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Effects of short-term very low-calorie diet on intramyocellular lipid and insulin sensitivity in nondiabetic and type 2 diabetic subjects

Cristina Lara-Castro[a,*], Bradley R. Newcomer[b], Jennifer Rowell[c], Penny Wallace[a], Sara M. Shaughnessy[d], A. Julian Munoz[a], Alanna M. Shiflett[a], Dana Y. Rigsby[a], Jeannine C. Lawrence[a], Daryl E. Bohning[e], Steven Buchthal[b], W. Timothy Garvey[a,d,f]

[a]*Department of Nutrition Sciences, University of Alabama, Birmingham, AL 35294, USA*
[b]*Department of Diagnostic and Therapeutic Sciences, University of Alabama, Birmingham, AL 35294, USA*
[c]*Department of Medicine, University of Alabama, Birmingham, AL 35294, USA*
[d]*Department of Medicine, Medical University of South Carolina, Charleston, SC 29403, USA*
[e]*Department of Radiology, Medical University of South Carolina, Charleston, SC 29403, USA*
[f]*Birmingham Veterans Affairs Medical Center, Birmingham, AL 35233, USA*

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Abstract

The study aimed to analyze the effects of a short-term very low-calorie diet (VLCD) on intramyocellular lipid (IMCL), total body fat, and insulin sensitivity in a group of obese nondiabetic and type 2 diabetic subjects. Seven untreated type 2 diabetic and 5 obese nondiabetic individuals were studied before and after a 6-day VLCD using proton magnetic resonance spectroscopy to quantify IMCL, dual-energy x-ray absorptiometry to assess body fat, and hyperinsulinemic-euglycemic clamps to measure peripheral insulin sensitivity. In both groups, decrements in total body fat mass and body mass index were small but statistically significant. In contrast, the diet resulted in a pronounced reduction in IMCL compared with baseline values in nondiabetic subjects (56% decrease) and type 2 diabetic subjects (40% decrease) ($P < .05$), and this was accompanied by an overall 9.3% increase in maximally stimulated glucose disposal rate ($P < .01$). Intramyocellular lipid was significantly correlated with insulin sensitivity ($r = -0.69$, $P < .01$) and waist circumference ($r = 0.72$ and 0.83, baseline and postdiet, respectively; both $P < .01$), but neither IMCL nor insulin sensitivity was related to measures of general adiposity such as body mass index, percentage of body fat, or total body fat (P = not significant). In conclusion, short-term VLCD is accompanied by small decrements in general adiposity, marked decrease in IMCL, and an increase in insulin sensitivity in nondiabetic and type 2 diabetic subjects. Therefore, rapid amelioration of insulin resistance by VLCD can be partially explained by loss of IMCL both in nondiabetic and type 2 diabetic subjects in the absence of substantial changes in total body fat. These observations are consistent with the idea that insulin resistance is more directly related to IMCL rather than to body fat per se.

1. Introduction

Skeletal muscle insulin resistance is a key metabolic abnormality that characterizes type 2 diabetes mellitus (T2DM) and metabolic syndrome, and elucidation of causal mechanisms is critical to the development of effective therapy and prevention. Obesity is commonly evoked as a cause or contributor to insulin resistance despite the fact that generalized obesity can only explain a relatively small portion of individual variability in insulin sensitivity in cross-sectional studies [1]. Nevertheless, there is a strong epidemiological link between obesity and the development of T2DM, and moderate weight reduction has consistently been reported to improve insulin sensitivity. This paradox points to the possibility that other factors, perhaps associated with obesity, may play a more direct role in the pathogenesis of insulin resistance. For example, relative upper body fat distribution, alterations in adipokine secretion, or increased intramyocellular lipid (IMCL) may directly influence insulin sensitivity in a manner that is only partially or indirectly related to overall adiposity. In support of this hypothesis, obese insulin-resistant adolescents were found to have greater quantities of

* Corresponding author. Tel.: +1 205 996 4014; fax: +1 205 934 7049.
E-mail address: larac@uab.edu (C. Lara-Castro).

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IMCL and visceral fat and lower adiponectin values compared with matched obese insulin-sensitive counterparts, despite similar degrees of adiposity [2].

