Journal of Nutrition and Health Sciences
Volume 5 | Issue 2
ISSN: 2393-9060

Research Article Open Access

Fuel Metabolism Following 3 Days on a Carbohydrate-Free Diet vs. 3 Days of Fasting in Men with Type 2 Diabetes: A Randomized Controlled Crossover Trial

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Citation: Nuttall FQ, Almokayyad RM, Gannon MC (2018) Fuel Metabolism Following 3 Days on a Carbohydrate-Free Diet vs. 3 Days of Fasting in Men with Type 2 Diabetes: A Randomized Controlled Crossover Trial. J Nutr Health Sci 5(2): 204. doi: 10.15744/2393-9060.5.204

Received Date: March 20, 2018 **Accepted Date**: April 28, 2018 **Published Date:** May 02, 2018

Abstract

Background: A 72-h fast results in a rapid decrease in circulating glucose to a lower level without a change in non-water body mass. Several metabolic adjustments are necessary. A nutrient-sufficient, carbohydrate (CHO)-free diet also has been reported to result in a decrease in glucose, and similar metabolic perturbations. However, direct comparisons are not available in subjects without, or with, type 2 diabetes (T2DM).

Objective: We are interested in the effect of variations in the diet on human physiology and metabolism in subjects with and without T2DM. Recently we have used two different approaches to maximally eliminate carbohydrate from the diet: a carbohydrate-free (CHO-free) diet and fasting. Our overall objective has been to compare the results when the metabolic fuel being oxidized is largely fat, without a significant loss of lean body mass.

Design: Using a crossover design, comparisons between a 72-h fast and ingestion of a CHO-free diet over 72 h were made in 7 male subjects with T2DM. Data also were compared to a baseline standard diet.

Results: Fasting resulted in a greater and more rapid weight loss than a CHO-free diet. Resting metabolic rate and fuel mix being oxidized were similar. Glucose/glycogen utilization, based on CHO oxidation, was similar between the two, but much greater than reported in subjects without diabetes. Calculated protein metabolism rates were similar.

Conclusions and Significance: In subjects with T2DM, metabolic adaptations to a CHO-free diet and a 72 h fast generally are similar. Both interventions result in a major decrease in circulating glucose concentration. Some, though not all, results are similar to those reported in subjects without diabetes. A decreased circulating glucose concentration and/or its rate of metabolism likely regulate these adaptations, which occur at a higher fasting glucose concentration in subjects with T2DM. The adaptation also spares hydrolysis of endogenous proteins.

Keywords: Weight Loss; High Fat Diet; Metabolic Rate; Glycogen; Fuel Oxidation

List of abbreviations: BMI: Body Mass Index; CHO: Carbohydrate; h: hour(s); IRB: Internal Review Board; IV: Intravenous; N/A: Not Applicable; nRQ: non-Protein RQ; RMR: Resting Metabolic Rate; RQ: Respiratory Quotient (CO_2/O_2); SDTU: Special Diagnostic & Treatment Unit (Similar to a Clinical Research Center); SEM: Standard Error of the Mean; T2DM: Type 2 Diabetes Mellitus; VistA: Veterans Health Information Systems and Architecture

Introduction

Our laboratory is interested in the effect of variations in the diet on human physiology and metabolism in subjects with and without type 2 diabetes (T2DM). We are particularly interested in determining several factors related to fuel metabolism.

Recently we have used two different approaches to maximally eliminate carbohydrate from the diet: a carbohydrate-free (CHO-free) diet and fasting. The metabolic effects of both have been compared in subjects with T2DM. Our overall objective has been to compare the results when the metabolic fuel being oxidized is largely fat, without a significant loss of lean body mass. Seventy-two hours of fasting is a time period over which a loss in non-water body mass is minimal [1]. Data from a 72-h fast were compared to 72-h macronutrient-sufficient, no carbohydrate, high fat diet in men with T2DM.

In the present report, weight changes, resting metabolic rate (RMR), plasma urea nitrogen, thyroid hormones, fuel oxidation, urine sodium, potassium, calcium, creatinine and urea nitrogen data are provided and discussed. The 24-hr glucose, insulin and glucagon responses [2] as well as the ghrelin and leptin responses [3] from these same subjects have been published.

