Original Article
OBESITY BIOLOGY AND INTEGRATED PHYSIOLOGY

Obesity

Glucose and Lipid Homeostasis and Inflammation in Humans Following an Isocaloric Ketogenic Diet

Michael Rosenbaum [1], Kevin D. Hall[2], Juen Guo[2], Eric Ravussin[3], Laurel S. Mayer[4], Marc L. Reitman [2], Steven R. Smith[5], B. Timothy Walsh[4], and Rudolph L. Leibel[1]

Objective: The objective of this study was to measure changes in glucose, lipid, and inflammation parameters after transitioning from a baseline diet (BD) to an isocaloric ketogenic diet (KD).
Methods: Glucose homeostasis, lipid homeostasis, and inflammation were studied in 17 men (BMI: 25-35 kg/m^2) during 4 weeks of a BD (15% protein, 50% carbohydrate, 35% fat) followed by 4 weeks of an isocaloric KD (15% protein, 5% carbohydrate, 80% fat). Postprandial responses were assessed following mixed-meal tests matched to compositions of the BD (control meal [CM]) and KD (ketogenic meal).
Results: Fasting ketones, glycerol, free fatty acids, glucagon, adiponectin, gastric inhibitory peptide, total and low-density lipoprotein cholesterol, and C-reactive protein were significantly increased on the KD. Fasting insulin, C-peptides, triglycerides, and fibroblast growth factor 21 were significantly decreased. During the KD, the glucose area under the curve was significantly higher with both test meals, and the insulin area under the curve was significantly higher only for the CM. Analyses of glucose homeostasis suggested that the KD insulin sensitivity decreased during the CM but increased during the ketogenic meal. Insulin-mediated antilipolysis was decreased on the KD regardless of meal type.
Conclusions: Switching to the KD was associated with increased cholesterol and inflammatory markers, decreased triglycerides, and decreased insulin-mediated antilipolysis. Glucose homeostasis parameters were diet dependent and test meal dependent.

Obesity (2019) **0**, 1-11. doi:10.1002/oby.22468

Introduction

Very low-carbohydrate, high-fat, ketogenic diets (KDs) have become increasingly popular for the treatment of obesity and type 2 diabetes (1). However, little is known about the effects of transitioning people from a high-carbohydrate diet to an isocaloric KD on glucose and lipid homeostasis or inflammation. Such very low-carbohydrate diets have been reported in some studies to result in decreased energy intake (EI) (2), improved glucose and lipid homeostasis, and decreased inflammatory biomarkers (3). However, most of these studies have been in outpatients in whom it is difficult to disassociate physiological dietary effects from those related to dietary adherence (4,5), even in controlled feeding studies in which all food is provided (6). Free-living diet studies do not investigate the effects of actually consuming diets but instead investigate the effects of the instructions to change diet in the prescribed way. To understand the metabolic effects of an isocaloric KD, an inpatient controlled feeding study is required.

We previously reported the effects of transitioning from 4 weeks of a 15% protein, 50% carbohydrate, and 35% fat baseline diet (BD) followed immediately by 4 weeks of an isocaloric 15% protein, 5% carbohydrate, and 80% fat KD on energy expenditure in 17 men with overweight or class I obesity (7). We also explored the changes in glucose and lipid homeostasis and in inflammatory markers in this population to test the hypothesis that these comorbidity risk factors were significantly affected by dietary macronutrient content.

[1] Departments of Pediatrics and Medicine, Division of Molecular Genetics, Columbia University Irving Medical Center, New York, New York, USA. Correspondence: Michael Rosenbaum (mr475@cumccolumbia.edu) [2] National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland, USA [3] Pennington Biomedical Research Center, Baton Rouge, Louisiana, USA [4] Department of Psychiatry, New York State Psychiatric Institute, Columbia University Irving Medical Center, New York, New York, USA [5] The Translational Research Institute for Metabolism and Diabetes, Orlando, Florida, USA.

Funding agencies: Nutrition Science Initiative (NuSI) was the primary funder for this study. NuSI convened the research team, helped formulate the hypotheses, and provided partial funding. NuSI and its scientific advisors were given the opportunity to comment on the study design and this manuscript, but the investigators retained full editorial control. This work was also supported, in part, by the Intramural Research Program of the National Institutes of Health (NIH), the National Institute of Diabetes and Digestive and Kidney Diseases (KDH, MLR), NIH UL1 TR00040 (Columbia Clinical and Translational Science Award, [MR, RL]), and Nutrition Obesity Research Center Grant P30DK072476 (ER).

Disclosures: The authors declared no conflict of interest.

Clinical trial registration: ClinicalTrials.gov identifier NCT01967563.

Additional Supporting Information may be found in the online version of this article.

Received: 12 December 2018; **Accepted:** 22 February 2019; **Published online 6 May 2019. doi:10.1002/oby.22468**

Methods

Subjects and study protocol

As previously reported (7), 17 men without diabetes and with BMI between 25 and 35 kg/m^2 were inpatients at 4 study sites (Table 1), where they resided on metabolic wards without access to food other than that provided within this study. Subjects spent 23 h/d for 2 consecutive days each week in a respiratory chamber. An initial weight maintenance energy requirement estimate was made as 1.5 times resting energy expenditure obtained by indirect calorimetry during the screening process. Subjects initially received the BD (15% protein, 50% carbohydrate, 35% fat), and caloric intake was further adjusted to achieve energy balance to within 5% of the average daily energy expenditure measured by the respiratory chamber based on the average of the two 23-hour chamber stays during that week of the BD (range to completion: 2-3 weeks). Subjects underwent 2 consecutive 23-hour chamber stays on the same days each week throughout the study. After 4 weeks of EI stability on the BD, subjects were switched to an isocaloric KD (15% protein, 5% carbohydrate, 80%) for 4 weeks. The last 2 weeks of each diet were designated as the "test period" to allow subjects time to demonstrate persistent agreement between dietary intake and chamber calorimetry during the BD and to allow accommodation for the switch to the KD.

The study protocol was approved by the institutional review boards of the National Institute of Diabetes and Digestive and Kidney Diseases (ClinicalTrials.gov identifier NCT01967563), the Pennington Biomedical Research Center (2013-3-PBRC), Columbia University Medical Center (IRB-AAAL7113), and the Translational Research Institute for Metabolism and Diabetes (FH IRB-493675) and was consistent with guiding principles for research involving humans (8). Written informed consent was obtained from all subjects.

Diets

Diets consisted of 7-day rotating menus using NUTRITIONIST PRO software (version 1.3; First Databank Inc., The Hearst Corp., San Bruno, California). The energy and macronutrient composition for each day was verified by chemical analysis (Covance Laboratories, Madison, Wisconsin). Food was prepared in the Pennington Biomedical Research Center metabolic kitchen, frozen, and shipped to the study sites, where meals were prepared for consumption according to standardized procedures and addition of fresh produce. Both menus contained minimal quantities of processed food, and despite the large differences in macronutrient composition, the ratios of refined to unrefined sugars were similar between the diets. This control of macronutrient quality permitted examination of the effects of the relative macronutrient contents without confounding due to differences in the types of nutrients (refined vs. unrefined sugars, simple vs. complex carbohydrates, saturated vs. mono- or polyunsaturated fatty acids) used. Quality of protein was monitored by using similar protein sources on corresponding BD and KD menu days (e.g., white-meat chicken on the BD and dark-meat chicken on the KD). Sample menus have been reported previously (7).

TABLE 1 Subject characteristics and weight changes during study

	Age (y)	Enrollment weight (kg)	Week 2 weight (kg)	Week 4 weight (kg)
BD	33.6 (7.3)[a]	89.1 (15.9)	**88.1 (15.6)**[b,c]	**87.4 (15.4)**[c]
KD			**85.7 (14.9)**[d]	85.1 (14.6)

Data are mean (SD).
Subjects consistently lost weight during study, indicating that they were in negative energy balance. Previous studies of body composition and energy expenditure during study indicate that magnitude of this negative energy balance was approximately 60 kcal/d (43).
[a]$P<0.001$ vs. all other time points.
[b]$P<0.005$ vs. BD week 4.
[c]$P<0.001$ vs. KD week 2 or week 4.
[d]$P<0.01$ vs. KD week 4.
BD, baseline diet; KD, ketogenic diet.