In recent years, new evidence has shed insights on the interrelationship involving IMCL and the development of insulin resistance associated with abnormalities in fatty acid metabolism. For example, healthy subjects showed a decrease in insulin sensitivity and an increase in IMCL after short-term intralipid infusion [3,4], and conversely, long-term hypocaloric diets or malabsorption induced by biliopancreatic diversion were found to lead to enhanced insulin sensitivity and decreased IMCL [5-7]. These observations have suggested a causal effect of IMCL on the development of insulin resistance. However, the link between IMCL and insulin resistance in T2DM is not so clear. Petersen et al [8] found that obese and insulin-resistant type 2 diabetic patients had improved insulin sensitivity despite no significant decrease in IMCL after a moderate hypocaloric diet (1200 kcal/d) over an average of 7 weeks. Tamura et al [9] found that weight loss produced by a moderate hypocaloric diet alone (25-30 kcal/kg per day) over 2 weeks reversed hepatic steatosis but did not improve insulin sensitivity or affect IMCL. Thus, in these 2 studies involving type 2 diabetic patients, moderate hypocaloric diets per se did not significantly decrease IMCL over 2 to 7 weeks. However, there have been no studies examining whether short-term substantial caloric restriction would affect IMCL accumulation and insulin sensitivity in type 2 diabetic or nondiabetic subjects. The aim of the current study was to test whether a short-term very low-calorie diet (VLCD; 700 kcal/d for 6 days) would significantly decrease IMCL in both type 2 diabetic and nondiabetic subjects and to use this perturbation to study the interrelationships between IMCL, insulin sensitivity, and generalized adiposity.

2. Research design and methods

2.1. Subject characteristics

We studied 12 (10 females, 2 males) overweight and obese subjects with and without T2DM [10], and the clinical characteristics of the study group are listed in Table 1. Mean hemoglobin A_{1c} in T2DM was 8.6 ± 1.3. Before the study, all subjects with T2DM (n = 7) were being treated with diet or sulfonylurea and/or metformin but were withdrawn from therapy for at least 3 weeks and followed on an outpatient basis. Mean baseline weight had to be stable (±3%) for at least 3 months before study, and none of the study subjects engaged in regular exercise. None of the volunteers had cardiovascular, renal, or hepatic disease, and all were chemically euthyroid. No subjects were ingesting any pharmacologic agents known to affect carbohydrate homeostasis, lipids, or lipoprotein metabolism. Protocols were approved by the institutional review board, and written informed consent was obtained from every subject.

2.2. Hypocaloric feeding protocol

After medical screening, volunteers were admitted as inpatients to the General Clinic Research Center (GCRC) in the afternoon and, over the next 3 days, equilibrated on an isocaloric diet containing 50% carbohydrate, 30% fat, and 20% protein with the help of a registered dietician who worked with

Table 1
Changes in body composition and metabolic parameters at baseline and after 6-day VLCD in the study groups

	Nondiabetic subjects (n = 5[a], females)		Type 2 diabetic subjects (n = 7[a], 5 females/2 males)	
	Before	After	Before	After
Age	38 ± 12		43 ± 6	
BMI (kg/m^2)	36 ± 5	35 ± 5 *	37 ± 7	35 ± 7 **
Fat mass (kg)	44 ± 10	43 ± 10	45 ± 19	44 ± 18
Lean body mass (kg)	43 ± 4	42 ± 4	58 ± 10	56 ± 9
% Fat	49 ± 5	49 ± 5	41 ± 11	41 ± 11
Waist (cm)	99 ± 10	96 ± 9 *	117 ± 14	113 ± 15 *
IMCL (a.u.)	7.9 ± 3.0	3.6 ± 2.0 **	18.6 ± 13.7	11.2 ± 10.9 *
Fasting plasma glucose (mmol/L)	5.05 ± 0.55	4.77 ± 0.22	11.93 ± 4.44 †	9.82 ± 3.71
Fasting plasma insulin (pmol/L)	144 ± 72	84 ± 18	90 ± 72	78 ± 72
GDR (mg · kg^{-1} · min^{-1})	13.3 ± 2.2	14.7 ± 1.8	6.4 ± 1.5 †	7.5 ± 1.1
HOMA-IR	5.5 ± 2.6	3.8 ± 1.4 *	6.9 ± 4.0	5.4 ± 5.1 *
Adiponectin (μg/mL)	7.4 ± 1.8	7.8 ± 0.8	7.8 ± 2.1	6.2 ± 2.1
RQ	0.85 ± 0.02	0.79 ± 0.06	0.78 ± 0.05 †	0.80 ± 0.02
Fat oxidation (g · kg lbm^{-1} · day^{-1})	1.3 ± 0.2	1.9 ± 0.6	2.0 ± 0.7 †	1.8 ± 0.1
Glucose oxidation (g · kg lbm^{-1} · day^{-1})	3.7 ± 1.2	2.5 ± 1.4	1.5 ± 1.2 †	1.9 ± 0.7
FFA (mmol/L)	400 ± 120	680 ± 90 **	570 ± 100	710 ± 180 *

a.u. indicates arbitrary units; RQ, respiratory quotient; lbm, lean body mass; FFA, free fatty acids.