Materials and Methods

Design and Setting

This is a randomized, crossover study design with a four-week washout period. On one occasion seven male subjects received a calorie-sufficient, CHO-free diet for 72 h consisting of <3% carbohydrate, 15% protein, ~85% fat (Table 1). On another occasion, all subjects starved for 72 h. This was preceded by a standard diet consisting of 55% carbohydrate, 15% protein, 30% fat. Recruitment began in June of 2009; follow-up was completed in June of 2010.

Day 1	
Breakfast	Scrambled eggs (with butter, heavy cream, cheese), Fried salt pork
Lunch	Lettuce salad with cheese, cooked egg, ham, turkey, Ranch dressing Diet gelatin with whipped topping
Dinner	Ground beef with butter, chili seasoning Cooked asparagus with Hollandaise sauce Diet gelatin with whipped topping
Day 2	
Breakfast	Scrambled eggs (with butter, heavy cream, cheese) Fried salt pork
Lunch	Egg salad on lettuce leaf (eggs, mayo, green onion, dill pickle) Diet Gelatin with whipped topping
Dinner	Polish sausage with cheese Cottage cheese with 1000 island dressing Lettuce salad with Caesar dressing, Parmesan cheese Broccoli with Hollandaise sauce
Day 3	
Breakfast	Fried eggs in butter Sausage links Tomato juice
Lunch	Tuna salad on lettuce leaf (tuna, mayo, celery, green onion, Sun-flower seeds) Diet gelatin with whipped topping
Dinner	Beef frankfurters Lettuce salad with tomato and Blue Cheese dressing Dill pickle Mousse

*Adjusted as necessary to provide required calories for each individual, keeping the macronutrient content at ~15% protein, 85% fat

Table 1: Foods for Carbohydrate-Free Diet*

Each subject was provided with a standardized dinner (55% CHO, 15% protein, and 30% fat), to be ingested at home at 1800 h the day before admission. Thereafter, only water was allowed until admission to our clinical research unit (Special Diagnostic and Treatment Unit, SDTU) the next morning.

Subjects reported to the SDTU at 0700 h. An indwelling IV catheter was inserted; blood samples were obtained at 0730, 0745 and 0800 h for baseline determinations. Subjects received a standard breakfast, lunch and dinner at 0800, 1200 and 1800 h, respectively. Blood samples were obtained every 15 minutes after each meal for the first h, every 30 minutes after the second and third h and hourly after that until the next meal or until 0800 the next morning. Urine was collected over 24 h. This represented day 1 of the study (control).

On the second day, subjects were asked to starve for 72 h, or were provided with the carbohydrate-free meals at 0800, 1200 and 1800 h each day during the 72 h. This represented days 2-5. Randomization occurred according to enrollment as follows: Subjects 1, 3, 5, and 7 fasted first; subjects 2, 4 and 6, received the CHO-free diet first. A list of representative foods for the CHO-free diet is shown in Table 1. All 7 subjects completed both arms of the study without harm or unintended effects.

Ingestion of water was encouraged. Black coffee, tea without sugar or cream, and calorie-free beverages were allowed. Activity was limited to quiet diversions such as reading or watching TV. Blood samples were obtained during the first and last 24 h of the 72-h intervention. Urine also was collected during the first and last 24 h as well. The subjects were under supervision the entire time.

Weight and blood pressure was determined daily in the AM, as was indirect calorimetry.

Participants

All subjects met the American Diabetes Association criteria for the diagnosis of type 2 diabetes [4]. Patient characteristics have been published previously [2]. Briefly, the mean age was 60 years (range 49-72); mean BMI was 31±2 kg/m^2 (range 25-38). Written informed consent was obtained from all subjects. The study was approved by the Internal Review Board (IRB), which also serves as the Ethics Committee, Department of Veterans Affairs, Minneapolis VA Health Care System and registered at ClinicalTrials.gov: NCT01469104.

Assays

Weight was determined on a digital scale (Scalitronix, White Plains, NY) in pajamas; blood pressure was determined using an automatic Dinemap Instrument (Critikon/Mediq, Pennsauken, NJ); indirect calorimetry (RMR and non-protein respiratory quotient (nRQ) was determined using a Medgraphics CPX Express apparatus (Medical Graphics Corp, St. Paul, MN), at 0800 h and over a 30-minute period.