Laboratory

Subjects underwent weekly fasting blood draws for assessment of ketosis, lipids, glucose and fatty acid homeostasis, and inflammation (Table 1). During the second week of EI stability on the BD (defined as week 4), subjects underwent 2 isocaloric mixed-meal tests (MMTs) at ~9 AM, at least 12 hours after their last meal. The test meal consisted of 20% of total daily prescribed energy with macronutrient distribution identical to the BD (designated as a "control meal" [CM]); and at least 3 days later, subjects received a meal of equal calories providing the same macronutrient distribution as the KD (designated as a "ketogenic meal" [KM]). Plasma and serum samples for glucose, insulin, free fatty acids (FFAs), β-hydroxybutyrate, and triglycerides were obtained at −10, −5, 0, 5, 10, 15, 30, 45, 60, 90, and 120 minutes relative to completion of meal consumption. Subjects were then switched to an isocaloric KD (15% protein, 5% carbohydrate, 80% fat) for 4 weeks. During week 4 on the KD, subjects underwent MMT. The order of MMTs on the KD was KM first, followed at least 3 days later by the CM. Assays are described in online Supporting Information.

Calculations and statistical analyses

Data are presented as mean (SD). The primary outcome variables of these biochemical studies were those relevant to glucose homeostasis and circulating lipids, in contrast to earlier studies that focused primarily on energy expenditure (7).

In order to justify the examination of multiple variables relevant to glucose homeostasis without necessitating post hoc adjustments for multiple comparisons (9), assessments of areas under the curve (AUCs) relative to their fasting premeal concentrations for insulin (relative $AUC_{insulin}$) and glucose (relative $AUC_{glucose}$) during MMTs were assessed prior to analysis of other variables relevant to glucose homeostasis. Because there were significant dietary macronutrient effects on insulin and glucose during MMTs (see *Results*), additional, more detailed analyses of glucose homeostasis were performed. Molecules subsequently studied were those that might mechanistically affect or reflect gluconeogenesis (e.g., glucagon) (10), insulin sensitivity (cortisol, FFA (11,12), and fibroblast growth factor 21 [FGF21] (13)), insulinogenesis (gastric inhibitory peptide [GIP] (14), glucagon-like peptide 1 [GLP-1] (15), and peptide YY [PYY] (16)), and inflammation (C-reactive protein [CRP] and interleukin-6 [IL-6]) as well as relative AUCs for triglycerides (relative $AUC_{triglyceride}$), FFAs (relative AUC_{FFA}), and β-hydroxybutyrate (relative $AUC_{\beta\text{-hydroxybutyrate}}$). Similarly, analyses of cholesterol subfractions (high-density lipoprotein [HDL] and low-density lipoprotein [LDL]) and triglycerides were not made until a significant diet effect on total cholesterol was noted.

There are few studies of the effects of isocaloric KDs on β-cell function and insulin sensitivity or fasting versus postprandial assessments of these variables. To address these issues, fasting measurements were used to calculate the homeostatic model assessment (HOMA) of insulin

resistance (IR) (primarily hepatic insulin sensitivity), HOMA-β (insulin release), and adipocyte IR (ADIPO-IR) (17), along with the fasting Belfiore Index (18). Glucose and insulin responses to MMTs were determined using postprandial relative AUCs. Absolute $AUC_{glucose}$ and $AUC_{insulin}$ were used in the calculation of the MMT Belfiore Index ($Belfiore_{MMTglucose}$) (18) normalized using the mean absolute values of $AUC_{glucose}$ and $AUC_{insulin}$ during the CM on the BD. The Matsuda Index—previously validated against clamp studies and in both oral glucose tolerance tests and MMTs (19)—was also calculated as a postprandial index of insulin sensitivity. The Insulinogenic Index (20) and the Insulin Secretion-Sensitivity Secretion Index 2 were chosen to assesses, respectively, β-cell function using isolated time points (0 and 30 minutes) and the absolute AUC data, as well as uncorrected and corrected data for variations in insulin sensitivity (20) from MMT results.

Adipose tissue sensitivity to insulin-mediated changes in circulating FFAs (integrating simultaneous effects on lipolysis and esterification) was calculated from the Belfiore Index ($Belfiore_{MMTFFA}$) (18) and compared with results from the ADIPO-IR, which is based on fasting data. Equations and additional calculations are presented in Table 2.

Within-subjects comparisons were made by ANOVA with repeated measures in which either diets (BD or KD) or MMTs (CM or KM) were used as covariates. To increase the likelihood that all biochemical values were "stable" within study periods, regression equations were calculated comparing values for fasting blood concentrations

TABLE 2 Equations used to calculate indices of insulin secretion, glycemic insulin sensitivity, and FFA-flux insulin sensitivity based on fasting data and mixed-meal tolerance test data

Name	Fasting/ during MMT	Calculation
Measures of insulin secretion in response to glucose		
HOMA-β (20)	Fasting	$360 \times FPI / FPG - 63$
Insulinogenic Index (20)	MMT	$Ins_{30} - FPI / G_{30}$
ISSI-2 (20)	MMT	$AUC_{insulin} / AUC_{glucose} \times$ Matsuda Index
Measures of glycemic insulin sensitivity or resistance		
HOMA-IR (20)	Fasting	$FPG \times FPI / 405$
$Belfiore_{FASTING}$	Fasting	$2 / ([nFPG \times nFPI] + 1)$
Matsuda Index (19)	MMT	$10{,}000 / \sqrt{([FPG \times FPI] \times [\bar{I} \times \bar{G}])}$
$Belfiore_{MMTglucose}$ (18)	MMT	$2 / ([nAUC_{insulin} \times nAUC_{glucose}] + 1)$
Measures of FFA-flux insulin sensitivity[a]		
ADIPO-IR (17)	Fasting	$FPI \times FPFFA$
$Belfiore_{MMTFFA}$ (18)	MMT	$2 / ([nAUC_{insulin} \times nAUC_{FFA}] + 1)$

[a]Magnitude of suppression of FFA circulating concentrations in response to insulin.
ADIPO-IR, adipocyte insulin resistance; AUC, absolute area under the curve during mixed-meal tests; $Belfiore_{FASTING}$, fasting Belfiore Index; $Belfiore_{MMTFFA}$, adipose tissue sensitivity to insulin-mediated changes in circulating free fatty acids calculated from Belfiore Index; FFA, free fatty acid; FPFFA, fasting plasma free fatty acid; FPG, fasting plasma glucose (mg/dL); FPI, fasting plasma insulin (μIU/mL); G, mean plasma glucose during testing; G_{30}, plasma glucose 30 minutes following glucose load; Ins30, plasma insulin 30 minutes following glucose load; MMT, mixed-meal test; HOMA, homeostatic model assessment; IR, insulin resistance; ISSI-2, Insulin Secretion-Sensitivity Secretion Index 2; $Belfiore_{MMTglucose}$, mixed-meal test Belfiore Index; $\bar{I}$, mean insulin value over entire MMT; $\bar{G}$, mean glucose value over entire MMT; nAUC, normalized area under the curve used for Belfiore indices where AUC for test meal is divided by mean AUC for baseline MMT on baseline diet; nFPG and nFPI, normalized fasting plasma glucose and insulin concentrations, respectively, for use in $Belfiore_{FASTING}$ index where fasting concentrations are divided by mean fasting concentrations during baseline diet.

