Low-Carbohydrate Diet Alters Intracellular Glucose Metabolism But Not Overall Glucose Disposal in Exercise-Trained Subjects

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Dietary composition has been strongly implicated as an important determinant of in vivo insulin sensitivity. However, the metabolic alterations associated with extreme changes in diet have not been well described. We compared glucose metabolism after a standard diet ([STD] 35% fat, 51% carbohydrate, and 14% protein) with the effects of a 3-week adaptation to a low-carbohydrate, high-fat diet ([LCD] 75% fat, 8% carbohydrate, and 17% protein). Ten healthy men were studied using the euglycemic clamp technique, indirect calorimetry, and percutaneous vastus lateralis muscle biopsies for analysis of glycogen synthase (GS) and pyruvate dehydrogenase (PDH) activities in the basal and insulin-stimulated states. Insulin-stimulated glucose disposal was unchanged (STD 46.1 ± 4.3 *v* LCD 46.0 ± 4.3 μmol/kg · min, P = NS), but marked alterations in the routes of glucose disposal were noted. Insulin-stimulated glucose oxidation (G_{ox}) was markedly reduced following LCD (STD 18.6 ± 1.9 *v* LCD 8.23 ± 1.9 μmol/kg · min, P = .0001), and nonoxidative glucose metabolism (G_{nox}) was enhanced by LCD (STD 24.9 ± 0.9 *v* LCD 38.9 ± 4.3 μmol/kg · min, P = .03). Following LCD, both the total and active forms of PDH (PDH_t and PDH_a) were significantly depressed. After LCD, GS activities ($FV_{0.1}$, %I, and $A_{0.5}$) were unaffected in the basal state, but were greater than for STD (P = .004) after insulin stimulation. The apparent increase in the sensitivity of GS to activation by insulin following LCD correlated strongly with maximal O_2 consumption ([$\dot{V}O_2$max] r = .97, P = .001), suggesting that physical conditioning interacted with the metabolic impact of LCD. In summary, LCD did not induce changes in net glucose disposal. However, LCD decreased insulin-stimulated G_{ox}, with a correspondingly diminished activity of PDH, the rate-limiting enzyme for G_{ox}. There was a reciprocal increase in insulin-stimulated G_{nox} concomitant with increased activation of GS, which was consistent with increased potential for glycogen synthesis.

PERIPHERAL INSULIN resistance has been demonstrated to be a prominent feature of both obesity and non–insulin-dependent diabetes mellitus.[1,2] Previous studies indicated that insulin resistance in skeletal muscle contributes to the glucose intolerance of these conditions.[3] This decrease in insulin action results from the combined impact of decreased cell-surface insulin receptors and decreased activity of various postbinding steps in the insulin action cascade.[4-6] Impairment of receptor-associated tyrosine kinase activity, decreased activity of the glucose transport system,[7,8] and decreased flux of glucose through both the glycogenic and oxidative pathways have been described.[9-12]

Over 50 years ago, Himsworth[13] proposed that dietary composition was an important determinant of insulin sensitivity. Short-term clinical trials have shown that feeding a high-carbohydrate, low-fat diet to both healthy and diabetic subjects leads to an improvement in insulin sensitivity.[14,15] Similarly, animal studies have demonstrated decreased cellular insulin action in conjunction with ingestion of a high-fat, low-carbohydrate diet.[16]

The current study was undertaken to assess the effects of a low-carbohydrate, high-fat diet (LCD) on insulin sensitivity and intracellular glucose processing in healthy subjects. Based on studies reported by Randle et al,[17-19] the increase in fatty acid oxidation induced by LCD should lead to a decrease in glucose oxidation in skeletal muscle. Thus, our working hypothesis was that long-term ingestion of LCD would lead to a decrease in insulin-stimulated glucose disposal in conjunction with a significant decrease in glucose oxidation.

SUBJECTS AND METHODS

General Approach

Subjects were studied using a repeated-measures design, with each subject serving as his own control while first consuming a standardized "normal" diet (STD), followed by the experimental LCD. STD testing was performed first because of the diet's anticipated similarity in macronutrient composition to the normal free-living diets of the subjects. This similarity between STD and free-living diets was confirmed by a 3-day diet record maintained for each subject.