Serum/plasma/urine data: creatinine, TSH, T_4, T_3, sodium, potassium, calcium, urea nitrogen and glucose were determined by an automated method on an Abbott Architect ci 8200 analyzer (Abbott Park, IL).

Plasma was analyzed for urea nitrogen over two 24-h periods (from 0800 on day 1 and 0800 on day 4). Some blood samples were not available during the overnight h for one subject while ingesting the CHO-free diet. Therefore, that complete data set for the plasma urea nitrogen 24-h profiles is presented for 6 subjects.

Dietary content of protein, fat and carbohydrate was estimated using diet analysis modules "Nutritionist Pro" (Axxya Systems LLC, Stafford, TX,) and "VistA" (Veterans Health Information Systems and Architecture; Department of Veterans Affairs, Veterans Health Administration, US Government). Both are based on United States Department of Agriculture (USDA) databases.

Calculations

The quantification of the carbohydrate (glucose) being oxidized was calculated based on the indirect calorimetry data. In the post-absorptive state, i.e., 14 h overnight without food, it was assumed that the glucose utilized came from endogenous sources (glycogen metabolism and gluconeogenesis) [5]. Two quantification methods were used. In the first, the fraction of energy utilization due to carbohydrate was determined using the nRQ data. In the second, quantitative excretion of potassium was used. During glycogenolysis in the liver, release of an amount of intracellular water containing 0.45 mmoles of potassium/g of glycogen has been reported [6,7]. This potassium enters the circulation, and in an equilibrium state, would be excreted in the urine. Thus, we have used the quantity of potassium in the urine as a surrogate for net glycogen/glucose utilization as fuel.

The caloric value of glycogen is 4.1 Kcal/g compared to glucose which is 3.7 Kcal/g [8]. Thus, the amount of glucose contributed from glycogen is increased by 11%.

Non-protein fuel oxidation was determined from published tables based on nRQ [9]. Protein fuel oxidation was calculated based on the 24-h urinary urea nitrogen excretion [10].

Protein metabolized generally is calculated by quantifying the total daily nitrogen lost. Urine urea nitrogen excretion makes up the great bulk, but small amounts are lost in the feces and from skin desquamation. In our study only the urinary urea nitrogen excretion was quantified and used to calculate protein metabolism (1 g N=6.25 g protein) [8].

Area Determination

The net and total integrated 24-h area responses were calculated with a computer program based on the trapezoid rule [11].

Statistics

Statistics were determined with Prism 4 software by Graphpad (LaJolla, CA) using paired Student's t-test. A p-value less than 0.05 was the criterion for significance. Data are presented as the mean±SEM.

Results

Body Weight

The mean initial body weight was 97 kg and 96 kg at the beginning of the CHO-free and fasting arms, respectively, indicating stability of the body weight over ~5 weeks. The mean body weight decreased from 97 to 95 kg after ingesting a carbohydrate-free diet for 72 h [2]. The weight loss was essentially linear.

During fasting, the mean body weight decreased from 96 to 93 kg 72 h later. In contrast to the linear weight loss with the CHO-free diet, the majority of the weight loss occurred within the first 24 h of fasting (Table 2).

CHO-Free	DAY 1**	DAY 2	DAY 3	DAY 4	DAY 5
	Standard Diet	Start of CHO-free	24 Hours CHO-free	48 Hours CHO-free	72 Hours CHO-free
Caloric Intake (Kcal/day)	2464 ±126	2436±116	2436±116	2436±116	N/A
Weight (kg)*	96.7±6	96.7±6	96.2±6	95.7±6	95.3±6+§
Pulse (BPM)	65±3	64±2	65±4	66±4	67±6
BP/S (mmHg)	130±7	126±6	123±6	123±6	120±2
BP/D (mmHg)	79±4	73±2	72±3	71±2	73±2§
RMR (Kcal/day)	1803±133	1867±154	1829±110	1701±68	1661±65
nRQ	0.85±0.01	0.84±0.02	0.80±0.01	0.80±0.01	0.79±0.01
% CHO Oxidized	50.7	47.2	33.4	33.4	29.9
% Fat Oxidized	49.3	52.8	66.6	66.6	70.1
Grams glucose[1]		238			134
Grams glucose[2]		249			165