TABLE 3 Comparisons of fasting plasma concentrations of morning ketones and FFAs in 17 subjects during isocaloric KD and BD

	BD		KD		Correlations between BD and KD			Diet effect
	Week 3	Week 4	Week 3	Week 4	$R_{BD\ weeks\ 3\ and\ 4}$	$R_{KD\ weeks\ 3\ and\ 4}$	$R_{mean\ BD\ vs.\ KD}$[a]	Mean diff. KD-BD[b]
Acetoacetate (mmol/L)	0.10 (0.03)	0.10 (0.03)	0.81 (0.48)	0.83 (0.40)	**0.56, $P=0.026$**	**0.86, $P<0.001$**	0.22, N.S.	**0.70 (0.44), $P<0.001$**
β-hydroxybutyrate (mmol/L)	0.09 (0.02)	0.11 (0.02)	0.77 (0.49)	0.77 (0.45)	**0.56, $P=0.024$**	**0.92, $P<0.001$**	0.24, N.S.	**0.71 (0.44), $P<0.001$**
FFAs (mmol/L)	0.44 (0.12)	**0.52 (0.10)**[c]	0.76 (0.17)	0.84 (0.18)	0.28, N.S.	0.44, $P=0.07$	−0.03, N.S.	**0.32 (0.18), $P<0.001$**
Glycerol (mg/L)	6.6 (1.7)	6.2 (1.5)	10.2 (2.8)	10.4 (3.1)	**0.50, $P=0.014$**	**0.48, $P=0.05$**	0.13, N.S.	**3.7 (2.8), $P<0.001$**

Data are mean (SD).
[a]Correlations between mean values for weeks 3 and 4 on BD with the mean values for weeks 3 and 4 on KD.
[b]Comparisons between KD and BD were made using mean of values obtained at last 2 weeks (designated as weeks 3 and 4).
[c]$P<0.05$ vs. week 3 on same diet.
BD, baseline diet; CM, control meal; diff., difference; FFA, free fatty acid; KD, ketogenic diet; N.S., not significant.

during the last 2 weeks of each diet. To determine whether measures obtained during the BD were predictive of those observed during the KD, regression equations were calculated comparing the mean values of fasting concentrations over the last 2 weeks of the BD with those during the last 2 weeks ingesting the KD. Analyses of the relationship of background diet and MMT type on the relationship of fasting measures of insulin sensitivity to measures derived from the MMT were made by multiple linear regression analysis in which the dynamic (MMT-derived) measure was the dependent variable and the fasting measures, diet type, and meal type were each treated as dichotomous variables.

Statistical significance was prospectively defined as $P\alpha < 0.05$. Values differing from the respective means by more than 3 SD were prospectively excluded.

Results

Subjects

Subjects (Table 1) were in a state of negative energy balance due to inadvertent underfeeding throughout the study. Weight decreased by 0.8 (0.2) kg ($P = 0.002$) during the last 15 days of the BD and 0.2 (0.1) kg (not significant) during the last 15 days of the KD (7). Energy expenditure by chamber calorimetry was the primary outcome variable of this study and was approximately 60 kcal/d higher on the KD, predominantly because of early changes in energy expenditure (7).

Fasting ketones, FFAs, and glycerol

Acetoacetate, β-hydroxybutyrate, and glycerol concentrations were stable and significantly correlated between weeks 3 and 4 within each diet, indicating stable rates of ketosis. FFA values were significantly higher on week 4 of the BD compared with week 3 without significant interweek correlation on either diet. Plasma ketones, FFAs, and glycerol were all significantly higher during the last 2 weeks of the KD compared with the BD. Fasting plasma ketones, FFAs, and glycerol measured during the BD were not significantly correlated with those measured during the KD (Table 3).

Glucose and FFA homeostasis

Fasting insulin, glucose, and C-peptide values were stable and significantly correlated between weeks 3 and 4 and within each diet (Tables 4-6). We reported previously (7) that absolute insulin secretion, as estimated by 24-hour urinary C-peptide excretion, was significantly decreased during the KD. Figure 1 and Table 6 illustrate that the absolute and relative $AUC_{glucose}$ values were significantly higher during the CM and KM on the KD ($P = 0.0001$) and that the absolute and relative $AUC_{insulin}$, but not the $AUC_{glucose}$, values were significantly higher during the CM on the KD ($P = 0.0002$), suggesting that insulin sensitivity was decreased during the CM (higher glucose and higher insulin) and probably decreased (higher glucose despite no significant decline in insulin) during the KM in subjects during the KD.

Absolute (Figure 1) and relative (Table 6) $AUC_{C\text{-}peptide}$ values were significantly higher during the CM on the KD (both $P < 0.0001$), suggesting that the insulin differences during the CM between KD and BD were the result of increased insulin secretion rather than reduced clearance. Table 6 indicates that the $AUC_{glucose}$ relative to premeal values was significantly higher during both MMTs on the KD, and the relative

TABLE 4 Measures relevant to glucose homeostasis in subjects on isocaloric KD and BD

	BD		KD		Correlations[a]			Diet effect[b]
	Week 3	Week 4	Week 3	Week 4	$R_{BD\ weeks\ 3\ and\ 4}$	$R_{KD\ weeks\ 3\ and\ 4}$	$R_{mean\ BD\ vs.\ KD}$	Mean diff. KD-BD
Glucose (mg/dL)	82 (7)	81 (4)	81 (7)	81 (6)	**0.63, $P = 0.006$**	**0.73, $P < 0.001$**	**0.68, $P = 0.003$**	0 (5), N.S.
Insulin (μIU/mL)[c]	7.8 (4.5)	7.1 (4.6)	6.1 (3.7)	6.1 (3.7)	**0.81, $P < 0.001$**	**0.73, $P < 0.001$**	**0.90, $P < 0.001$**	**−1.6 (1.9), $P = 0.006$**
C-peptide (ng/mL)[c]	1.51 (0.52)	1.43 (0.59)	1.13 (0.49)	1.13 (0.45)	**0.82, $P < 0.001$**	**0.79, $P < 0.001$**	**0.94, $P < 0.001$**	**−0.34 (0.21), $P < 0.001$**

Data are mean (SD).
Glucose homeostasis based on fasting plasma concentrations during weeks 3 and 4 of BD and KD in 16 subjects. In 1 subject during the last week of KD, insulin and glucose concentrations were more than 3 SD above the mean, suggesting that the subject was not fasting. Glucose and insulin values from time zero on the morning of mixed-meal tolerance test (Table 5) in same week were substituted for this 1 data set in this 1 subject. Exclusion of the subject did not affect significance of data presented in this table. One subject was excluded because 1 set of fasting plasma insulin concentrations was more than 3 SD above the mean.
[a]Correlations between mean values for weeks 3 and 4 of BD with mean values for weeks 3 and 4 of KD.
[b]Comparisons between KD and BD were made using mean of values obtained in last 2 weeks (designated as weeks 3 and 4).
[c]Data reported in only 16 of 17 subjects because of an outlier whose fasting insulin was 40 μIU/mL and whose C-peptide level was 4.4 nmol/L in week 4. Because these values were more than 3 SD above the mean, they were not included in analysis. If they were included, then the differences between fasting insulin and C-peptides on KD and BD were not statistically significant.
BD, baseline diet; diff., difference; KD, ketogenic diet; N.S., not significant.

TABLE 5 Molecules relevant to insulin and glucose homeostasis

	BD		KD		Correlations[a]		Diet effect[b]
	Week 3	Week 4	Week 3	Week 4	$R_{BD\ weeks\ 3\ and\ 4}$	$R_{KD\ weeks\ 3\ and\ 4}$	$R_{mean\ BD\ vs.\ KD}$	Mean diff. KD-BD
Glucagon (pg/mL)	101 (34)	**85 (33)[c]**	127 (39)	119 (33)	**0.71, $P=0.002$**	**0.73, $P=0.001$**	**0.60, $P=0.015$**	**25 (29), $P=0.003$**
Adiponectin (μg/mL)	3.74 (1.70)	3.47 (1.67)	6.31 (2.98)	6.59 (3.06)	**0.93, $P<0.001$**	**0.96, $P<0.001$**	**0.81, $P<0.001$**	**2.84 (1.91), $P<0.001$**
GIP (pg/mL)	48.7 (21.2)	47.6 (21.3)	59.1 (25.7)	59.5 (28.0)	**0.85, $P<0.001$**	**0.73, $P<0.001$**	**0.73, $P=0.001$**	**11.1 (17.3), $P=0.017$**
GLP-1 (pM)	4.97 (4.83)	4.70 (4.82)	5.83 (6.36)	5.54 (6.72)	**0.99, $P<0.001$**	**0.97, $P<0.001$**	**0.98, $P<0.001$**	0.85, N.S.
FGF21 (pg/mL)[c]	82 (27)	75 (29)	58 (17)	58 (21)	**0.57, $P=0.044$**	**0.91, $P<0.001$**	−0.04, N.S.	**−23 (30), $P=0.022$**
PYY (pg/mL)	71.8 (17.9)	67.6 (17.3)	72.0 (15.4)	67.9 (19.9)	**0.58, $P=0.014$**	**0.47, $P=0.060$**	**0.64, $P=0.006$**	0.2, N.S.
Cortisol (units)	11.4 (2.9)	10.2 (2.6)	12.1 (3.0)	11.1 (2.1)	**0.64, $P=0.006$**	0.35, N.S.	**0.80, $P<0.001$**	0.8, N.S.
CRP (mg/L)	1.17 (0.93)	1.37 (1.01)	1.70 (0.96)	1.55 (0.89)	**0.95, $P<0.001$**	**0.84, $P<0.001$**	**0.75, $P<0.001$**	**0.45 (0.72), $P=0.021$**
IL-6 (pg/mL)	1.20 (0.50)	1.21 (0.58)	1.41 (0.90)	1.13 (0.59)	**0.67, $P=0.004$**	**0.65, $P=0.005$**	**0.86, $P<0.001$**	0.10, N.S.