Subjects

Ten healthy, physically fit men without family histories of diabetes were studied. Their mean age was 27 ± 2 years (± SE), mean body mass index 24 ± 1 kg/m^2, mean maximal oxygen consumption ($\dot{V}O_2$max) 47.4 ± 3.8 mL/kg per minute, mean fasting glucose 4.9 ± 0.1 mmol/L, and mean fasting serum insulin 36 ± 6 pmol/L. No subject was taking any medication known to affect glucose metabolism. All studies were approved by the Committee on Studies Involving Human Subjects and were performed at the General Clinical Research Center (GCRC) at the University of

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California, San Diego, Medical Center. Informed consent was obtained from each subject before enrollment. Individuals who volunteered for this study were dedicated, with an intellectual interest in the results. Although subjects were allowed out of the study unit to exercise, their activities were monitored as closely as possible.

Dietary Manipulation

Each subject was studied initially after consuming the STD (51% carbohydrate, 14% protein, and 35% fat) for 3 weeks (Table 1). Daily caloric intakes were adjusted to maintain body weight. Following a period of at least 1 week, subjects were switched to the LCD (8% carbohydrate, 17% protein, and 75% fat) for 21 days, and then the studies were repeated. All meals were prepared in the metabolic kitchen of the GCRC. Compliance with the LCD dietary regimen was ensured by (1) careful selection of subjects through verifying their interest after a detailed explanation of the anticipated rigors of the study, (2) daily compliance logs, and (3) ascertainment that no additional carbohydrate intake occurred during the LCD period through confirmation by a marked increase in fasting serum free fatty acid (FFA) concentrations and maintenance of positive tests for urine ketones monitored regularly by first morning voids.

Exercise

During 6 weeks of dietary intervention, subjects were permitted to leave the hospital to continue their usual exercise (one triathlete, three long-distance bicyclists, three runners [>25 mi/wk], one runner [<12 mi/wk], and two runners/weight lifters [<15 mi/wk]). Daily exercise logs were maintained by the subjects, allowing scrutiny of their daily physical activity. Subjects were also instructed not to exercise within the 48 hours preceding their clamps. This included even light exercise such as climbing stairs.

Study Protocol

Euglycemic clamp. In vivo insulin action was assessed by the euglycemic, hyperinsulinemic glucose clamp technique as previously described,[20,21] in conjunction with indirect calorimetry and needle biopsies of the vastus lateralis. After an overnight fast, a primed (60 μCi)-continuous (0.6 μCi/min) infusion of ^{3}H-3-glucose (New England Nuclear, Boston, MA) was initiated and continued for the duration of the study. After 2 hours (to allow tracer equilibration), a primed-continuous insulin infusion was started at a rate of 40 mU/m^2 per minute and continued for 180 minutes. A 20% dextrose solution was infused at a variable rate to keep the glucose level at 5 mmol/L, based on plasma glucose determinations performed at 5-minute intervals. Rates of glucose appearance (R_a) and disappearance (R_d), based on tracer kinetics, were calculated for 10-minute intervals using Steele equations for non–steady-state conditions.[22,23] If an isotopically determined glucose R_a was less than the concurrent glucose infusion rate, the rate of exogenous glucose infusion was substituted in the determination of glucose turnover. Hepatic glucose output (HGO) was assessed as R_a minus glucose infusion rate for each period. Mean R_a, R_d, and HGO values were calculated during the final 40 minutes of the basal and insulin-stimulated periods.

Table 1. Dietary Composition of STD and LCD

Parameter	STD	LCD
Calories		
Per day	3,511 ± 155	3,509 ± 155
Per kilogram	46 ± 1	45 ± 1
Carbohydrate (%)	51	8
Dietary fiber (g)	30 ± 1	9 ± 1
Protein (%)	14	17
Fat (%)	35	75
Saturated	35	33
Monounsaturated	42	37
Polyunsaturated	23	30
Cholesterol (mg/d)	907 ± 40	1,690 ± 75

Indirect calorimetry. Indirect calorimetry measurements (Deltatrac Metabolic Monitor; Sensormedics, Anaheim, CA) were performed in six subjects during the final 40 minutes of the basal and insulin-stimulated states. Minute averages of O_2 consumption and CO_2 production were used to calculate glucose and fat oxidation rates (G_{ox} and L_{ox}, respectively) according to the equations reported by Frayn.[24] G_{ox} and L_{ox} were corrected for protein oxidation using urinary nitrogen excretion from timed urine collections performed during the euglycemic clamp studies. Rates of nonoxidative glucose metabolism (G_{nox}) were determined by subtracting G_{ox} from R_d.