[a] Except for GDR, where n = 5 (before) and n = 4 (after), for both nondiabetic and type 2 diabetic subjects.

* $P < .05$, significantly different from baseline within the group.

** $P < .01$, significantly different from baseline within the group.

† $P < .05$, significantly different from nondiabetic subjects.

the patients during the length of the protocol. Baseline studies were performed including anthropometrics, oral glucose tolerance test, dual-energy x-ray absorptiometry (DXA) for body composition, proton magnetic resonance spectroscopy assessment of IMCL, indirect calorimetry, and a hyperinsulinemic clamp study to assess insulin sensitivity. On day 4 of admission, the patients started with the VLCD containing 700 kcal/d, with the same macronutrient distribution as in the initial diet. Patients remained on this diet for 6 days. At the end of this period, the metabolic studies performed at baseline were repeated to assess effects of the dietary intervention. During these repeat measurements, the VLCD was continued to ensure that the subjects were maintained in a negative energy balance state. Throughout the study, participants remained as inpatients and all meals were prepared and provided by the metabolic kitchen of the GCRC.

2.3. Oral glucose tolerance test

Standard 75-g oral glucose tolerance tests were performed after a 12-hour overnight fast [10]. Among the 5 nondiabetic subjects, 4 subjects were classified as having a normal oral glucose tolerance test result and 1 was found to have impaired fasting glucose. Seven patients had T2DM.

2.4. Insulin sensitivity

In vivo insulin sensitivity was assessed using the euglycemic-hyperinsulinemic glucose clamp technique at a maximally effective steady-state serum insulin concentration as previously described [11]. Briefly, after a 12-hour fast, a catheter was inserted into the brachial vein to administer insulin, glucose, and KPO_4. A dorsal hand vein was cannulated in a retrograde manner and kept in a warming device (65°C) to provide arterialized venous blood for sampling. To maximally stimulate glucose uptake and suppress hepatic glucose production, we administered regular insulin (Humulin; Eli Lilly, Indianapolis, IN) at a rate of 200 $mU \cdot m^{-2} \cdot min^{-1}$, producing a mean steady-state insulin concentration of 3480 ± 138 pmol/L, which is maximally effective for stimulating glucose uptake into skeletal muscle [11]. Serum glucose was clamped at 5.0 mmol/L for at least 3 hours, and maximal glucose uptake for each individual was calculated from the mean glucose infusion rate over the final three 20-minute intervals. Whole-body glucose uptake was calculated based on the glucose infusion rate corrected for changes in the glucose pool size, assuming a distribution volume of 19% body weight and a pool fraction of 0.65. Glucose uptake was normalized per kilogram lean body mass (excluding bone mass) determined by DXA to yield the glucose disposal rate (GDR) per kilogram of lean body mass. Lower GDR values indicate greater insulin resistance. During initial baseline studies, only 10 of 12 subjects were able to complete the hyperinsulinemic-euglycemic clamp and only 8 of those 10 subjects were able to complete this test at the end of the dietary period because of problems with venous access.

2.5. Homeostatic model assessment insulin sensitivity index

The homeostatic model assessment index was used to determine the levels of insulin resistance (HOMA-IR) using fasting insulin and glucose levels and the formula [glucose (in millimoles per liter) × insulin (in microunits per milliliter)]/22.5. The HOMA index was calculated for all subjects before and after intervention and is presented to complement the data on hyperinsulinemic clamps that were not available for all patients as explained above.

2.6. Intramyocellular lipid

Intramyocellular lipid was quantified using proton magnetic resonance spectroscopy. Because of logistical circumstances, different magnetic resonance scanners were used to study the nondiabetic and diabetic subjects. For any given subject, all measurements were performed on the same scanner and with the same acquisition parameters, which permitted baseline and postdiet comparisons in these individuals. For instance, all studies on type 2 diabetic subjects (n = 7) were run on a 1.5-T clinical magnetic resonance scanner with a commercially provided ^1H transmit/receive knee coil (Philips Gyroscan 1.5 T ACS-NT PT6000 with actively shielded compact gradients and the ^1H spectroscopy software package f/ACS-NT; Philips Medical Systems, Bothell, WA). All studies on this 1.5-T system used a point-resolved spectroscopy (PRESS) single-voxel acquisition sequence in which 3 water-suppressed PRESS voxels (echo time [TE] = 33 milliseconds, repetition time [TR] = 5000 milliseconds, 2 × 2 × 1 cm^3 voxel size) were acquired from 3 different locations in the soleus. These locations were chosen to give an overall characterization of soleus muscle lipid content. Separate non–water-suppressed spectra were also collected under fully relaxed conditions so that the water signal could be used as an amplitude reference (TE = 33 milliseconds, TR = 5000 milliseconds, 2 × 2 × 1 cm^3 voxel size). On all follow-up studies in this group of patients, the location of the PRESS voxels was kept constant.