Data are mean (SD).
[a]Correlations between mean values for weeks 3 and 4 of BD with mean values for weeks 3 and 4 of KD.
[b]Comparisons between KD and BD were made using mean of values obtained in last 2 weeks (designated as weeks 3 and 4).
[c]FGF21 values reported for only 12 subjects, as values for remaining subjects were below limits of detection for assay (50 pg/mL).
BD, baseline diet; CRP, C-reactive protein; diff., difference; FGF21, fibroblast growth factor 21; GIP, gastric inhibitory peptide; GLP-1, glucagon-like peptide 1; IL-6, interleukin-6; KD, ketogenic diet; N.S., not significant; PYY, peptide YY.

$AUC_{insulin}$ was higher after the CM on the KD. The $Belfiore_{MMTglucose}$ Index indicated that the KD resulted in impaired insulin sensitivity during the CM test, whereas the Matsuda Index reflected improved insulin sensitivity during the KM test. Fasting tests of insulin sensitivity were not significantly different between diets. There were no significant effects of diet on any indices of insulinogenesis.

During the KM, the relative AUC for both glycerol and triglycerides was higher during the KD (Table 6). Insulin-mediated antilipolysis was reduced during the KD, whether calculated from fasting data (ADIPO-IR) or based on the MMT ($Belfiore_{MMTFFA}$).

With the exception of relative $AUC_{glucose}$ during the MMT on the BD, measurements of glucose and insulin concentrations and other molecules relevant to glucose and FFA homeostasis were highly correlated between BD and KD phases of the study.

Cytokines, inflammatory markers, and other molecules

The inflammatory cytokines (CRP and IL-6), adiponectin, PYY, the insulinogenic and lipoprotein lipase-activating GIP, and the insulinogenic GLP-1 and FGF21 were stable and significantly correlated between weeks 3 and 4 and within each diet (Table 5). Circulating concentrations of CRP, adiponectin, glucagon, and GIP were significantly higher, whereas FGF21 was significantly decreased during the KD. PYY was not significantly changed. In all cases except FGF21, values during the BD were highly correlated with those measured during the KD.

Lipids

Fasting total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides were all stable and significantly correlated between weeks 3 and 4 and within each diet (Figure 2, Table 7). Fasting total and HDL cholesterol were significantly higher, and triglycerides were significantly lower during the KD. The KM led to increased relative $AUC_{triglyceride}$, regardless of the prevailing diet, and the relative $AUC_{triglyceride}$ during the KM was increased on the KD. Fasting concentrations of total, HDL, and LDL cholesterol (but not triglycerides) on the BD were highly correlated with values during the KD.

Correlations between different measures of fuel use

Correlational analyses between fasting and MMT-derived indices of fuel use were examined for diet and meal effects (see online Supporting Information). We found highly significant correlations of fasting and dynamic indices of insulin sensitivity and FFA flux, but not insulin secretion, suggesting that extrapolations of fasting to postprandial measures of insulin-mediated glucose disposal are dependent on both current diet and acute meal type.

Discussion

We examined biochemical parameters relevant to insulin sensitivity, inflammation, and dyslipidemia during consumption of a BD, whose relative composition of fat, carbohydrates, and protein was similar to the average American diet, and following the transition to an

TABLE 6 relative AUCs for plasma concentrations of glucose, insulin, FFAs, glycerol, triglycerides, and β-hydroxybuturate during a morning-meal tolerance test in 17 subjects and calculations of insulin sensitivity and islet cell function

	BD		KD		Correlations[a]				Diet effect[b]	
	CM test	KM test	CM test	KM test	R_{meal} on BD	R_{meal} on KD	R_{diet} on CM	R_{diet} on KM	CM test	KM test
Relative $AUC_{glucose}$ (mg/dL × min)	685 (687)	**−102 (595)**[c]	2,004 (1,112)	**713 (1,113)**[c]	**0.69, $P=0.002$**	0.36, N.S.	0.03, N.S.	**0.52, $P=0.03$**	**1,319 (1,280), $P<0.001$**	**815 (951), $P=0.003$**
Relative $AUC_{insulin}$ (μIU/mL × min)	2,525 (1,386)	**1,401 (1,037)**[c]	3,570 (2,277)	**1,348 (728)**[c]	**0.67, $P=0.003$**	**0.65, $P=0.004$**	**0.59, $P=0.012$**	**0.62, $P=0.008$**	**1,045 (1,761), $P=0.026$**	53 (845), N.S.
Relative $AUC_{C\text{-}peptide}$ (ng/mL × min)	242 (87)	**131 (73)**[c]	377 (191)	**136 (66)**[c]	**0.79, $P<0.001$**	**0.55, $P=0.022$**	**0.55, $P=0.021$**	0.44, N.S.	**135 (160), $P=0.003$**	5 (74), N.S.
Relative AUC_{FFA} (mmol/L × min)	−16.0 (8.9)	**3.3 (10.2)**[d]	−14.3 (19.0)	**1.9 (17.4)**[d]	−0.03, N.S.	0.18, N.S.	−0.06, N.S.	0.46, N.S.	−1.7 (20.6), N.S.	1.4 (15.7), N.S.
Relative $AUC_{triglyceride}$ (mg/dL × min)	2,454 (1,355)	**5,768 (3,052)**[d]	4,346 (3,801)	**7,201 (3,334)**[d]	**0.67, $P=0.003$**	**0.60, $P=0.011$**	0.22, N.S.	**0.65, $P=0.005$**	451 (1,776), N.S.	**1,433 (2,690) $P=0.043$**
Relative $AUC_{glycerol}$ (mg/L × min)[e]	298 (272)	**651 (419)**[d]	849 (950)	**1,374 (804)**[d]	0.24, N.S.	**0.77, $P<0.001$**	0.33, N.S.	**0.68, $P<0.005$**	406 (754), N.S.	**636 (469), $P<0.001$**
Relative $AUC_{FFA/glycerol}$	−0.010 (0.20)	−0.001 (0.031)	−0.021 (0.19)	−0.019 (0.072)	−0.20, N.S.	0.10, N.S.	0.28, N.S.	**0.65, $P=0.009$**	0.127 (0.251), N.S.	−0.019 (0.059), N.S.
Calculated indices of β-cell function										
HOMA-β (fasting)[f]	128 (50)	130 (54)	137 (161)	103 (155)	**0.63, $P=0.007$**	00.37, N.S.	0.11, N.S.	**0.59, $P=0.013$**	9 (163), N.S.	−27 (131), N.S.
Insulinogenic Index	0.33 (0.22)	**0.18 (0.13)**[d]	0.40 (0.18)	**0.17 (0.10)**[c]	0.48, $P=0.053$	0.39, N.S.	**0.49, $P=0.045$**	0.48, $P=0.051$	−0.07 (0.21), N.S.	−0.01 (0.12), N.S.
ISSI-2	−21.7 (243.0)	14.4 (91.2)	22.8 (27.3)	1.8 (134.1)	0.02, N.S.	−0.04 (N.S.)	0.26, N.S.	0.08, N.S.	−44.4, (300.3), N.S.	−12.6 (159.7), N.S.
Calculated indices of glycemic insulin sensitivity										
HOMA-IR (fasting)[f]	1.4 (0.8)	1.5 (0.9)	1.3 (0.7)	**1.3 (1.5)**	**0.75, $P<0.001$**	**0.78, $P<0.001$**	**0.71, $P=0.0014$**	**0.70, $P=0.0016$**	−0.1 (0.6), N.S.	−0.2 (1.1), N.S.
$Belfiore_{FASTING}$[f]	1.06 (0.07)	1.09 (0.07)	1.09 (0.07)	**1.21 (0.09)**	**0.81, $P<0.001$**	**0.82, $P<0.001$**	**0.74, $P<0.001$**	**0.83, $P<0.001$**	0.03 (0.05), N.S.	**0.13 (0.05), $P=0.031$**
Matsuda Index	11.4 (6.4)	**14.4 (8.7)**[d]	10.0 (6.0)	**18.6 (10.4)**[c]	**0.86, $P<0.001$**	**0.81, $P<0.001$**	**0.84, $P<0.001$**	**0.75, $P<0.001$**	−1.4 (3.3), N.S.	**4.2 (6.9), $P=0.022$**
$Belfiore_{MMTglucose}$	1.05 (0.07)	**1.27 (0.07)**[d]	0.90 (0.07)	**1.30 (0.06)**[d]	**0.84, $P<0.001$**	**0.62, $P=0.008$**	**0.72, $P=0.001$**	**0.77, $P<0.001$**	**−0.16 (0.04), $P=0.002$**	0.03 (0.05), N.S.
Calculated indices of FFA-flux insulin sensitivity										
ADIPO-IR	25.8 (13.9)	26.2 (17.4)	38.5 (25.9)	30.6 (26.4)	**0.73, $P=0.001$**	**0.55, $P=0.021$**	0.45, $P=0.067$	**0.79, $P<0.001$**	**12.7 (23.1), $P=0.037$**	4.5 (16.6), N.S.
$Belfiore_{MMTFFA}$	1.06 (0.24)	1.14 (0.30)	0.73 (0.29)	1.00 (0.25)	**0.77, $P<0.001$**	**0.71, $P=0.0017$**	**0.65, $P=0.005$**	**0.71, $P=0.0013$**	**−0.33 (0.22), $P<0.001$**	**−0.14 (0.21), $P=0.015$**