Each subject's aerobic capacity was assessed at entry onto the study on an electronically braked cycle ergometer, using sequential 20-W increments in workload every minute until the subject was unable to maintain the predetermined pedal speed. Measurement of oxygen consumption was performed using a Sensormedics MTS 4400.

Muscle biopsy. At the end of both the basal and insulin-stimulated periods, percutaneous needle biopsies of the vastus lateralis muscle were performed using Bergstrom needles[25] under local anesthesia induced with 1% Xylocaine (lidocaine; Astro Pharmaceuticals). Muscle tissue was frozen in liquid nitrogen within 15 seconds for subsequent analysis of pyruvate dehydrogenase (PDH) and glycogen synthetase (GS) activities. Muscle glycogen level was measured on some residual samples. However, insufficient samples for most of the subjects produced inconclusive data and are not reported here.

Analytical Methods

Metabolite and hormone determinations. Plasma glucose and lactate concentrations were measured using a YSI analyzer (Yellow Springs Instruments, Yellow Springs, OH). Glucose specific activity was determined on perchloric acid extracts of plasma. FFA concentration was measured by the method reported by Novak.[26] Glucagon[27] and insulin[28] levels were measured by standard radioimmunoassay techniques.

Muscle PDH and GS extraction. Extraction of muscle for enzyme assays was performed by a modification of the method reported by Hagg et al.[29] Muscle samples of approximately 50 mg were weighed while frozen and homogenized in an ice-cold buffer consisting of 2.0 mmol/L 1,4-dithiothreitol, 20 mmol/L NaF, 2 mmol/L EDTA, and 50 mmol/L potassium phosphate, pH 7.4. Fluoride was included to inhibit PDH phosphatase activity[30] to maintain PDH in its in situ phosphorylation state; all steps were performed at 4°C. The crude extract was then divided into two tubes and centrifuged at 20,000 × *g* for 20 minutes to form pellets. One pellet was suspended in 2 mL of the previous buffer for determination of the active fraction of PDH (PDH_a). The other pellet was suspended in 2 mL of the buffer without NaF for determination of total PDH activity (PDH_t). The pellets were again centrifuged at 20,000 × *g*. The resulting pellets were homogenized in 0.75 mL of the buffers with 0.16% Triton X-100 added to solubilize mitochondrial membranes. Protein contents of these extracts were determined by the method reported by Lowry et al.[31]

Determination of PDH activity. The activity of PDH was measured by a modification of the method reported by Hagg et al,[29] based on the technique reported by Blass et al.[32] As previously

described, 75-μL suspensions of muscle pellets were preincubated for 30 minutes at 37°C in either of two solutions.[33] After preincubation, the reactions were started by addition of 25 μL of the following concentrations of cofactors and substrate: β-NAD 5.0 mmol/L, coenzyme A 1.0 mmol/L, thiamine pyrophosphate 1.0 mmol/L, dithiothreitol 2.0 mmol/L, 1-^{14}C-pyruvic acid 0.14 mmol/L (New England Nuclear, Boston, MA), unlabeled pyruvic acid 0.66 mmol/L, and potassium phosphate 50 mmol/L, pH 7.4.

The tubes were immediately capped, and reactions were allowed to proceed for 4 minutes at 37°C. The resulting $^{14}CO_2$ was collected for 30 minutes as previously described.[33] Blank activities were determined using boiled muscle extract. Results were expressed as nanomoles of pyruvate oxidized per milligram extract protein per minute.

GS assay. GS was assayed by a modification of the method reported by Thomas et al.[34] Muscle samples were weighed frozen and homogenized (50 mg tissue/mL buffer) at 4°C in a buffer consisting of 20 mmol/L EDTA, 25 mmol/L NaF, and 50 mmol/L Tris hydrochloride, pH 7.8. The crude extract was then centrifuged at 20,000 × *g* for 20 minutes, and the resulting supernatant was diluted 1:5 with the same buffer. Determination of GS activity was performed as previously described.[33] Blank activities were assayed using boiled extract. Enzyme activity was expressed as nanomoles of ^{14}C incorporated into glycogen per milligram extract protein per minute.