In a similar manner, all studies in obese nondiabetic subjects (n = 5) were run on a 4.1-T whole-body imaging and spectroscopy system interfaced to a Bruker console (Bruker Instruments, Billerica, MA). All subjects' legs were positioned inside a laboratory-built ^1H birdcage coil with the knee in extension and the ankle in a neutral position. Intramyocellular lipid was measured on this system using a commercially provided slice selective 2-dimensional magnetic resonance spectroscopic imaging sequence. Details concerning this technique and the reproducibility of these methods have been previously published [12]. In brief, after the acquisition, a series of 36 spectra from a 6 × 6-voxel region of interest was summed to provide a single spectrum that represented a 2.25-mL area of soleus muscle. This single summed spectrum is what was analyzed for each subject. On all follow-up studies in this group of patients, the magnetic resonance spectroscopic imaging slice location and the location of the summed region of interest were kept constant.

All spectra were analyzed by fitting the peak positions and areas through time-domain fitting using Java-based magnetic resonance user interface [13]. The same fitting procedures were used regardless of the system used for acquiring the spectra and all fitting models and sets of prior knowledge information have been previously published [14-17]. To account for day-to-day variation in system performance, our protocol normalized the IMCL peak amplitudes to the corresponding internal water peak amplitude in that same muscle location. This is similar to the methodology described by Krssak et al [18]. All peak areas in this study are expressed in arbitrary units per pixel area relative to internal water and have been corrected for relaxation effects by using published T_1's and T_2's for water and IMCL at 1.5 T [19] and 4 T [20]. Using the combination of the internal water as an intensity reference with correcting for T_1 and T_2 relaxation effects allows us to compare the 1.5- and 4.1-T measurements of IMCL in our subject groups.

2.7. Indirect calorimetry

After an overnight fast, resting energy expenditure, fat oxidation, and carbohydrate oxidation were measured by indirect calorimetry using a Deltratrac metabolic monitor (Deltratrac II, SensorMedics, Yorba Linda, CA) as previously described [21]. Measurements began after 30 minutes of rest while supine in bed. Expired air was collected using the adult-size ventilated canopy system for 20 minutes after a 10-minute equilibration. Whole-body oxygen consumption and carbon dioxide production were calculated by measuring gradients across the face and the flow rates of air using Haldane transformation. Rates of lipid and carbohydrate oxidation were determined from the respiratory quotient and normalized per kilogram of metabolically active body mass, as previously described [21].

2.8. Anthropometric and body composition

Body mass index (BMI) was calculated as the weight in kilograms divided by the square of height in meters (kg/m^2). Fat distribution was assessed by waist and hip circumferences (cm) using a tension-controlled tape measure by Novel Products (Rockton, IL). Dual-energy x-ray absorptiometry scanning was performed using DPX-L (Lunar Radiation, Madison, WI) with the use of software version 1.33 (Lunar) and provided body composition measures including total body fat, percentage of body fat, and lean body mass independent of bone mass. Lean body mass was used to normalize rates of glucose disposal and fuel oxidation.

2.9. Other assays

Plasma glucose was measured with the glucose oxidase method using a glucose analyzer (YSI 2300; Yellow Springs Instruments, Yellow Springs, OH). Serum insulin levels were measured using an electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). In our laboratory, this assay has a mean intra-assay coefficient of variation of 5% and a mean interassay coefficient of variation of 6%. Plasma adiponectin levels were measured using an enzyme-linked immunosorbent assay kit (Linco Research, St Charles, MO). Plasma free fatty acids were measured using a non-esterified fatty acids (NEFA) C kit (Wako Chemicals, Richmond, VA) as described by the manufacturer; the intraassay coefficient of variation is 0.8%.