Data are mean (SD).
Morning-meal tolerance test used within-subjects fixed intake of 20% of total daily caloric intake of CM or KM caloric load while subjects ingesting BD or KD. Calculations of insulin sensitivity and islet cell function based on fasting values and those derived from MMT data.
[a]Correlations between mean values for weeks 3 and 4 of BD with mean values for weeks 3 and 4 of KD.
[b]Comparisons between KD and BD were made using mean of values obtained in last 2 weeks (designated as weeks 3 and 4).
[c]$P<0.001$ vs. control meal study.
[d]$P<0.05$ vs. control meal study.
[e]$n=16$ because of incomplete data set in 1 subject.
[f]$n=16$ because of an outlier whose fasting insulin was 40 μIU/mL and whose C-peptide level was 4.4 nmol/L in week 4. Because these values were more than 3 SD above the mean, they were not included in analysis. If data from this subject was included, differences between groups in $Belfiore_{FASTING}$ Index were no longer significant.
ADIPO, adipocyte; BD, baseline diet; $Belfiore_{FASTING}$, fasting Belfiore Index; $Belfiore_{MMTglucose}$, mixed-meal test Belfiore Index; CM, control meal; FFA, free fatty acid; HOMA, homeostatic model assessment; IR, insulin resistance; ISSI-2, Insulin Secretion-Sensitivity Secretion Index 2; KD, ketogenic diet; KM, ketogenic meal; MMT, mixed-meal test; N.S., not significant; relative AUC, relative area under the curve.

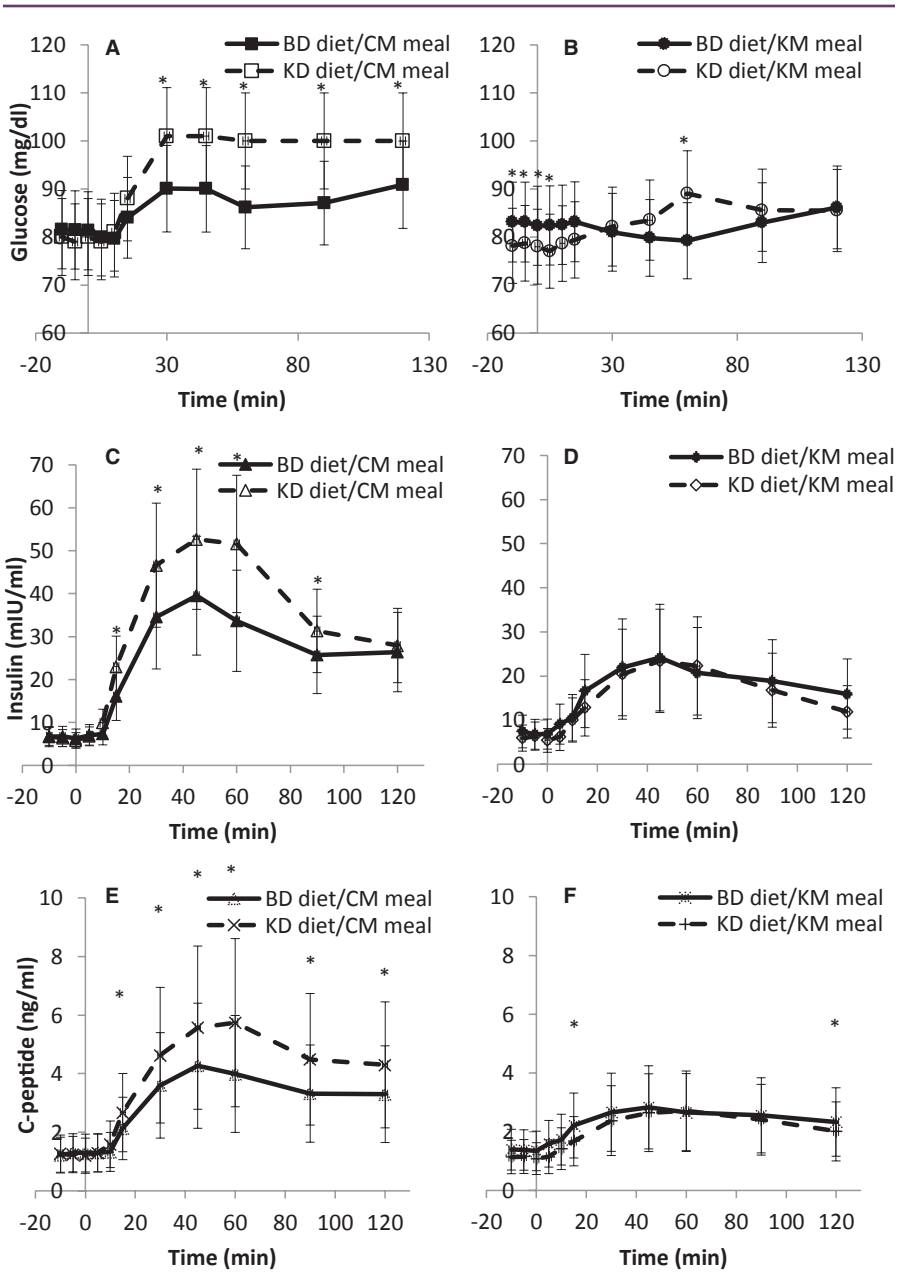

Figure 1 Results of morning mixed-meal tests. Glucose, insulin, and C-peptide excursions during the control meal (CM) with composition matched to the baseline diet (BD) (solid lines, solid markers) were significantly greater during the ketogenic diet (KD) than during the BD. After an isocaloric ketogenic meal (KM) (dashed lines, open markers), only the glucose excursion was significantly increased during the KD, and C-peptide levels were significantly lower on the KD at 15 minutes and 120 minutes. *Diet difference, $P<0.05$. See *Methods* for compositions of BD and KD. Data are mean (SD). (**A**) Glucose excursion during morning meal testing with a CM meal. (**B**) Glucose excursion during morning meal testing with a KM meal. (**C**) Insulin excursion during morning meal testing with a CM meal. (**D**) Insulin excursion during morning meal testing with a KM meal. (**E**) C-peptide excursion during morning meal testing with a CM meal. (**F**) C-peptide excursion during morning meal testing with a KM meal.