Performing the assay with a range of glucose-6-phosphate (G6P) concentrations allowed determination of $A_{0.5}$ for G6P (the concentration of G6P that half-maximally stimulates GS) and the V_{max}. The activity of GS assayed at 0.1 mmol/L G6P divided by the activity of GS at 10 mmol/L G6P is termed the fractional velocity ($FV_{0.1}$) and is a sensitive measurement of changes in GS activity.[35] The percent activity independent of G6P (%I) is determined in the absence of G6P.

Data Analysis

Data are presented as the mean ± SE. Statistical analysis was performed using a two-tailed Student's *t* test for paired and unpaired data, Wilcoxon's signed-rank test for nonparametric data, and ANOVA. Significance was defined at *P* less than .05. Calculations and data analyses were performed using the Clinfo Data Base Management and Analysis System of the GCRC in conjunction with the BMDP (Los Angeles, CA) statistical software package.

RESULTS

The composition of the two diets as determined by computer analysis (Nutritionist III; N-Squared Computing, Salem, OR) is shown in Table 1. The mean caloric intake necessary to maintain weight was 45 ± 1 kcal/kg. Due to the nature of the LCD, intake of fiber was approximately 70% lower during LCD.

Three weeks of consuming the LCD led to a substantial decrease in mean fasting serum insulin level, from 36 ± 6 to 12 ± 2 pmol/L for STD and LCD, respectively (P = .02). However, the corresponding mean fasting plasma glucose concentration was unchanged by diet (STD 4.9 ± 0.1 *v* LCD 5.1 ± 0.1 mmol/L, P = NS).

Euglycemic Insulin Clamp Studies

Insulin infusion resulted in mean steady-state serum insulin concentrations of 456 ± 24 pmol/L during STD and 402 ± 12 pmol/L during LCD clamp (P = NS). During the final 40 minutes of insulin infusions, mean plasma glucose concentrations were 4.9 ± 0.1 mmol/L for both STD and LCD (P = NS), with respective coefficients of variation of 5.1% and 4.5%.

HGO

HGO was unaffected by the dietary intervention. Basal HGO during STD was 11.0 ± 0.6, versus 10.1 ± 0.2 μmol/kg per minute during LCD (P = NS). In response to insulin infusion, isotopically determined HGO was −3.9 ± 2.2 for STD and −2.9 ± 0.9 μmol/kg per minute during LCD (P = NS).

Peripheral Glucose Disposal

The effect of eucaloric fat substitution on R_d is shown in Fig 1. Basal R_d was unchanged by dietary manipulation (STD 11.1 ± 0.6 *v* LCD 10.2 ± 0.2 μmol/kg per minute, P = NS). Insulin infusion resulted in a fourfold increase in R_d, to 46.1 ± 4.0 μmol/kg per minute during STD and 46.0 ± 4.3 μmol/kg per minute during LCD.

Indirect Calorimetry

Although LCD induced no change in basal or insulin-stimulated R_d, substantial alterations in the routes of intracellular glucose metabolism were seen (Fig 2). In the basal state, net G_{ox} decreased from 7.8 ± 1.1 after STD to 2.5 ± 1.3 μmol/kg per minute after LCD (P = .02). Net G_{ox} during insulin infusion was 56% lower during LCD, reaching only 8.3 ± 1.4 μmol/kg per minute, as compared with 18.6 ± 1.4 μmol/kg per minute during STD (P = .0001).

G_{nox}, which primarily represents glycogen formation,[16] tended to be higher during the basal state following LCD (8.1 ± 1.3 μmol/kg per minute) as compared with STD (3.3 ± 1.4 μmol/kg per minute, P = .09). However, this increase was not significant based on the data from six subjects undergoing indirect calorimetry. In response to