2.10. Statistical analyses

All data are given as means ± SD unless otherwise indicated. Logarithmic transformation was used for normalization of the variables of interest when appropriate. The correlations between baseline insulin sensitivity and different metabolic and anthropometric variables were examined using Spearman correlation coefficients. Changes in outcomes of interest before and after diet were compared using paired *t* test. These analyses were performed separately for the entire group, and in subgroups consisting of nondiabetic and type 2 diabetic subjects. The SAS program version 8.0 (SAS Institute, Cary, NC) was used for analyses. Differences were accepted as significant at $P < .05$.

3. Results

3.1. Baseline characteristics

A total of 12 subjects were studied, 5 nondiabetic and 7 with T2DM. Baseline and postdiet characteristics of the study subjects are shown in Table 1.

3.2. Effects of a 6-day VLCD in nondiabetic and type 2 diabetic subjects

Over the course of the 6-day diet, obese nondiabetic subjects lost an average of 2.3 kg ($P < .05$) and type 2 diabetic subjects lost an average of 3.7 kg of body weight ($P < .01$). Table 1 shows mean baseline and postdiet levels of anthropometric and metabolic variables for nondiabetic and type 2 diabetic subjects. Body mass index and waist circumference decreased modestly after the diet; BMI decreased by 2.5% ($P < .05$) and 5% ($P < .01$) in nondiabetic and type 2 diabetic subjects, respectively, and was accompanied by decreases in waist circumference of around 3% in both groups (both $P < .05$). Importantly, the diet produced more profound decrements in IMCL in all patients ranging from 16% to 74% compared with baseline values. These decrements averaged 56% ($P = .006$) and 40% ($P = .04$) in nondiabetic and type 2 diabetic subjects, respectively, as shown in Fig. 1. Changes in IMCL in these 2 groups are also outlined in Table 1. In the entire group, these changes in body composition and IMCL were accompanied by a significant increase in insulin sensitivity, characterized by a 14% increase in maximally stimulated GDR from 9.8 ± 4.0 to 11.1 ± 4.1 mg/kg lean body mass per minute at the end of the dietary period ($P < .01$) (Fig. 1). Significant increments in insulin sensitivity were also documented in both nondiabetic and type 2 diabetic subjects by HOMA-IR as shown in

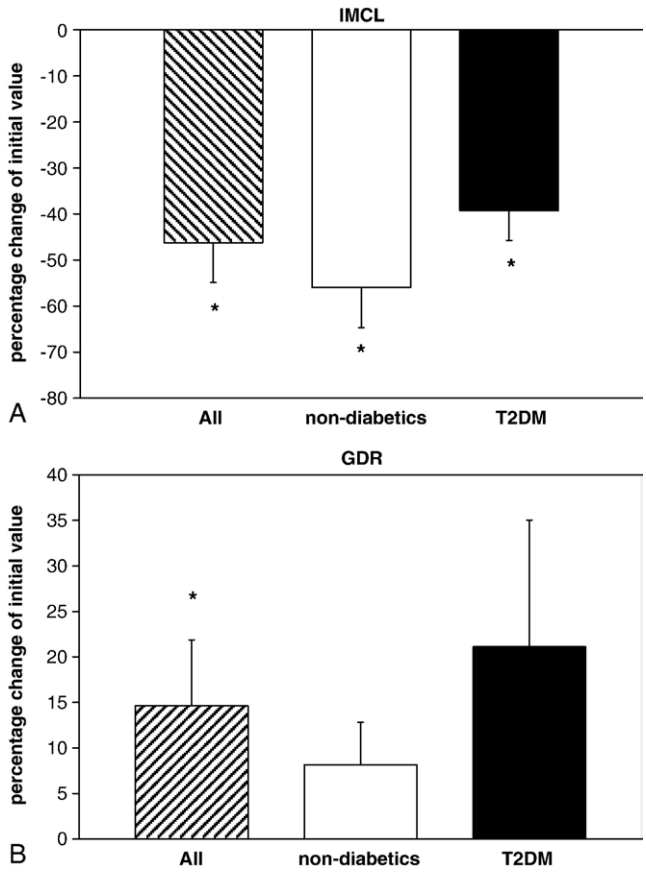

Fig. 1. Percent change in IMCL and GDR in all subjects (n = 12), nondiabetic subjects (n = 5), and type 2 diabetic subjects (n = 7) after short-term VLCD. Intramyocellular lipid was assessed in the soleus muscle of each individual using proton nuclear magnetic resonance spectroscopy, and GDR was assessed using the euglycemic-hyperinsulinemic glucose clamp technique. *$P < .05$, significantly different from baseline values.