isocaloric KD as part of a study of the effects of diet composition on energy homeostasis (7). We found that switching from the BD to the KD resulted in significantly increased circulating concentrations of fasting ketones, glycerol, FFAs, glucagon, adiponectin, GIP, total and LDL cholesterol, and CRP and decreased fasting insulin, C-peptides, triglycerides, and FGF21. MMTs indicated that the $AUC_{glucose}$ was significantly higher during the KD, regardless of whether the test meal was CM or KM, and the $AUC_{insulin}$ and $AUC_{C\text{-}peptide}$ during the KD were significantly higher during the CM. Measures of glucose homeostasis derived from the MMT suggested that the KD impaired

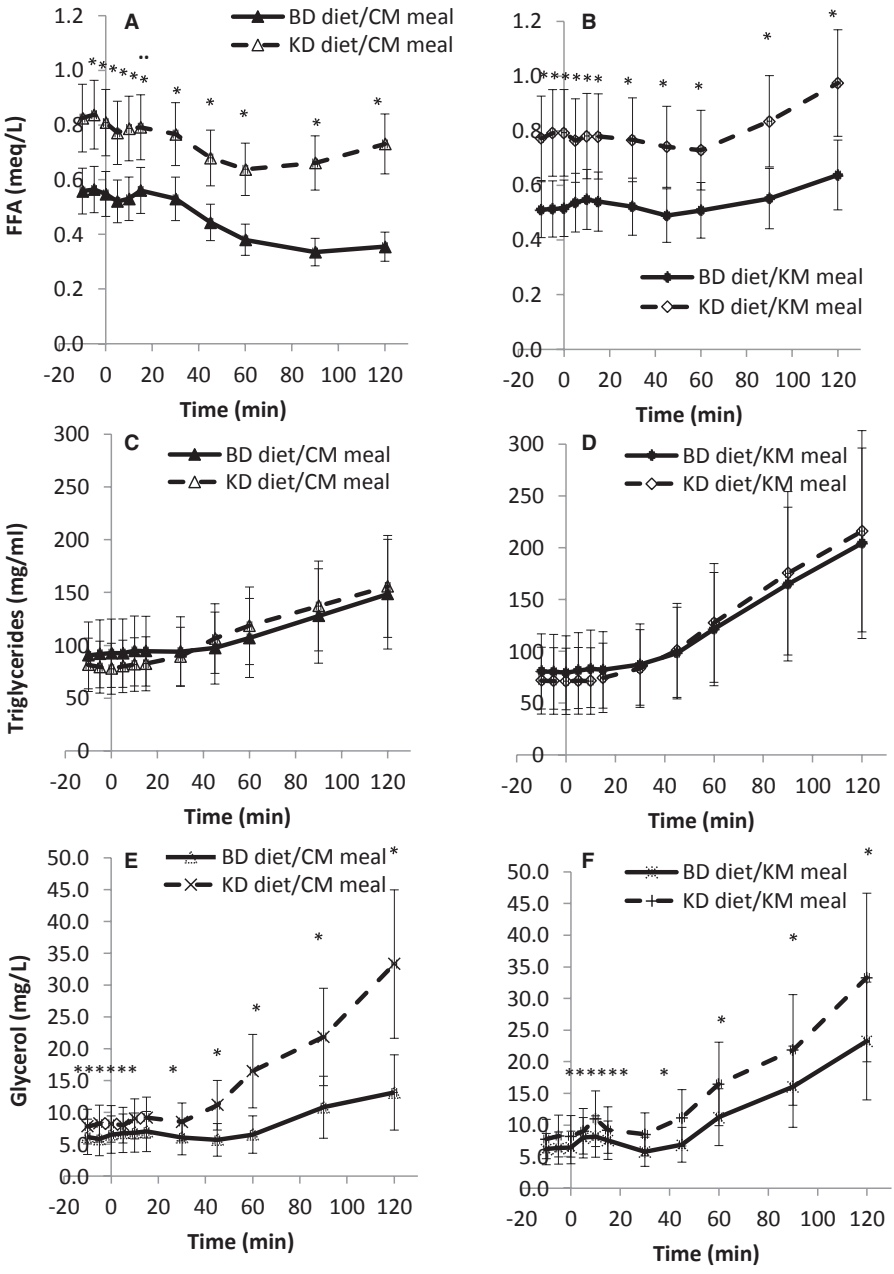

Figure 2 Lipids during the control meal (CM) and ketogenic meal (KM) mixed-meal tolerance tests. Free fatty acids (FFAs) and glycerol were significantly greater at all time points on the ketogenic diet (KD). Data are mean (SD). (**A**) FFA excursion during morning meal testing with the CM. (**B**) FFA excursion during morning meal testing with the KM. (**C**) Triglyceride excursion during morning meal testing with the CM. (**D**) Triglyceride excursion during morning meal testing with the KM. (**E**) Glycerol excursion during morning meal testing with the CM. (**F**) Glycerol excursion during morning meal testing with the KM.

insulin sensitivity in response to the CM and improved sensitivity in response to the KM. Insulin-mediated antilipolysis during the KD was decreased following both MMTs.

Similar to the authors of the present study, Jebb et al. (21) found no effects of isocaloric low-glycemic-index diets versus high-glycemic-index diets on insulin sensitivity over a 4-week period. Partsalaki et al. (22) examined the effects of an ad libitum KD versus a low-calorie diet (30%-35% fat, 50%-55% carbohydrate; 500-kcal/d deficit) in children with obesity and found that although there was greater weight loss on the KD, there were no significant differences in insulin sensitivity beyond those attributable to weight loss. Some studies have reported significant adverse effects of overfeeding of high- versus low-carbohydrate diets on insulin sensitivity during weight gain (23) and on hepatic

TABLE 7 Comparisons of lipid values in 17 subjects ingesting isocaloric KD and BD

	BD		KD		Correlations[a]			Diet effect[b]
	Week 3	Week 4	Week 3	Week 4	$R_{BD\ weeks\ 3\text{-}4\ and\ 4}$	$R_{KD\ weeks\ 3\text{-}4\ and\ 4}$	$R_{mean\ BD\ and\ KD}$	Mean diff. KD-BD
Total plasma cholesterol (mg/dL)	190 (28)	184 (28)	213 (46)	208 (36)	**0.87, $P<0.001$**	**0.93, $P<0.001$**	**0.93, $P<0.001$**	**9 (12), $P=0.009$**
Triglycerides (mg/dL)	106 (28)	102 (26)	85 (33)	82 (29)	**0.96, $P<0.001$**	**0.88, $P<0.001$**	0.26 (N.S.)	**–21 (35), $P=0.026$**
HDL cholesterol (mg/dL)	44 (11)	43 (10)	46 (15)	44 (10)	**0.96, $P<0.001$**	**0.93, $P<0.001$**	**0.92, $P<0.001$**	2 (5), N.S.
LDL cholesterol (mg/dL)	125 (27)	122 (29)	150 (38)	148 (33)	**0.91, $P<0.001$**	**0.95, $P<0.001$**	**0.84, $P<0.001$**	**26 (19), $P<0.001$**

Data are mean (SD).
Total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol were stable within each of the 2 testing periods during BD and KD.
[a]Correlations between mean values for weeks 3 and 4 of BD with mean values for weeks 3 and 4 of KD.
[b]Comparisons between KD and BD were made using mean of values obtained in last 2 weeks (designated as weeks 3 and 4).
BD, baseline diet; diff., difference; HDL, high-density lipoprotein; KD, ketogenic diet; LDL, low-density lipoprotein; N.S., not significant.

insulin sensitivity during weight regain (24), suggesting that both weight change and diet composition significantly alter carbohydrate effects on glucose homeostasis. Other studies, some more recent (25) and others dating back more than 75 years (26), have reported that low-carbohydrate diets, but not necessarily KDs, may result in impaired glucose tolerance in some individuals. The overall lack of a clear, generalizable health benefit of one diet over the other is similar to that recently reported by Gardner et al. in subjects studied over 12 months on low-carbohydrate versus low-fat weight loss diets (27).

