.83	0.626*	18.13±6.20	17.91±6.32	0.798*
AST (IU/l)	19.54±4.40	18.75±4.67	0.223**	19.61±4.05	18.84±4.79	0.387**
ALP (IU/l)	140.17±28.19	129.95±20.86	0.034*	141.16±33.89	149.20±31.10	0.065*
Blood Pressure						
SBP (mmHg)	12.46±1.21	12.57±1.65	0.703*	12.10±1.97	12.41±2.06	0.161*
DBP (mmHg)	7.48±0.80	7.52±0.72	0.791*	7.12±0.83	7.18±1.03	0.675*
Dietary Intake						
Energy intake (kcal)	2291.38±340.98	2166.05±347.36	<0.001*	2267.67±349.59	2296.19±404.43	0.379*
Dietary carbohydrate (g/day)	315.21±57.96	308.12±68.49	0.332*	312.72±62.61	332.78±55.07	0.024*
Dietary fat (g/day)	82.57±22.61	72.47±19.27	<0.001*	84.43±20.65	82.29±21.05	0.489*
Dietary protein (g/day)	75.42±15.45	70.43±16.82	0.059*	76.94±21.32	74.29±19.33	0.247*

*P-values for Paired *t*-test within groups comparison, **P-values for Wilcoxon test within groups comparison. *P-values for Paired t-test within groups comparison, **P-values for Wilcoxon test within groups comparison. BMI=Body mass index; FM=Fat mass; SMM=Skeletal muscle mass; VFA=Visceral fat area; WC=Waist circle; FBS=Fasting blood sugar; HbA1c: glycated hemoglobin A; LDL-C=Low-density lipoprotein cholesterol; HDL-C=High-density lipoprotein cholesterol; ALT= alanine aminotransferase; AST= aspartate aminotransferase; ALP= Alkaline Phosphatase; HOMA-IR=Homeostasis model assessment of

insulin resistance; QUICKI= The quantitative insulin sensitivity check index; SBP=Systolic blood pressure; DBP=Diastolic blood pressure; SD=Standard deviation.