Fasting	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
	Standard Diet	Start of Fast	24 Hours Post fast	48 Hours Post fast	72 Hours Post fast
Caloric Intake (Kcal/day)	2464±126	0	0	0	N/A
Weight (kg)*	96.2±6	96.2±6	94.4±6	94.0±6	93.1±6+§
Pulse (BPM)	68±4	67±3	69±5	73±6	73±5
BP/S (mmHg)	136±8	121±4	122±4	122±8	113±3
BP/D (mmHg)	79±3	74±2	73±2	74±3	68±2+§
RMR (Kcal/day)	1724±112	1866±76	1661±107	1609±126	1572±101
nRQ	0.82±0.02	0.85±0.01	0.82±0.01	0.79±0.01	0.78±0.02
% CHO Oxidized	40.3	50.7	40.3	29.9	26.3
% Fat Oxidized	59.7	49.3	59.7	70.1	73.7
Grams glucose[1]		256			112
Grams glucose[2]		224			146

Values are means±SEM; **Standard Diet=Control (55% CHO, 15% protein, 30% fat)
*Day 1 and Day 5 weight published previously [2].
+ = P<0.05 Day 5 vs Day 2; § = P<0.05 CHO-free day 5 vs. Fasting day 5
[1]Calculated from RMR & nRQ
[2]Calculated from Urine Potassium
Abbreviations: kg=kilograms; BPM=beats per minute; mmHg=millimeters of mercury; BP/S=blood pressure/systolic; BP/D=blood pressure diastolic; Kcal/day=kilocalories/day; nRQ=non-protein respiratory quotient; RMR=resting metabolic rate; CHO=carbohydrate
Note: That the data were obtained at 0800 hr. and thus are representative of the caloric intake from the previous day.
Table 2: Physiologic Data (n=7)

Blood Pressure

The CHO-free diet, as well as fasting, resulted in a small decrease in blood pressure. However, only the decrease in diastolic pressure when the subjects had fasted was significant (Table 2).

Calculated Food Energy Intake

The mean calculated food energy intake when the subjects ingested the standard diet on day one was 2464 Kcal/24 h for both arms of the study. When ingesting the CHO-free diet it was 2436 Kcal/24 h (Table 2).

Indirect Calorimetry: Resting Metabolic Rate (RMR)

The calculated RMR decreased from 1867 Kcal/day at 0800 hr on the morning of the first day of the CHO-free diet (day 2) to 1661 Kcal/day following 72 h of the CHO-free meals (Δ = -206 Kcal/day). The average RMR for days 3-5 was 1730 Kcal/day. The calculated mean daily caloric intake was 2436 Kcal/day. Thus the average excess caloric intake over RMR was 706 Kcal/day (29%) (Table 2).

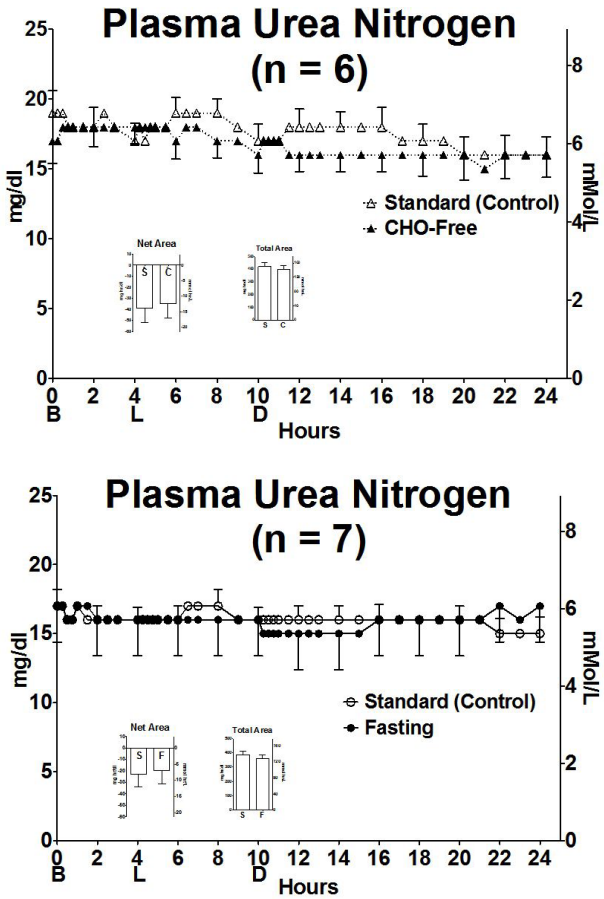
On the first day of the fast (day 2), the calculated RMR was 1866 Kcal/day. Following 72 hrs of fasting, it decreased to 1572 Kcal/day (Δ = -294 Kcal/day). The average RMR for days 3-5 was 1614 Kcal/day, and nearly all of the decrease occurred on the first day.