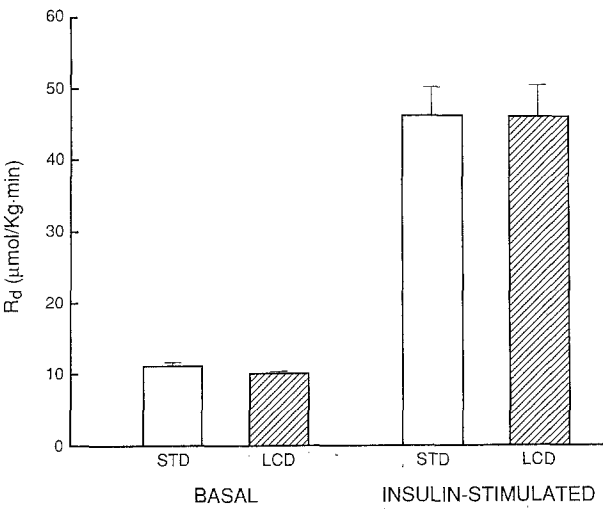

Fig 1. Basal and insulin-stimulated R_d in 10 subjects during STD and after 3 weeks of LCD. R_d was determined at euglycemia (5 mmol/L) in the basal state and in response to a 40-mU/m²/min insulin infusion. R_d is plotted as μmol/kg per minute, and is shown as the mean ± SEM.

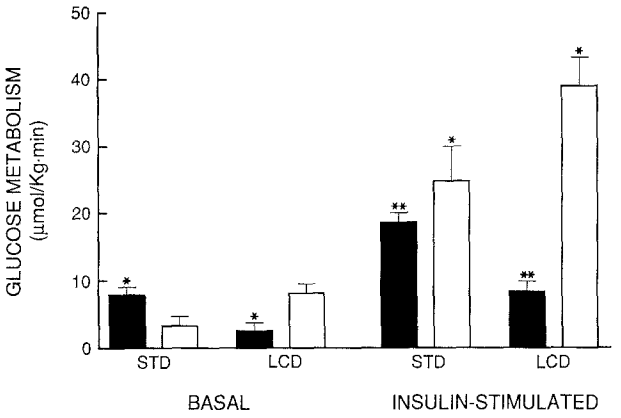

Fig 2. Basal and insulin-stimulated intracellular glucose metabolism in six subjects during STD and after 3 weeks of LCD. Using indirect calorimetry, G_{ox} (■) was determined under the same conditions outlined in Fig 1. G_{nox} (□) was calculated as the difference between R_d and G_{ox}. Results are the mean ± SEM. *P < .05, STD v LCD; **P < .001, STD v LCD.

insulin infusion, G_{nox} was greater after LCD than after STD (38.9 ± 4.2 v 24.8 ± 5.2 μmol/kg per minute, P = .03).

Indirect calorimetry also showed differences in L_{ox} as a result of LCD adaptation (Fig 3). Although L_{ox} was not significantly altered in the basal state, infusion of insulin following STD produced an 80% decrease in L_{ox}. After 3 weeks of adaptation to LCD, insulin infusion produced only slight suppression of L_{ox}, to a rate comparable to that seen during the basal state of STD.

Despite the decreased ability of insulin to decrease net L_{ox} following LCD, circulating FFA levels were promptly suppressed by insulin infusion following both diets (0.17 ± 0.02 for STD v 0.20 ± 0.02 mmol/L for LCD, P = NS; Table 2). For six subjects who underwent indirect calorimetry, insulin-suppressed FFA concentrations were

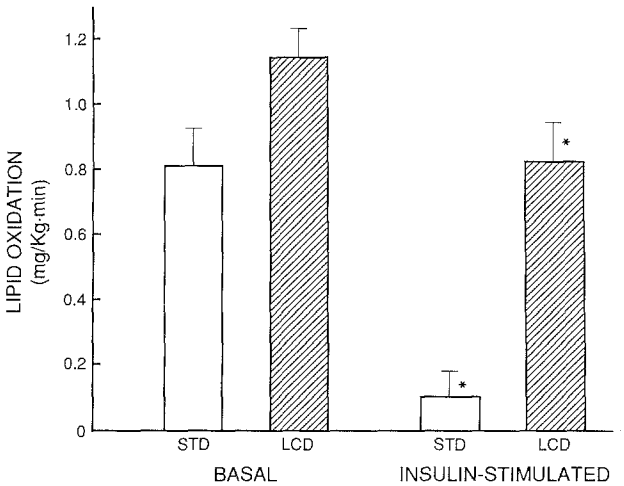

Fig 3. Basal and insulin-mediated suppression of net L_{ox} in six subjects undergoing indirect calorimetry during STD and after 3 weeks of LCD. L_{ox} expressed as mg/kg per minute was determined under the conditions described in Fig 1. Data are the mean ± SEM. *P < .005, STD v LCD.