Table 1. Overall, these metabolic and anthropometric changes occurred for all subjects regardless of diabetic status and were not proportionally different between these 2 groups. Levels of plasma free fatty acids increased significantly in nondiabetic subjects (from 400 ± 120 to 680 ± 90 μmol/L, $P < .01$) and type 2 diabetic subjects (from 570 ± 100 to 710 ± 180 μmol/L, $P < .05$), reflecting increased lipolysis secondary to caloric restriction. Whole-body rates of fat or carbohydrate oxidation did not change significantly after the intervention.

3.3. Relationships between insulin sensitivity and measures of regional and total adiposity before and after 6-day VLCD

Insulin sensitivity measured by GDR was found to be negatively correlated with IMCL ($r = -0.69$, $P < .01$) as shown in Fig. 2A and with waist circumference ($r = -0.59$, $P < .01$) as shown in Fig. 2B. In contrast, insulin sensitivity in these subjects was not correlated with BMI ($r = -0.12$, P = not significant [NS]; Fig. 2C). When analyzed before and after VLCD, the correlations between IMCL and insulin sensitivity showed a trend to significance ($r = -0.58$ and $r = -0.67$, before and after, respectively; both $P = .06$). Similarly, waist circumference showed borderline correlations with insulin sensitivity ($r = -0.55$ and -0.63, before and after, respectively; both $P = .09$). Intramyocellular lipid was highly correlated with waist circumference both at baseline and postdiet ($r = 0.72$ and 0.83, respectively; both

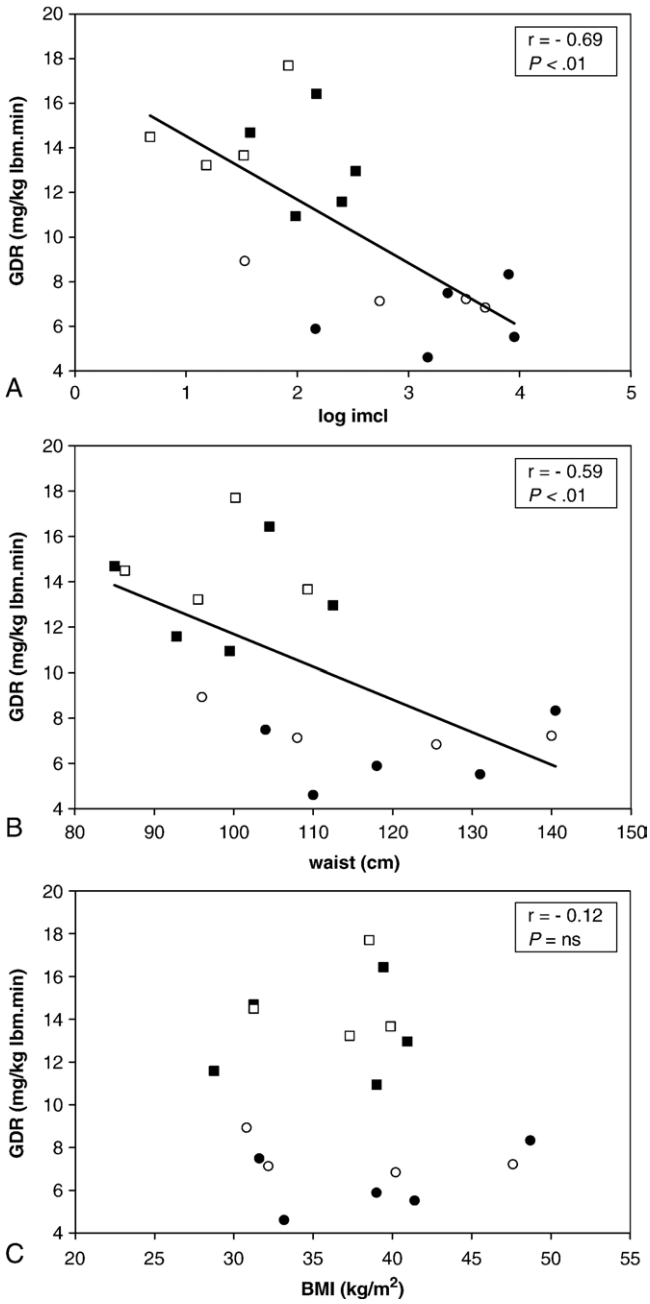

Fig. 2. Relationships between insulin sensitivity and IMCL, waist circumference, and BMI before and after VLCD in the group of nondiabetic and diabetic subjects. The figures show correlations between GDR (measured by hyperinsulinemic-euglycemic clamp) and IMCL (measured by proton nuclear magnetic resonance spectroscopy, A), waist circumference (B), and BMI (C). Circles represent diabetic subjects before (●) and after (○) VLCD; and squares represent nondiabetic subjects before (■) and after (□) VLCD.