Non-Protein Respiratory Quotient (nRQ)

The initial mean nRQs were 0.84 on the first day of the CHO-free diet and 0.85 when fasting. The non-protein fuel mix being oxidized based on published tables was 47% carbohydrate, 53% fat for the CHO-free diet and 51% carbohydrate, 49% fat with fasting [9]. The nRQ subsequently decreased to 0.79 at the end of the 72-hr CHO-free diet and 0.78 with fasting. At this time the calculated non-protein fuel mix being oxidized was 30% carbohydrate, 70% fat with the CHO-free diet and 26% carbohydrate, 74% fat with fasting. Thus, the nRQ data were similar whether the subjects fasted or ingested the CHO-free diet (Table 2).

Protein Metabolism

Plasma Urea Nitrogen (Figure 1): The overnight 0800 h fasting plasma urea nitrogen concentrations were similar for the Control pre CHO-free diet and the Control pre fasting arms of the study, 6.78 and 6.07 mMol/L, respectively. With the CHO-free diet or with fasting they were identical, 6.07 mMol/L. During the subsequent 24 h there was little change in concentration under any of the conditions. The net and total 24-h integrated area responses also were similar.



Top: (n=6) Mean±standard error of the mean (SEM) while ingesting the standard diet (i.e., control–open triangles) and for the last 24 hours (hours 48-72) of ingesting a CHO-free diet (closed triangles)
Bottom: (n=7) Mean±SEM while ingesting the standard diet (i.e., control–open circles) and for the last 24 hours (hours 48-72) of fasting (closed circles).
X-axis: 0 indicates 0800 hr. B, L, D indicate breakfast, lunch, and dinner meal times.
Y Axis: Left - concentration in English units, Right - concentration in Scientific Units.
Inserts: Net Area represents the mean±SEM integrated 24-hr area response, using the overnight fasting concentration as baseline. Total Area represents the mean±SEM integrated 24-hr area response, using zero as baseline.
S = Standard diet (control), C = CHO-free diet, F = Fasting
Figure 1: Plasma Urea Nitrogen. Twenty-four hour plasma urea nitrogen responses.

Urine Urea Nitrogen (Table 3): The initial 24 h quantitative urine urea nitrogen excretions also were similar. They decreased only modestly with ingestion of the CHO-free diet or with fasting. The decrease was identical in both arms of the study (1.6 g), however, significant only with fasting.

	Meals					
Urine	**Pre CHO-Free Standard Diet**	**CHO-Free 48-72 hr**	**Δ**	**Pre-Fasting Standard Diet**	**Fasting 48-72 hr**	**Δ**
****Volume (ml)**	3281±441	3012±311	-269	3213±439	2399±297*	-814
Sodium (mmol)	223±21	188±18	-35	193±23	68±8*	-125
Potassium (mmol)	101±8	67±8*	-34	91±7	59±5*	-32
Calcium (mg)	185±33	151±16	-34	198±26	110±14*	-88
Creatinine (mg)	1633±128	1702±177	69	1477±90	1681±84*	204
Urea Nitrogen (g)	13.7±1.2	12.1±1.3	-1.6	12.3 ± 0.9	10.7±0.9*	-1.6
****Glucose (g)**	23.5±14.0	0.26±0.1 ^^	-23.24	29.5±12.0	0.07±0.01*	-29.43