Table 5. Comparison the mean changes from baseline between four groups

Variables	Diet group with overweight (n=26)	Control group with overweight (n=27)	Diet group with normal weight (n=25)	Control group with normal weight (n=26)	*P*-trend	*P* Diet versus Control (overweight)	*P* Diet versus Control (normal weight)
Anthropometric Parameters							
Weight (kg)	-2.03±0.31	0.69±0.28	-1.18±0.32	0.40±0.35	<0.001*	<0.001*	<0.001*
BMI (kg/m²)	-0.75±0.12	0.25±0.10	-0.40±0.11	0.14±0.12	<0.001*	<0.001*	<0.001*
FM (kg)	-1.36±0.27	1.17±0.28	-0.71±0.24	0.96±0.29	<0.001**	<0.001**	<0.001**
SMM (kg)	-0.40±0.14	-0.20±0.14	-0.38±0.13	-0.26±.11	0.701*	0.305*	0.566*
VFA (cm³)	-6.34±1.41	7.49±1.81	-1.59±1.39	4.69±1.42	<0.001**	<0.001**	<0.005**
WC	-1.47±0.33	2.10±0.41	-0.68±0.43	1.52±0.44	<0.001*	<0.001*	<0.001*
Biochemical Parameters							
FBS (mg/dL)	-12.65±3.76	3.45±4.93	-6.37±2.67	1.44±5.86	0.010**	0.015**	0.233**
HbA1c (%)	-0.4±0.24	0.2±0.30	-0.6±0.16	-0.2±0.23	0.112**	0.031**	0.374**
Insulin (µu/ml)	-0.56±0.46	0.13±0.51	-0.72±0.47	0.65±0.29	0.104*	0.269*	0.029*
HOMA-IR	-4.79±3.39	0.84±2.75	-5.28±2.46	4.42±2.43	0.016**	0.163**	0.012**
QUICKI	0.01±0.00	-0.00±0.00	0.01±0.00	0.01±0.00	0.003*	0.018*	0.006*
Triglycerides (mg/dl)	-12.79±7.21	7.04±6.44	-19.66±6.84	-9.68±6.20	0.041*	0.039*	0.291*
Cholesterol (mg/dl)	-0.69±5.18	9.18±4.89	-3.75±5.84	8.04±4.93	0.221*	0.175*	0.120*
LDL-C (mg/dl)	-1.50±3.80	6.85±3.70	-5.05±3.46	8.00±4.01	0.010**	0.114**	0.018**
HDL-C (mg/dl)	0.04±0.98	0.84±1.55	0.29±0.90	2.24±0.89	0.157**	0.614**	0.226**
ALT (IU/l)	-1.95±1.27	-0.64±1.22	-0.39±.79	-0.21±0.83	0.652*	0.378*	0.910*
AST (IU/l)	-2.16±0.98	-0.84±0.88	-0.79±0.85	-0.76±0.76	0.612*	0.288*	0.986*
ALP (IU/l)	-3.38±5.02	9.62±4.56	-10.21±4.51	8.04±4.14	0.008*	0.042*	0.008*
Blood Pressure							
SBP (mmHg)	-0.46±-0.22	0.03±0.26	0.10±0.28	0.31±0.21	0.080**	0.148**	0.562**
DBP (mmHg)	-0.09±0.23	0.19±0.23	0.04±0.15	0.05±0.12	0.401**	0.300**	0.966**
Dietary Intake							
Energy intake (kcal)	-191.34±25.97	64.50±27.03	-125.33±25.91	28.52±31.84	<0.001*	<0.001*	<0.001*
Dietary carbohydrate (g/day)	-21.39±6.76	23.84±6.38	-7.09±7.16	20.05±8.31	<0.001*	<0.001*	0.009*
Dietary fat (g/day)	-5.20±3.51	-2.30±2.85	-10.10±2.26	-2.13±3.03	0.207*	0.487*	0.063*
Dietary protein (g/day)	-9.64±1.30	-2.79±2.16	-4.99±2.50	-2.65±2.24	0.066*	0.021*	0.438*

Data are presented as mean ± SE or percent of valid cases. *Analyzed by one-way ANOVA between four groups and POST HOC test was done by LSD method, **Analyzed by Kruskal-Wallis between four groups, SE=Standard error. BMI=Body mass index; FM=Fat mass; SMM=Skeletal muscle mass; VFA=Visceral fat area; WC=Waist circle; FBS=Fasting blood sugar; HbA1c: glycated hemoglobin A; LDL-C=Low-density lipoprotein cholesterol; HDL-C=High-density lipoprotein cholesterol; ALT= alanine aminotransferase; AST= aspartate aminotransferase; ALP= Alkaline Phosphatase; HOMA-IR=Homeostasis model assessment of insulin resistance; QUICKI= The quantitative insulin sensitivity check index; SBP=Systolic blood pressure; DBP=Diastolic blood pressure; SD=Standard deviation.

At the end of the study, we found a significant reduction in FBS concentration among participants in both diet groups (P=0.003 for overweight group and P=0.026 for normal weight group). In the comparison between groups, a significant difference in FBS level was seen only in overweight group with a low-calorie diet compared to overweight control group (P=0.015), and there was no significant difference between normal weight diet group compared to normal weight control group (P=0.233).

There was also a significant reduction in HbA1c level only in normal weight diet group (P = 0.001); however, reduction in HbA1c in overweight diet group was not significant (P = 0.053).

There was a significant increase in insulin sensitivity index (QUICKI) level only in overweight diet group (P =0.012); however, no significant changes were found in low-calorie diet normal weight group (P =0.053). Also, insulin resistance index (HOMA-IR) value was significantly decreased only in normal weight diet group (P =0.044); however, reduction in overweight diet group was not significant. In between-group comparisons, we observed significant changes in insulin levels and HOMA-IR only in normal weight low-calorie diet group compared to normal weight control group (P=0.029 and P=0.012, respectively). However, in term of QUICKI, in both of overweight and normal weight groups, low-calorie diets led a significant improvement compared to control diet group (P=0.018 for overweight group and P=0.006 for normal weight group).