$P < .01$). However, IMCL was not related to measures of total adiposity including total fat mass ($r = 0.32$ and 0.42, baseline and postdiet, respectively; P = NS) or BMI ($r = 0.45$, and 0.53 baseline and postdiet respectively, P = NS). Interestingly, the changes in IMCL were inversely correlated with the changes in plasma free fatty acids ($r = -0.71$, $P < .05$) because IMCL levels decreased, whereas free fatty acid levels increased as a result of the hypocaloric dietary intervention. Thus, greater decrements in IMCL were progressively associated with higher levels of circulating free fatty acids. Baseline plasma adiponectin levels did not differ from postdiet values (7.6 ± 1.8 vs 6.9 ± 1.8μg/mL, respectively).

4. Discussion

To the best of our knowledge, this is the first study investigating the effects of short-term caloric restriction on IMCL and the relationships between changes in IMCL and whole-body metabolism in this context. In addition, our data address the effects of a VLCD on IMCL in both type 2 diabetic and nondiabetic subjects. Results from this study indicate that IMCL was decreased concomitant with an increase in insulin sensitivity after a short-term diet providing 700 kcal/d and that these changes occurred in both diabetic and nondiabetic subjects. Furthermore, insulin sensitivity was significantly correlated with IMCL and waist circumference in the entire group. In contrast, diet-induced changes in IMCL and insulin sensitivity were accompanied by only small effects on total body fat and BMI, and these measures of general adiposity were not correlated with IMCL and insulin sensitivity. Therefore, increments in insulin sensitivity observed with short-term VLCD are associated with loss of IMCL in the absence of marked changes in total body fat.

Several intervention studies, varying in length from weeks to months, have explored the effects of mild to moderate caloric restriction on IMCL [3-9,22]. Most of these studies have focused on obese nondiabetic subjects who achieved weight loss through surgically induced malabsorptive procedures or through moderate dietary caloric restriction. Most of these studies established that IMCL was reduced in response to these interventions in conjunction with a parallel increase in insulin sensitivity. A cause-effect relationship between IMCL accumulation and insulin signaling has been previously hypothesized [6], and a feedback mechanism involving effects of intracellular triglycerides and long-chain fatty acyl coenzyme A on insulin action and glucose metabolism has been posited [23]. Fewer studies have addressed the effects of weight loss on IMCL and insulin sensitivity in T2DM and have only then used mild to moderate caloric restriction [5,8,9]. Petersen et al [8] found that type 2 diabetic subjects undergoing moderate weight reduction on a low-fat diet providing approximately 1200 kcal/d experienced hepatic but not peripheral changes in insulin sensitivity. These experimental conditions led to a marked reduction in hepatic triglyceride and an increase in hepatic insulin sensitivity with no changes in IMCL or peripheral glucose metabolism. In this study, the length of the dietary intervention was variable, between 3 to 12 weeks including a period of weight stabilization, and was targeted toward achievement of normoglycemia rather than a predefined amount of weight loss. Tamura et al [9] randomized subjects with T2DM to either diet (1700 kcal/d for 2 weeks) or diet plus exercise for 2 weeks. Again, in contrast to the current findings, the diet-only arm resulted in a decrease in intrahepatic lipid but did not affect IMCL or peripheral insulin sensitivity. These previous studies suggest that IMCL may not be responsive to hypocaloric diets in T2DM. Differences between previous intervention studies and the one reported here include degree of caloric restriction and length of the intervention. For instance, our 6-day inpatient protocol provided less calories (700/d) and was aimed to determine the short-term effect of a severe caloric restriction on levels of IMCL and its relation to insulin sensitivity. Overall, our findings suggest that a more robust caloric restriction may be necessary to initiate mobilization of IMCL stores and further activate signaling pathways aimed to improve muscle glucose uptake. Another pertinent factor in study design is whether the dietary intervention was followed by a period of weight stabilization before postdiet assessment. The study by Petersen et al did feature isocaloric equilibration, whereas the current study and the study by Tamura et al did not. In the current study, the observed decrements in IMCL on a VLCD reflect active mobilization of IMCL stores under predominantly negative energy balance conditions and illustrate the dynamic nature of this highly active lipid pool [3,4]. An important finding was that although obese and type 2 diabetic subjects are characterized by defects in substrate oxidation [7,24] including impaired lipid oxidation [25,26], we observed that both nondiabetic and type 2 diabetic subjects responded to a significant caloric restriction by decreasing IMCL stores. Under conditions of reduced energy intake resulting in lipolysis and elevated circulating free fatty acids, there is increased reliance on fat oxidation by muscle as the predominant source of fuel [27]. Our results indicate that IMCL responds immediately to caloric manipulation [28,29] and that IMCL represents a dynamic fuel compartment that provides readily available energy for muscle function in both type 2 diabetic and nondiabetic subjects.