Values are means±SEM
*P≤0.05 Standard vs Treatment by Student's t test
^^ P=0.02 by Wilcoxon signed rank test
**The average of the 2 "Pre" data sets for volume and glucose, and individual post data set for volume and glucose were published previously [2].
24 Hour collection for Standard (i.e. Control) was from 0800 day 1 to 0800 day 2
24 Hour collection for CHO-Free and Fasting was from 0800 day 4 to 0800 day 5
The creatinine excretion was extraordinarily low for the control diet before fasting, for an unexplained technical reason
Table 3: 24-Hour Urine Data (n=7)

Urine Creatinine (Table 3): The initial quantitative urine creatinine was lower at the beginning of the fasting arm of the study compared to that at the beginning of the CHO-free diet arm. This was a uniform finding, and likely was an assay error. Over the last 24 h the creatinine had only increased by 69 mg with the CHO-free diet but by 204 mg with fasting. Had the initial creatinine excretions been similar, the slight increase at the end of the study would have been similar.

Other Quantitative Urine Data (Table 3): The mean urine volumes as well as the sodium, potassium and calcium content were similar when the subjects ingested the standard diet on the two different occasions.
Both the CHO-free diet and fasting resulted in a decrease in the urine volume, sodium, potassium, and calcium excretion compared to the standard diet. In general the decrease was greatest when the subjects fasted. During the fast, all of the decreases compared to the standard diet were statistically significant. The potassium decreases were similar and statistically significant in both arms.

Plasma/Serum Data (Table 4): The circulating creatinine, TSH, and T_4 concentrations remained unchanged. The T_3 decreased in both arms of the study. The HbA1c and glucose data have been published previously and are included for the possible interest of the reader [2].

	Meals					
Test	**Pre CHO-Free (Standard Diet)**	**Post CHO-Free**	**Δ**	**Pre Fasting (Standard Diet)**	**Post Fasting**	**Δ**
Creatinine (mg/dl)	0.8±0.04	0.9±0.05	0.1±0.01	0.8±0.03	0.9±0.04	0.1±0.06
TSH (μU/ml)	1.8±0.4	2.0±0.6	0.2±0.4	2.1±0.7	1.9±0.6	-0.2±0.11
Free T_4 (μg/dl)	1.1±0.03	1.1±0.03	0	1.0±0.10	1.2±0.08 *	0.2±0.07
Total T_3 (ng/dl)	111±5	94±5*	-17±5	113±8	93±5*	-20±6
HbA1c (%)	7.9±0.4	7.8±0.5	-0.1±0.1	8.1±0.5	7.9±0.5	-0.2±0.1
**Glucose (mg/dl)	188±22	149±14*	-39±12	204±25	114±11*	-90±23

Values are means ± SEM, Standard Diet=Control (55% CHO 15% protein 30% fat)
* P < 0.05 Standard vs Treatment by Student's t test
Δ = Post minus Pre value
**Average of the data for Pre CHO-free and Pre Fasting Standard data for volume and glucose, and individual data for post volume and glucose published previously [2].
Abbreviations: TSH=Thyroid-stimulating hormone; T_4=thyroxine; T_3=triiodothyronine; HbA1c=Hemoglobin A1c
Table 4: Plasma/Serum Data (n=7)

Discussion

In people without diabetes, the weight loss of short-term fasting (~1 kg/day) was reportedly reproduced by a CHO-free diet [1,12]. It was associated in both metabolic states by a similar sodium-induced diuresis. Addition of dietary CHO resulted in prompt sodium retention. Diuresis-associated weight loss was greatest during the first 24 h, and generally lasted only 3-4 days. It was

associated with little change in potassium excretion, indicating little loss of non-fat body mass [12]. Later, this was documented by determination of total body potassium [13]. In contrast, in the present study, when the CHO-free diet was ingested, weight loss was linear and only 0.5 kg/day. This was associated with little change in urine volume or sodium excretion. A much greater weight loss occurred with fasting (1 kg/day). The majority occurred during the first 24 h, likely associated with a major initial sodium diuresis. Urine volume decreased 25% and sodium excretion decreased 65% by the last 24 h. Thus, these results were similar to those obtained previously by others [12]. Initially a major but similar glucosuria was present. It essentially disappeared by the last 24 h [2], which could complicate interpretation of the data.