The physiological significance of the apparent increased insulin sensitivity in response to a KD MMT versus the decreased insulin sensitivity in response to a BD MMT on the KD (both relative to the BD) cannot be determined from these data. The health consequences of improved insulin sensitivity as long as one remains on a KD may be small because of the consistently low circulating insulin concentrations on said diet. Similarly, the duration of the implied decreased insulin sensitivity in someone transitioning from a low-carbohydrate diet or KD to a higher-carbohydrate diet and the long-term health effects cannot be assessed at present.

Blunting of insulin-mediated antilipolysis on the KD should favor loss of body fat. However, the physiological significance of this finding is unclear because it seems unlikely that the additional effects of the KD to diminish insulin-mediated antilipolysis would add significantly to the high levels of lipolysis already occurring as a result of low circulating insulin concentrations. In fact, loss of body fat was not increased during the KD period as compared with the BD period, as previously reported (7).

The increases in plasma adiponectin during the KD would presumably promote insulin sensitivity (28) but may reflect the state of negative energy balance and weight loss rather than an effect of reduced carbohydrate ingestion per se. Other studies of moderate carbohydrate restriction (<30% total calories) without weight loss, with caloric restriction (−500 kcal/d for 4-6 weeks, a greater negative energy balance and weight loss than in the present study) have not found a significant carbohydrate effect on circulating adiponectin (29). There are, to our knowledge, no studies of the effects of a weight maintenance KD on adiponectin in humans other than a study of 10 children with refractory epilepsy due to glucose transporter 1 deficiency, which found no significant changes in circulating adiponectin after 3 months of a KD (30).

Low-protein diets in humans and mice have been shown to result in increased FGF21 (31), whereas calorie-restricted KDs have been reported to decrease FGF21 (32). In this isocaloric study, as the protein content of the BD and KD remained constant, the observed decrease in FGF21 on the KD most likely reflected the KD (33). Reduced FGF21 could be associated with decreased glucose uptake by adipocytes during carbohydrate restriction, as has been reported in mice (13), and may be ameliorated by ketosis (34). Circulating concentrations of FGF21 on either diet were not significantly correlated with any fasting or MMT-derived indices of insulin secretion or sensitivity.

A recent meta-analysis (35) suggested that a KD during weight loss results in a small but significant decrease in appetite. Studies during weight maintenance using a low-carbohydrate diet have yielded varied results (36,37). The lack of differences between diets in in circulating concentrations of PYY and GLP-1 suggests that any effects of a KD on appetite are not mediated by these molecules.

Circulating concentrations of CRP, but not the inflammatory cytokine IL-6, were significantly higher on the KD. Ketosis in mice has been reported to increase tissue-specific (liver and white adipose tissue) expression of inflammatory cytokines (e.g., tumor necrosis factor alpha, IL-6, and macrophage markers (38)) and to downregulate the FGF receptor (39). Outpatient studies of low-carbohydrate diets in humans have found significant increases in plasma CRP but not IL-6 (5,40). In subjects with type 2 diabetes, advice to follow a lower-carbohydrate diet decreased IL-6 but not CRP concentrations (5,41).

During the KD, our subjects had higher fasting levels of plasma total and LDL cholesterol but had lower triglycerides. Interestingly, the $AUC_{triglyceride}$ values were higher during the KD regardless of the composition of the MMT, but the KM led to significantly greater postprandial triglyceride excursion (peak − baseline) on both diets. Furthermore, the $AUC_{triglyceride}$ after the KM was further increased during the KD period; this result differs from those of previous studies showing that adaptation to a high-fat/low-carbohydrate diet decreases postprandial triglycerides following a high-fat meal (42). Perhaps the limited duration of the MMTs in the current study was not sufficient to reflect improvements in triglyceride clearance associated with adaptation to a high-fat diet.

With the exception of triglycerides and FGF21, all BD measures of insulin sensitivity, molecules affecting glucose homeostasis,

and plasma lipids were highly predictive of values obtained during the KD. These correlations suggest that although the rank order of propensity toward adiposity-related comorbidities within a given population on a similar diet remains stable, the absolute risk of adiposity-related comorbidities can be modified, but not eliminated, by diet modification.

A strength of this study is that it was conducted in an inpatient environment with assured dietary and behavioral compliance, as evident in biochemical/endocrine test stability. This investigation is limited by its examination of an extremely low-carbohydrate (ketogenic) diet that differed only in relative macronutrient proportions, not macronutrient quality. The results cannot be extrapolated to low-carbohydrate, non-ketogenic diets; to diets with specific restrictions on types of sugars or fats; or to the effects of dietary macronutrient content on comorbidities over longer periods of time. The lack of a randomized crossover design fails to control for possible significant effects of testing order (5). The unintentional weight loss may have biased the results toward improved insulin sensitivity, lipids, and inflammatory markers in subjects on the KD (5).

In summary, this study shows that switching from a BD to an isocaloric KD is associated with the following: increased plasma LDL cholesterol but decreased triglycerides, increased plasma CRP but decreased FGF21, reduced glycemic insulin sensitivity to a meal with normal carbohydrate content, improved insulin sensitivity to a KM, and impaired antilipolysis insulin sensitivity regardless of meal type. Importantly, even drastic changes in diet macronutrient composition did not affect the rank order of an individual's risk factors for metabolic disease. Those subjects with the highest and lowest risk reflected by glucose homeostasis, inflammation, and circulating lipid concentrations maintained the same relative rank order regardless of diet. O

Acknowledgments

Jeff Volek and Brittanie Volk from Beyond Nutrition Inc. helped design the study diets in collaboration with the investigators and the study dietitians: Courtney Brock, Amber Courville, Wahida Karmally, Pamela Legowski, and Renee Puyau. Serge Cremers, Mary Walter, and Peter Walter assayed the blood samples. Crystal Brown, Emma Crayner, Lilian Howard, Karen Jones, Kalle Liimatta, Elinor Naor, Stacy Shankleton, Monica Skarulis, Celeste Waguespack, and Laura Yannai were study coordinators and research support staff. Dympna Gallgher (Columbia University Irving Medical Center) was responsible for the detailed body composition analyses. We are most grateful to the study subjects who volunteered to participate in this demanding protocol.