As summarized in Tables 3 and 4, at the end of the study, lipid profiles (TG, Chol and LDL-C) were significantly reduced in both diet groups; however, in between-group comparisons, serum TG level was significantly decreased only in overweight diet group (P = 0.039) (Table5). In term of LDL-C concentration, we found a significant reduction in low-calorie normal weigh group compared to normal weight control group (P=0.018). No significant differences were found between groups in term of HDL-C and total cholesterol in both normal weight and overweight groups (P>0.05).

Liver enzyme levels were reduced after 12 weeks of calorie restriction. AST reduction was significant in overweight diet group (P=0.034). Also, ALP reduction was significant in normal weight diet group (P=0.034); however, reduction of other enzymes was not significant in diet groups (P>0.05). The between-group comparisons showed significant differences in ALP level between diet groups and control groups (P=0.042 for overweight group and P=0.008 for normal weight group).

At the end of the study, SBP was significantly reduced in overweight diet group (P=0.046); however, reduction in DBP was not significant (P=0.690). SBP and DBP were not significantly changed in normal weight diet group (P>0.05).

No significant differences were observed between diet groups and control groups in these parameters (P>0.05).

As summarized in Tables 3 and 4, at the end of the study, participants in intervention groups had significantly lower calorie intake than those in control groups (P<0.001). Dietary carbohydrate intake was significantly reduced in overweight diet group during the intervention (P=0.004); however, reduction in other dietary factors was not significant (P>0.05). Dietary fat intake was significantly reduced in normal weight diet group during the intervention (P<0.05), but reduction in other dietary factors was not significant (P>0.05). Considering between-group comparisons, there were significant differences in total energy intake and carbohydrate intake between diet groups and control groups (P<0.05); however, differences in other dietary factors between diet groups and control groups were not significant (P>0.05).

Discussion

Managing diabetes with no side effects is one of the challenging topics in the medical nutrition therapy of diabetes. One of the most effective ways for improving metabolic control in diabetes patients is weight loss[22]. According to the latest guidelines of American Diabetes Association (ADA) in 2022, losing weight by about 7% and increasing physical activity to 150 minutes per week is an important part of diabetes prevention program (DPP)[23]. In the present study, we evaluated the effects of calorie restriction on biochemical and anthropometrical variables among the two groups of normal and overweight patients with T2DM.

In this study, we found that significant calorie restriction could significantly reduce weight along with some anthropometric parameters such as BMI, FM, VFA, and WC in diet groups compared to control groups. The results showed that overweight diet group experienced more significant decrease in anthropometric parameters than normal weight diet group. One possible reason for more decrease of anthropometric indices in overweight group was their higher motivation for weight loss. Also, FM was higher in overweight people, which made them respond faster to weight loss diet. In line with our findings, it has been reported in other previous studies that calorie-restricted diet was more effective in obese and overweight people than in normal weight subjects in term of anthropometric variables[24, 25]. Headland et al. in a systematic review and meta-analysis study showed that adherence from low-calorie diets led to about 5 to 8.5 kg weight loss after 6 months in adult obese participants[26]. In other study conducted among obese or overweight participants, it was reported that prescribing diets with 500 kcal less than estimated required energy led to an average weight loss about 4.5 kg after 5 months of intervention[27]. Also, Banasik et al. found that adherence from a very low-calorie diet (800 kcal/day) for 28 days among obese patients with T2DM caused a significant weight loss (5±0.5 pounds) and a considerable reduction in visceral adipose tissue[28]. Comparison of the results obtained from this study and those of above-mentioned studies showed that the observed weight losses were lower than those mentioned above. At baseline, there were no significant differences among the four groups in term of FM and SMM. However, at the end of the study, we found significant reductions in FM and VAF among obese calorie-restricted group than other groups. The amount of FM reduction in diet groups was higher than that in control groups due to limited calorie intake in interventions groups. However, there were not significant differences in SMM changes among groups. Several studies have investigated the effect of various types of weight loss diets on FM and FFM, and some of them have compared the effect of a weight loss diet alone compared to that of a weight loss diet plus exercise on the amount of FM and FFM. Redman et al. evaluated the effects of diet alone (25% energy deficit; n=12) and diet plus aerobic exercise (each 12.5% energy deficit; n=12) for six months in overweight men and women. They found that after six months of intervention, weights had declined by 10.4±0.9% for diet alone group and 10.1±0.9% for diet plus exercise group, about 8.3 kg for both groups. There were no between-