The RMR, as well as body weight, at the initiation of each arm of the study was similar when the subjects ingested a mixed diet, although the data were obtained weeks apart. At the end of the study the RMR had decreased by 11% with the CHO-free diet and 16% with fasting, a statistically non-significant difference. We are not aware of comparative RMR data in people with T2DM between a short-term fast and a low or CHO-free diet. Thus, whether the current data are typical of the subjects with T2DM in general, remains to be determined. In contrast to the modest decrease noted in these men with T2DM, in normal young men and women, an increase in RMR has been reported with short-term fasting or no change after fasting for 7 days [14-18]. With a CHO-free diet, Bergstrom et al reported a decrease in RMR in fit young men [19]. However, others reported no change in normal subjects [20-22].

In subjects without diabetes, many authors have reported that a CHO-free or a very low CHO diet, or fasting, induced a decrease in plasma T_3 [22-27]. As reported by the current Senior Author, the decrease in T_3 can be observed within 8 h when CHO-free meals are ingested [28]. In our subjects, a decrease in total T_3 was present and was similar when ingesting the low-CHO diet or fasting.

To our knowledge, there are only limited nRQ data obtained in normal subjects ingesting a CHO-free diet. In 7 males the calculated fuel mixture being oxidized was 12% CHO, 88% fat after 4 days [20]; similar data were reported in 6 males [19]. Thus, when CHO was not available, fuel consumption was essentially from fat. With fasting, a low CHO: high fat oxidation ratio (~10% CHO: 90% fat) has been reported frequently [14-17,25]. Our data indicate CHO still provided a significant component to the oxidized fuel and was similar in each arm. For unknown reasons, these subjects with T2DM had not completely adapted to a typical fat-derived fuel mixture. Circulating glucose concentrations also were higher than in normals [2]. Whether this could result in a greater glucose production rate is a consideration.

In normal subjects, the gluconeogenesis rate reportedly remains unchanged with a short-term CHO-free diet or with fasting [29,30]. The predominant change in glucose production is due to a change in the net rate of glycogen oxidation. In the present study, the net glucose utilization (glycogen-dependent glucose oxidized) was estimated by two different methods. The first was based on the RMR and nRQ data, the second on the 24-h urinary potassium excretion. Results were similar. Traditionally oxidation of liver glycogen as fuel is considered to occur during the first 1-2 days of fasting. If glycogen is depleted, the only source of CHO fuel is through recycling of glucose via the Cori cycle, amino acids from protein, plus a small amount derived from fat. In normal people, needle biopsy data indicated that liver glycogen was indeed rapidly (24 h) reduced during short-term fasting or ingestion of a CHO-free diet [31]. However, it was still present and became stable, but at a low level, and was not re-accumulated until CHO was ingested [31]. Similar data were obtained by others later using NMR spectroscopy to quantify liver glycogen. This was compared to the overall glucose production rate [32]. There was essentially no net glycogenolysis from 46-64 h of fasting, and 96% of glucose production was due to gluconeogenesis. Owen et al also reported that oxidation of CHO from glycogen was undetectable after 2 days of fasting in obese subjects, some with T2DM [27]. However, other indirect data suggest that considerable glycogen is still present in subjects with T2DM after 64-72 h of fasting and can be a source of fuel, if gluconeogenesis is impaired [32,33]. Glycogen may actually be increased after a 72-h fast [34]. In addition, inhibition of gluconeogenesis by ethanol administration did not result in hypoglycemia [35], which would be expected if glycogen was not present and mobilizable as fuel [33]. The amount of glucose released by glucagon injection also was reported to be much greater in subjects with T2DM after a 3 day fast [34]. Our data based on RMR and nRQ calculations, also are compatible with liver glycogen being increased and mobilizable as fuel in these subjects. The source of excessive glycogen stores and the mechanism by which a relatively high CHO utilization occurs when fasting or not ingesting CHO remains unexplained. Our data indicate it cannot be due to an accelerated metabolism of protein.