Table 2. Muscle Enzyme and Serum Biochemical Responses to the Glucose Clamp

	Basal		Insulin-Stimulated	
Parameter	STD	LCD	STD	LCD
PDH_a	0.46 ± 0.11	0.004 ± 0.004†	0.82 ± 0.27	0.03 ± 0.02*
PDH_t	3.00 ± 0.63	0.51 ± 0.48*	4.99 ± 0.96	0.37 ± 0.15†
GS				
$FV_{0.1}$	0.16 ± 0.02	0.24 ± 0.04	0.28 ± 0.04	0.44 ± 0.04†
%I	1.0 ± 0.2	1.9 ± 0.6	2.3 ± 0.5	4.60 ± 0.8*
$A_{0.5}$	0.71 ± 0.11	0.43 ± 0.08	0.33 ± 0.08	0.13 ± 0.03*
Lactate				
(mmol/L)	0.88 ± 0.08	0.74 ± 0.05	1.09 ± 0.08	1.03 ± 0.07
FFA (mmol/L)	0.47 ± 0.06	0.62 ± 0.05*	0.17 ± 0.02	0.20 ± 0.02

NOTE. N = 10.
*P < .03.
†P < .003.

0.17 ± 0.02 and 0.18 ± 0.02 mmol/L for LCD and STD, respectively (P = NS).

Muscle Enzyme Determinations

PDH. Myocyte PDH activity was dramatically decreased following 3 weeks of carbohydrate restriction (Table 2). Following LCD, PDH_a was essentially undetectable in the basal state, and during insulin infusion it was less than 4% of the level recorded during STD. PDH_t was also significantly depressed both in the basal state (P = .007) and in response to insulin (P = .0005). Thus, similar effects of LCD ingestion were seen in vivo in terms of G_{ox} and in vitro with regard to the activity of PDH, which is the enzyme complex that catalyzes the rate-limiting step in the pathway for glucose oxidation.

GS. LCD ingestion had similar effects on in vivo G_{nox} and parameters of GS activation (Table 2). Before insulin infusion, there was no significant difference in the GS activation state between STD and LCD. Insulin-stimulated GS activation was much greater during LCD, as shown by the higher $FV_{0.1}$ (P = .002) and %I (P = .02) and the lower $A_{0.5}$ (P = .009).

ANOVA showed significant interactions between diet and insulin infusion on $FV_{0.1}$ (P = .001) and %I (P = .004), indicating that LCD increased the sensitivity of GS to stimulation by insulin.

Regression analysis demonstrated linear correlations between aerobic capacity and insulin-stimulated R_d following both the STD (r = .68, P = .03) and LCD (r = .75, P = .01). No significant correlations were seen between aerobic capacity and glucose oxidation or PDH activity under any condition tested. In contrast, a strong correlation (r = .97, P = .002) between $\dot{V}O_2max$ and insulin-stimulated G_{nox} following LCD was observed (Fig 4). In addition, $\dot{V}O_2max$ was correlated with insulin-stimulated GS V_{max} during STD (r = .63, P = .048) and during LCD (r = .73, P = .02). These observations strongly suggest that aerobic capacity is important in maintaining insulin-stimulated G_{nox} during a LCD diet.

DISCUSSION

Previous studies have strongly suggested that dietary composition is an important determinant of in vivo insulin

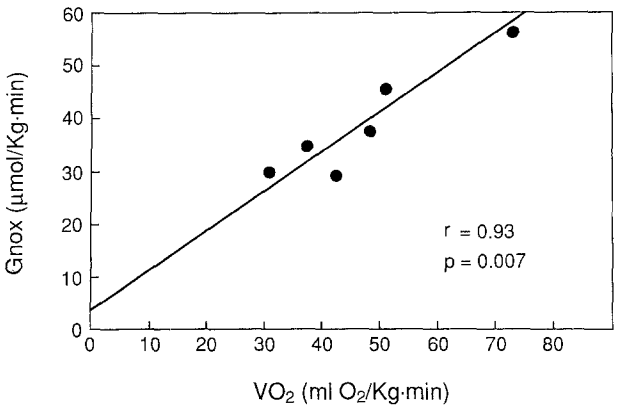

Fig 4. Insulin-stimulated G_{nox} following LCD plotted against $\dot{V}O_2max$ in six subjects undergoing indirect calorimetry. G_{nox} is expressed as µmol/kg per minute, and $\dot{V}O_2max$ is expressed as mL O_2 consumed/kg per minute.