group significant differences in changes from baseline in total body fat and FFM as measured by DXA[29]. In the present study, we found that calorie restriction and weight loss could contribute to improvement in glycemic indexes in diet groups compared with control groups. This is probably due to significant reductions in FM and WC in diet groups. It is established that metabolic abnormalities, such as diabetes complications and insulin resistance, are more frequently related with excessive abdominal adiposity [30]. At the end of the study, in term of glycemic profile, we found that overweight patients in calorie-restricted group had significantly lower FBS and HbA1c and improved QUICKI compared to control group. Also subjects in normal weight intervention group had lower insulin levels and HOMA-IR than control group. In term of lipid profile, we found significantly lower levels of only LDL-C in normal weight intervention group than control group. In line with our findings, Franz et al. in a meta-analysis study reported nonsignificant beneficial effects of weight loss interventions on HbA1c, lipids, or blood pressure. They found that most lifestyle weight-loss interventions in overweight or obese adults with T2DM resulted in weight loss <5% and did not result in beneficial metabolic outcomes. A weight loss of >5% appeared necessary for beneficial effects on HbA1c, lipids, and blood pressure[31]. The American Heart Association/American College of Cardiology/The Obesity Society Guideline also reported that weight losses of <5% lowered HbA1c by 0.2% to 0.3% and weight Losses of >5% lowered HbA1c by 0.6% to 1.0%, but the statistical significance of these data are not reported[32]. Also, based on the new guideline of ADA in 2023, overweight and obese diabetic patients should lose 7% of their weight in 6 months to improve their glycemic profile[33].
In 2016, Steven *et al.* revealed that following a very low-calorie diet (VLCD) for 8 weeks improved glycemic control in T2D patients[9]. They also found that weight loss normalized insulin sensitivity and fat content of liver. Another research revealed that a 6 months intervention with 25% calorie restricted diet led to significant reductions in BMI, FBS, blood pressure, WC, HbA1c, and LDL-to-HDL cholesterol ratio in patients with T2DM[34]. We did not find any significant differences between groups in term of SBP and DBP.

In the present study, we showed that calorie restriction in overweight and normal weight diabetic patients led to a significant reduction in ALP concentration compared to control groups. However, we did not find any significant differences among groups in term of AST and ALT. Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic complications of T2DM[35]. In line with our findings, Larson et al. reported that adherence from 6-month calorie restriction and exercise had no significant effects on ALT concentration and lipid profile among overweight and obese people[36]. However, Straznicky et al. in a randomized clinical trial among patients with metabolic syndrome showed that after 12 weeks adherence from a calorie-restricted diet, patients in intervention group had significantly lower levels of liver enzymes[36]. Part of the observed differences in liver enzymes was due to different composition of macronutrients in different studies. Some studies have shown that low-calorie diets with limited carbohydrates had a greater effect on reducing liver enzymes[37]. The limitations of this study must be considered. First, examination period was limited to 3 months and a balanced low-calorie diet was followed. In some studies, a period of 6 months or even 12 months has been used to evaluate the effect of low-calorie diets, which may be a reason for the discrepancies observed in our study compared to some other studies due to the duration of the intervention. Second, the sample size of the study was small. Therefore, for future research, it is recommended that larger populations and longer time periods be studied. Considering the findings of present trial and similar studies, it could be concluded that calorie restriction and weight loss were effective and safe interventions for diabetes management. Calorie restriction could be used as a complementary intervention in patients suffering from T2DM. In conclusion, the results of the present study revealed that short-term nutritional intervention with a balanced low-caloric diet could reduce body weight and total body fat, mainly found in abdominal region, and efficiently improve insulin sensitivity and decrease cardiovascular risk in normal and overweight patients with T2DM.

Conflict of interest

Author declares no conflict of interest.

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