Circulating urea nitrogen concentrations remained unchanged following mixed meals, CHO-free meals, or with fasting. Also, the concentration did not vary throughout 24 h, indicating lack of a meal-related effect of ingested protein, and lack of a circadian rhythm. With fasting it remained stable even though the urinary excretion decreased, suggesting a strong intrinsic system for regulating the circulating urea and presumably total body protein turnover. We are not aware of comparative data. In normal subjects with short-term fasting, urea nitrogen excretion reportedly remains the same as when ingesting mixed meals or increases modestly [36,37]. However, other authors reported a decrease [32,38,39]. After 72 h on a CHO-free diet, urine nitrogen excretion reportedly doubled in normal subjects [40]. Also, an increase was reported in normal subjects ingesting a CHO-free diet over 10 days [41]. The present results in subjects with T2DM are different. Urea nitrogen excretion was similar following mixed meals on 2 different occasions or with CHO-free meals, and was only modestly lower with fasting. Thus, the amount of protein used for fuel was similar regardless of the dietary changes. Creatinine excretion also was similar, compatible with a stable utilization of creatine by muscle, and maintenance of skeletal mass. If so, the excreted urea was the result of oxidation of non-structural proteins in the

so-called labile protein pool [25]. Maintenance of skeletal muscle mass with short-term fasting also has been observed in normal subjects [27].

Conclusion

We suggest that glucose sensing provides a mechanism for assuring an adequate type and amount of fuel for the brain and other organs under all possible variations in macronutrient ingestion, as well as when exogenous fuel is lacking. In the present study, in one case the fuel supply was exogenous while in the other it was endogenous, indicating the ability of the body to similarly regulate the mixture of fuels being oxidized regardless of source.

The study strengths are: a well-controlled observational study with 2 different methods of inducing a dietary carbohydrate deficiency, the metabolic stability of the subjects, and a separate control diet for each arm of the study. The study limitations are: the relatively small number of subjects and males only; thus the results may or may not be representative of subjects with T2DM, in general.

We were not able to find contemporary data in subjects without diabetes ingesting a carbohydrate-free diet to compare to our study. It is known that certain indigenous peoples (Greenland Eskimos, Central Plains Indians), explorers in the early 20th century, etc. were able to exist for long periods of time on a carbohydrate-free diet [42-44]. These were presumably normal healthy adults. However, detailed quantitative measurements of fuel metabolism are not available for these subjects.

While not directly related to the present study, there is a literature regarding the effect of high- or low-carbohydrate diets in normal subjects interested in improving athletic performance.

Since the 1960s, when techniques for muscle biopsies were popularized by Bergstrom *et al*, high muscle glycogen concentration has been associated with enhanced physical performance [19,45]. High carbohydrate diets, and/or carbohydrate loading are techniques used to increase muscle glycogen concentration.

An observation by Pernow and Saltin suggested that some "heavy exercise" *was* possible following reduction of muscle glycogen, provided that an adequate supply of non-esterified fatty acids (NEFAs) was available to the muscle [46]. Subsequently Phinney *et al*. reported that chronic ketosis, induced by dietary carbohydrate restriction, was not deleterious to physical performance [22,47]. Current literature exists in support of enhanced exercise performance with both high-carbohydrate and ketogenic diets (for example, [48-51]).

Acknowledgments

The authors thank our volunteers with type 2 diabetes, the staffs of the SDTU and the Clinical Chemistry Laboratory, Heidi Hoover, MS, RD, Research Dietitian and Linda Hartich, MT, Laboratory Technologist. Without the help and dedication of these individuals, the current studies would not have been possible.

Funding

Supported in part by Funds from the Department of Veterans Affairs. This material is the result of work supported with resources and the use of facilities at the Minneapolis VA Health Care System. The Department of Veterans Affairs had no involvement in the study design, the collection, analysis and interpretation of data, in the writing of the manuscript or in the decision to submit the manuscript for publication.

Author Contributions

RMA applied for and obtained IRB approval for the study, recruited and enrolled the subjects, determined the randomization schedule, obtained the blood specimens, performed the majority of the indirect calorimetry, and contributed to the data analysis. RMA was a Fellow in Endocrinology when these studies were done. His present address is: Park Nicollet Health Care System, 3800 Park Nicollet Blvd., St. Louis Park, MN 55416, USA. FQN and MCG obtained the funding, formulated and designed the study, performed the final analysis of the data and wrote the final manuscript. FQN is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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