sensitivity. In animal models, high-fat diets have been shown to induce insulin resistance, increase circulating insulin levels, and decrease cellular insulin binding in target tissues in conjunction with decreased activity of various intracellular pathways of glucose metabolism.[16,36] Randle et al[17,19] have suggested that increased availability of circulating FFA leads to a preferential increase in L_{ox} in muscle tissue. As a result, G_{ox} is inhibited due to end-product inhibition and phosphorylation of the PDH enzyme complex.[30] Inhibition of PDH activity by PDH kinase has been attributed to altered ratios of intramitochondrial acetyl coenzyme A/reduced acetyl coenzyme A and adenosine triphosphate/diphosphate.[30]

Over 50 years ago, Himsworth[13] demonstrated that increasing dietary carbohydrate from 10% to 30% of the daily caloric intake was associated with improved insulin sensitivity. Similar observations have been reported by other investigators in both nondiabetic subjects and subjects with impaired glucose tolerance.[14] These studies used diets that obtained over 60% of total daily calories from carbohydrate sources. More recently, Borkman et al[37] have reported that increasing dietary carbohydrates from 31% to 55% of total daily caloric allotment failed to alter overall insulin sensitivity. Therefore, it is possible that the metabolic effects of adding carbohydrates to the diet may vary depending on the degree of carbohydrate restriction initially present.

Dietary fiber has also been shown to increase insulin sensitivity in normal and diabetic subjects.[38] Due to the nature of the LCD used in this study, dietary fiber intake was greatly reduced. At the outset of this study, we postulated that by reducing fiber intake and replacing it with fat, a significant portion of the total daily carbohydrate intake would lead to decreased in vivo insulin sensitivity, due in part to a decrease in G_{ox} by muscle. However, we did not demonstrate deterioration in insulin-stimulated R_d following LCD in this young, physically fit population. Using steady-state insulin levels in the midphysiologic range, mean R_d was almost exactly the same during both dietary periods.

Although mean R_d did not change in response to LCD, pronounced alterations in intracellular glucose metabolism were observed. As predicted, rates of G_{ox} were significantly lower in the basal and insulin-stimulated states following adaptation to LCD. After LCD, G_{ox} decreased by 68% in the basal state and by 56% during hyperinsulinemia. These significant decreases in G_{ox} were paralleled by decreases in skeletal muscle PDH_t and PDH_a activities. These observations strongly suggest that alterations in the activity of this rate-limiting step for the oxidative pathway contribute to the decreases in G_{ox} observed in vivo following LCD.

Simultaneous assessments of L_{ox} indicated that this metabolic process was increased by 39% and 820% in the basal and insulin-stimulated states following LCD. In contrast, circulating FFA levels were suppressed to similar values during the insulin-stimulated state. This suggested that either the flux of fatty acids into cells was increased or that oxidation of intracellular lipid stores continued throughout the insulin-stimulated period following LCD.

The combination of decreased G_{ox}, decreased PDH activity, and a marked increase in L_{ox} is consistent with the Randle hypothesis.[17,19] If flux through the glycolytic pathway were unchanged by diet, this sequence of events should increase intracellular pyruvate and lactate production, leading to efflux of these metabolites from skeletal muscle. However, plasma lactate levels during the insulin-stimulated state were not increased following LCD (Table 2). There are several possible explanations for this apparent discrepancy. First, since the fluxes of lactate, pyruvate, and alanine were not assessed, it is possible that efflux of these metabolites from muscle was increased in conjunction with increased removal from the circulation. A second alternative is that flux through the glycolytic pathway to pyruvate may have also been decreased secondary to alterations in the activity of phosphofructokinase. Finally, substrate may have been diverted into the glycogenic pathway at the level of G6P, limiting the amount of substrate available to enter the glycolytic pathway. Our inconclusive muscle glycogen data did not help to clarify this possibility. Measurement of these factors in future studies would help to clarify the mechanism.