References

1. Abbasi J. Interest in the ketogenic diet grows for weight loss and type 2 diabetes. *JAMA*. 2018;319:215-217.
2. Boden G, Sargard K, Homko C, Mozzoli M, Stein T. Effect of a low-carbohydrate diet on appetite, blood glucose levels, and insulin resistance in obese patients with type 2 diabetes. *Ann Intern Med*. 2005;142:403-411.
3. O'Brien K, Brehm B, Seeley R, et al. Diet-induced weight loss is associated with decreases in plasma serum amyloid a and c-reactive protein independent of dietary macronutrient composition in obese subjects. *J Clin Endocrinol Metab*. 2005;90:2244-2249.
4. Hall K. A review of the carbohydrate-insulin model of obesity. *Eur J Clin Nutr*. 2017;71:323-326.
5. Ebbeling C, Swain J, Feldman H, et al. Effects of dietary composition on energy expenditure during weight loss maintenance. *JAMA*. 2012;307:2627-2634.
6. Das S, GIlhooly C, Golden J, et al. Long-term effects of 2 energy-restricted diets differing in glycemic load on dietary adherence, body composition, and metabolism in CALERIE: a 1-y randomized controlled trial. *Am J Clin Nutr*. 2007;85:1023-1030.
7. Hall K, Chen K, Guo J, et al. Energy expenditure and body composition changes after an isocaloric ketogenic diet in overweight and obese men. *Amer J Clin Nutr*. 2016;104:324-333.
8. American Physiological Society. Guiding principles for research involving animals and human beings. *Am J Physiol Regul Integr Comp Physiol*. 2002;283:R281-R283.
9. Neuhauser M. How to deal with multiple endpoints in clinical trials. *Fundam Clin Pharmacol*. 2006;20:515-523.
10. Unger R, Cherrington A. Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. *J Clin Invest*. 2012;122:4-12.
11. Abraham S, Rubino D, Sinali N, Ramsey S, Nieman L. Cortisol, obesity, and the metabolic syndrome: a cross-sectional study of obese subjects and review of the literature. *Obesity (Silver Spring)*. 2013;21:E105-E117.
12. Szendroedi J, Frossard M, Klein N, et al. Lipid-induced insulin resistance is not mediated by impaired transcapillary transport of insulin and glucose in humans. *Diabetes*. 2012;61:3176-3180.
13. Murata Y, Nishio K, Mochiyama T, et al. Fgf21 impairs adipocyte insulin sensitivity in mice fed a low-carbohydrate, high-fat ketogenic diet. *PLoS One*. 2013;8:e69330. doi:10.1371/journal.pone.0069330
14. Beck B. Gastric inhibitory polypeptide: a gut hormone with anabolic function. *J Mol Endocrinol*. 1989;2:169-174.
15. D'Alessio DA, Kahn SE, Leusner CR, Ensinck JW. Glucagon-like peptide 1 enhances glucose tolerance both by stimulation of insulin release and by increasing insulin-dependent glucose disposal. *J Clin Invest*. 1994;93:2263-2266.
16. Boey D, Sainsbury A, Herzog H. The role of peptide YY in regulating glucose homeostasis. *Peptides*. 2007;28:390-395.
17. Søndergaard E, De Ycaza A, Morgan-Bathke M, Jensen M. How to measure adipose tissue insulin sensitivity. *J Clin Endocrinol Metab*. 2017;102:1193-1199.
18. Belfiore F, Iannello S, Volpicelli G. Insulin sensitivity indices calculated from basal and OGTT-induced insulin, glucose, and FFA levels. *Mol Genet Metab*. 1998;63:134-141.
19. Lorenzo C, Haffner S, Kuusisto J, Laakso M. Fasting and OGTT-derived measures of insulin resistance as compared with the euglycemic-hyperinsulinemic clamp in nondiabetic Finnish offspring of type 2 diabetic individuals. *J Clin Endocrinol Metab*. 2015;100:544-550.
20. Patarrao RS, Lautt WW, Macedo MP. Assessment of methods and indexes of insulin sensitivity. *Revista Portuguesa de Endocrinologia, Diabetes e Metabolismo*. 2014;9:65-73.
21. Jebb S, Lovegrove J, Griffin B, et al. Effect of changing the amount and type of fat and carbohydrate on insulin sensitivity and cardiovascular risk: the RISCK (Reading, Imperial, Surrey, Cambridge, and Kings) trial. *Amer J Clin Nutr*. 2010;92:748-758.
22. Partsalaki I, Karvela A, Spiliotis B. Metabolic impact of ketogenic diet compared to a hypocaloric diet in obese children and adolescents. *J Pediatr Endocrinol Metab*. 2012;24:697-704.
23. Lagerpusch M, Enderle J, Eggeling B, et al. Carbohydrate quality and quantity affect glucose and lipid metabolisms during weight regain in healthy men. *J Nutr*. 2013;143:1593-1601.
24. Hron B, Ebbeling C, Feldman H, Ludwig D. Hepatic, adipocyte, enteric and pancreatic hormones: response to dietary macronutrient composition and relationship with metabolism. *Nutr Metab (Lond)*. 2017;14:44. doi:10.1186/s12986-017-0198-y
25. Numao S, Kawano H, Endo N, et al. Short-term low carbohydrate/high-fat diet intake increased postprandial glucose and glucagon-like peptide-1 levels during an oral glucose tolerance test. *Eur J Clin Nutr*. 2012;66:926-931.
26. Sweeney JS. Dietary factors that influence the dextrose tolerance test: a preliminary study. *Arch Intern Med (Chic)*. 1927;40:818-830.
27. Gardner C, Trepanowski J, Del Gobbo L, et al. Effects of low-fat vs low-carbohydrate diet on 12-month weight loss in overweight adults and the association with genotype pattern or insulin secretion: the DIETFITS Randomized Clinical Trial. *JAMA*. 2018;319:667-679.
28. Neuhouser M, Schwartz Y, Wang C, et al. A low-glycemic load diet reduces serum C-reactive protein and modestly increases adiponectin in overweight and obese adults. *J Nutr*. 2012;142:369-374.
29. Rajaie S, Azadbakhy L, Saneei P, Khazaei M, Esmaillzadeh A. Comparative effects of carbohydrate versus fat restriction on serum levels of adipocytokines, markers of inflammation, and endothelial function among women with the metabolic syndrome: a randomized cross-over clinical trial. *Ann Nutr Metab*. 2013;63:159-167.
30. Bertoli S, Neri I, Trentani C, et al. Short-term effects of ketogenic diet on anthropometric parameters, body fat distribution, and inflammatory cytokine production in GLUT1 deficiency syndrome. *Nutrition*. 2015;31:981-997.
31. Laeger T, Henagan T, Albarado D, et al. FGF21 is an endocrine signal of protein restriction. *J Clin Invest*. 2014;124:3913-3922.
32. Christodoulides C, Dyson P, Sprecher D, Tsintzas K, Karpe F. Circualting fibroblast growth factor 21 is induced by peroxisome proliferator-activated receptor agonists but not ketosis in man. *J Clin Endocrinol Metab*. 2009;94:3594-3601.
33. Hill C, Berthoud H, Munzberg H, Morrison C. Homeostatic sensing of dietary protein restriction: a case for FGF21. *Front Neuroendocrinol*. 2018;51:125-131.

34. Fazeli P, Lun M, Kim S, et al. FGF21 and the late adaptive response to starvation in humans. *J Clin Invest*. 2015;125:4601-4611.
35. Gibson A, Seimon R, Lee C, et al. Do ketogenic diets really suppress appetite? A systematic review and meta-analysis. *Obes Rev*. 2015;16:64-76.
36. Veldhorst M, Westerterp K, van Vught AMW-P. Presence or absence of carbohydrates and the proportion of fat in a high-protein diet affect appetite suppression but not energy expenditure in normal-weight human subjects fed in energy balance. *Br J Nutr*. 2010;104:1395-1405.
37. Stubbs R, Mazian N, Whybrow S. Carbohydrates, appetite and feeding behavior in huamns. *J Nutr*. 2001;131:2775S-2781S.
38. Garbow J, Doherty J, Schugar R, et al. Hepatic steatosis, inflammation, and ER stress in mice maintained long term on a very low-carbohydrate ketogenic diet. *Am J Physiol Gastrointest Liver Physiol*. 2011;300:G956-G967.
39. Asrih M, Altirriba J, Rohner-Jeanrenaud F, Jornavyaz F. Ketogenic diet impairs FGF21 signaling and promotes differential inflammatory responses in the liver and white adipose tissue. *PLoS One*. 2015;10:e0126364. doi:10.1371/journal.pone.0126364
40. Song X, Kestin M, Schwarz Y, et al. A low-fat high-carbohydrate diet reduces plasma total adiponectin concentrations compared to a moderate-fat diet with no impact on biomarkers of systemic inflammation in a randomized controlled feeding study. *Eur J Nutr*. 2015;55:237-246.
41. Jonasson L, Guldbrand H, Lundberg A, Nystrom F. Advice to follow a low-carbohydrate diet has a favourable impact on low-grade inflammation in type 2 diabetes compared with advice to follow a low-fat diet. *Ann Med*. 2014;46:182-187.
42. Volek J, Phinney S, Forsythe C, et al. Carbohydrate restriction has a more favorable impact on the metabolic syndrome than a low fat diet. *Lipids*. 2009;44:297-309.
43. Hall K, Bemis T, Brychata R, et al. Calorie for calorie, dietary fat restriction results in more body fat loss than carbohydrate restriction in people with obesity. *Cell Metab*. 2015;22:427-436.