Although IMCL was severely depleted after the short-term dietary intervention in both type 2 diabetic and nondiabetic subjects, these changes were accompanied by only small changes in general adiposity, which was assessed by BMI and measures of total fat mass and percentage of body fat. Moreover, before and after the intervention, measures of generalized adiposity were not correlated with either IMCL or insulin sensitivity, probably as a result of the small sample size of the study. On the other hand, upper body fat

distribution as reflected by waist circumference was borderline correlated with insulin sensitivity and did vary as a function of IMCL. These observations suggest that measures of regional adiposity, IMCL, and abdominal fat, rather than measures of generalized adiposity, might be more pathophysiologically relevant to insulin resistance. However, 2 functional aspects of adipose tissue do not appear to be involved in the loss of IMCL and increase in muscle glucose disposal in response to the diet. First, IMCL was reduced despite an increase in circulating free fatty acids and free fatty acid availability, with no measurable change in basal rates of lipid and carbohydrate oxidation. Infusion of lipid emulsions has been previously shown to immediately induce insulin resistance together with augmentation in IMCL [3,4]; however, in the current setting on the hypocaloric diet, increments in free fatty acids brought about by lipolysis during hypocaloric feeding were associated with a decrease rather than an increase in IMCL. The absence of change in whole-body basal lipid oxidation indicates that IMCL was reduced by preferential oxidation of IMCL over circulating free fatty acids in skeletal muscle and supports previous observations indicating that free fatty acids taken up by resting muscle are not oxidized directly but probably enter the intramuscular pool, which is the immediate source of oxidized lipid substrate [30]. Secondly, circulating levels of the adipokine, adiponectin, were unchanged after the VLCD diet. Other studies have demonstrated that adiponectin levels are increased after substantial weight loss; however, in the current study, short-term caloric restriction itself, without major loss of body fat, was not sufficient to increase adiponectin concentrations. Our results are in agreement with a few other studies showing that small to moderate weight loss after conventional caloric restriction was not correlated with changes in adiponectin levels [31,32]. In contrast, studies reporting major weight loss after bariatric surgery consistently report increases in adiponectin levels [33,34] supportive of the idea that more severe or chronic weight loss may be necessary to alter plasma adiponectin. In our study, the absence of correlations between adiponectin and insulin sensitivity or IMCL suggests that under short-term caloric restriction, adiponectin does not play a direct role in altering peripheral insulin sensitivity or IMCL levels.

The current results underscore the relevance of IMCL as a target for treatment and prevention of T2DM and the metabolic syndrome. Limitations of the study include the small sample size when nondiabetic and type 2 diabetic subjects are considered as separate subgroups. Also, studies at the end of the dietary period were performed while subjects were continued on the hypocaloric diet without a period of isocaloric equilibration. It will be important to observe whether the decrease in IMCL due to short-term VLCD is retained during isocaloric stabilization and to examine the relationship between IMCL and insulin sensitivity under these conditions. In addition, we did not assess hepatic fat content changes and their contribution to the improved metabolic condition, as well as the role of other markers with the potential to affect insulin sensitivity and cellular energy balance (ie, tumor necrosis factor α, adenosine monophosphate kinase).

In conclusion, short-term VLCD resulted in marked decrements in IMCL together with increases in insulin sensitivity, in both nondiabetic and type 2 diabetic subjects. At the same time, the dietary intervention produced small to minimal changes in measures of general adiposity, such as BMI, percentage of body fat, or total fat mass, which were not related to the effects on IMCL or insulin sensitivity. Adiponectin levels did not change in response to the diet, and circulating fasting free fatty acid levels were elevated indicative of increased delivery to skeletal muscle. Therefore, the increase in insulin sensitivity and reduction in IMCL reflected responses that were primarily for skeletal muscle and could not be explained by effects originating in adipose tissue as reflected by adiponectin secretion or free fatty acid availability. By inference, a partial component of the increase in insulin sensitivity observed after longer-term diets may be related to changes in IMCL and other fat depots than to reductions in total body fat. Thus, after short-term VLCD, IMCL appears to be an important correlate of insulin sensitivity in both nondiabetic and type 2 diabetic subjects.

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