A decrease in G_{ox} would be expected to decrease in vivo insulin sensitivity following LCD. However, the expected decrease in R_d was not observed. Since G_{nox} is determined as the difference between R_d and G_{ox}, a reciprocal increase in G_{nox} was calculated. The increase in insulin-stimulated G_{nox} following LCD was accompanied by a corresponding increase in the GS activation state, as reflected by an increase in $FV_{0.1}$ and %I and a decrease in $A_{0.5}$. These findings, although surprising, are consistent with earlier studies of the effect of insulin in combination with fatty acids on rat skeletal muscle glucose metabolism.[39] In this model, elevated FFA in conjunction with insulin concentrations in the mid-physiologic range resulted in increased skeletal muscle glucose utilization and increased glucose flux toward glycogen. Although FFA concentrations in our subjects were suppressed by insulin infusion, the continued high rate of net L_{ox} and the increased activation state of GS suggest that similar changes in intracellular metabolism may have been induced by LCD.

In agreement with our data, two recent publications have demonstrated that during moderate hyperinsulinemia, main-

taining FFA concentrations through infusion of lipid emulsions results in decreased rates of G_{ox}[40,41] and PDH activity.[41] However, both articles reported that GS activity was decreased in muscle biopsies obtained at the end of the euglycemic clamps with the coincident infusion of lipid emulsion. Boden et al[40] hypothesized that during the initial 3 to 4 hours of their 6-hour lipid/insulin infusion study, when glucose disposal was normal but G_{ox} was decreased, glucose that entered the muscle was shunted to glycogen, which resulted in eventual saturation of muscle glycogen storage capacity. This saturation of glycogen storage capacity then decreased GS activity, G_{nox}, and glucose uptake during the final hour of their clamp. It is our contention that in our subjects, physical training coupled with carbohydrate deprivation increased GS activity and resulted in increased G_{nox}. It is unknown if such results would have persisted had our euglycemic clamp been extended for longer periods.

Although insulin-stimulated GS activation was increased following LCD, linear regression analysis failed to demonstrate significant relationships between G_{nox} and any of the parameters of GS activation. This lack of a correlation between in vivo and in vitro parameters is puzzling, but likely related to the fact that in vitro measurements reflect the GS activation state rather than the absolute activity of the enzyme. It is also possible that other processes, such as glucose transport, were rate-limiting for G_{nox}, or that intracellular levels of G6P were increased following LCD due to decreased flux of substrate through the oxidative pathway. Since G6P is an allosteric activator of GS, this alteration would exert a direct stimulatory effect on GS.[42,43] If any of these processes were operative, the relationship between G_{nox} and GS would be obscured.

The correlation between aerobic capacity and the calculated $\dot{V}_{max}$ for GS during both the STD and LCD periods is of interest. These observations suggest that the degree of physical conditioning influences the functional capacity of GS. In addition, a strong correlation was observed between aerobic capacity and insulin-stimulated G_{nox} following LCD ($r = .93$, $P = .007$). Since the increase in insulin-stimulated G_{nox} following LCD appears to be responsible for maintaining R_d and since GS represents the rate-limiting enzyme for the majority of G_{nox}, these results suggest that physical conditioning is capable of influencing the metabolic impact of dietary carbohydrate restriction via activation of GS. One should be cautious in extrapolating the results of this study to less physically fit populations. In addition, increasing total caloric intake by increasing dietary fat, rather than the eucaloric substitution of fat for carbohydrate may result in different findings. The most likely mediator responsible for the observed adaptations was the relatively low insulin concentration at rest and in response to LCD. With this in mind, an additional caution is warranted: increasing the carbohydrate content in LCD may result in responses that are different from those reported.

In summary, the results of the present study indicate that eucaloric substitution of fat for dietary carbohydrate does not uniformly lead to a decrease in overall insulin sensitivity in young, physically fit subjects. While the expected decrease in PDH activity and G_{ox} occurred, an equal and opposite increase in G_{nox} evolved, presumably due to an increase in GS activation, resulting in no significant change in the rate of insulin-stimulated glucose disposal. The increase in G_{nox} was highly correlated with aerobic capacity of the subjects, suggesting that physical conditioning influenced the metabolic effects of the dietary intervention. Thus, it appears that in normal individuals physical conditioning and dietary composition interact in modulating carbohydrate metabolism.

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