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High-fat/low-carbohydrate diets regulate glucose metabolism via a long-term transcriptional loop

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Abstract

Insulin sensitivity is characterized by insulin-stimulated glucose metabolism in skeletal muscle. We hypothesized that carbohydrate metabolism and storage might be under transcriptional control. To test this hypothesis, we fed insulin-sensitive males (glucose disposal rate, 14.7 ± 4.1 mg/kg fat-free mass [FFM] per minute) an isoenergetic high-fat/low-carbohydrate diet (HF/LCD) for 3 days with muscle biopsies before and after intervention. Oligonucleotide microarrays revealed a total of 369 genes of 18 861 genes on the arrays were differentially regulated in response to diet (Bonferonni adjusted $P < .01$). A similar experiment was conducted in mice with a 3-week intervention using a control group and an HF/LCD group to offset the lack of a control group within the human cohort. As part of an analysis of results previously published from this data set, 7 genes in the carbohydrate metabolism pathway changed in response to the HF/LCD, and 3 genes were confirmed by quantitative reverse transcriptase–polymerase chain reaction: fructose-2,6-biphosphatase 3 (PFKFB3), pyruvate dehydrogenase kinase, isoenzyme 4 (PDK4), and glycogen synthase 1 (muscle). In a separate experiment, we fed C57Bl/6J mice an HF/LCD for 3 weeks and found that the same glucose metabolism genes were changed by ~70% on average. Fructose-2,6-biphosphatase 3 and pyruvate dehydrogenase kinase, isoenzyme 4 increased and glycogen synthase 1 (muscle) decreased. Combined, these results suggest a mechanism whereby HF/LCD regulates the genes necessary for glucose utilization and storage vis-á-vis transcriptional control.

1. Introduction

Type 2 diabetes mellitus affects more than 110 million people worldwide, and type 2 diabetes mellitus associated with obesity is reaching epidemic proportions [1,2]. Many pathophysiologic processes have been implicated in diabetes, such as pancreatic beta-cell dysfunction, as well as defects in insulin signaling, carbohydrate utilization, and mitochondrial metabolism [3,4]. The earliest detectable abnormality in people at risk for type 2 diabetes mellitus is insulin resistance in skeletal muscle. The ability of insulin to activate signal transduction events, alter gene expression of selected genes [5], and stimulate muscle glycogen synthesis is an integral part of the body's response to macronutrient intake.

The physiologic purpose of alterations in fuel selection is to channel energy to and from the appropriate storage compartments under all circumstances that may confront the organism. An obligate need exists to regulate glycogen within a relatively narrow window. Studies conducted in subjects with type 2 diabetes mellitus and in healthy subjects to determine the fate of glucose after it is taken up by muscle cells demonstrate that muscle glycogen synthesis, along with glycolysis, is an important pathway in skeletal muscle glucose metabolism [6]. Muscle cells adjust the fuels they oxidize to match substrate supply, signals from the endocrine and neural systems, and the adenosine triphosphate required for contraction. The supply of substrate to muscle tissue depends upon several factors including the dietary macronutrient content, the storage of nutrients in liver and fat, capillary recruitment and transport, and in the case of triglycerides, the release of free fatty acids by the enzymatic activity of lipoprotein lipase at the surface of the cell. The adjustment of substrate oxidation to the local nutrient concentrations occurs through at least 3 mechanisms: minute-to-minute changes in the activity of enzymes that direct carbohydrate and fat into oxidation or storage [6],

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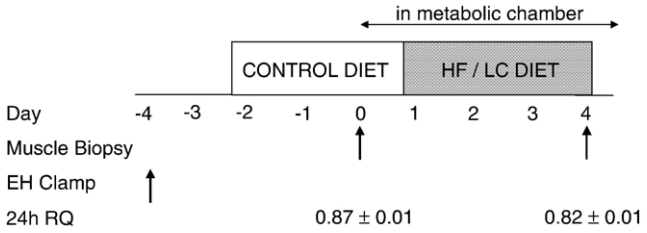

Fig. 1. Study design. Subjects consumed a prescribed control diet (35% fat, 49% carbohydrate, 16% protein) followed by an EH clamp and a muscle biopsy. They were switched to a prescribed HF/LCD (50% fat, 34% carbohydrate, 16% protein) and placed in metabolic chamber for a total of 4 days where fatty acid oxidation was measured by RQ at days 0 and 4. They finished with a muscle biopsy on day 4.

the activation of signaling pathways such as protein kinase C (PKC) [7] and NFκB [8], and through long-term regulatory systems that involve changes in gene transcription and hence the cellular machinery driving the first 2 processes. The ability of insulin to regulate glucose homeostasis, that is, insulin sensitivity, is closely related to these regulatory pathways, justifying further attempts to unravel the details of these regulatory systems. Strong experimental evidence exists for each of these pathways, particularly for the regulation of substrate utilization and insulin sensitivity through enzyme activity [9] and signaling pathways [7]. Less data are available to implicate transcriptional pathways as key components of this autoregulatory loop in glucose homeostasis. Accordingly, it is an important area of diabetes research that warrants focus and attention. We believe the relevance relates to transcriptional control of substrate utilization more than to insulin-stimulated glucose uptake. This is particularly true for the relationship between transcriptional control of substrate utilization and expression of pyruvate dehydrogenase kinase, isoenzyme 4 (PDK4)/pyruvate dehydrogenase (PDH)/fructose-2,6-biphosphatase 3 (PFKFB3). We previously described the effects of a short-term HFD to reduce the expression of genes related to oxidative phosphorylation within the skeletal muscle of young, healthy volunteers [10]. We chose insulin-sensitive males based on their $V \cdot O_2$max, which correlates with glucose disposal rate (GDR) in men at baseline (data not shown). A similar experiment was conducted in mice with a 3-week intervention using a control group and a high-fat/low-carbohydrate diet (HF/LCD) group to offset the lack of a control group within the human cohort. The purpose of this research was to expand that investigation and to identify the transcriptional responses for other metabolic systems such as carbohydrate oxidation and storage, as well as fatty acid oxidation.

2. Research design and methods

2.1. Study population and design

ADAPT is a short-term intervention study designed to examine the ability to increase fat oxidation after consuming an isoenergetic HF/LCD [11,12]. Initial studies of the effects of this diet on genes involved in oxidative phosphorylation have been published elsewhere [10]. These studies extend and refocus those investigations on the regulation of carbohydrate metabolism. Ten healthy young men, aged 23.0 ± 3.1 years and with a body mass index of 24.3 ± 3.0 kg/m^2, were chosen from the larger study population based on a high aerobic capacity and insulin sensitivity and underwent physical examination, medical laboratory tests, and anthropometry. Participants presented to the Pennington Biomedical Research Center inpatient unit (Fig. 1) on day −4 at 07:00 PM and fasted overnight. The following day they ate a weight-maintaining (35% fat, 49% carbohydrate, 16% protein) diet prepared by the metabolic kitchen at 12:30 PM as part of the euglycemic-hyperinsulinemic (EH) clamp recovery procedure. On day −3, an EH clamp was performed, and this diet was continued on days −2, −1, and 0. On day 1, participants ate a similar weight-maintaining 35% fat/49% carbohydrate diet, and total daily energy expenditure (EE), fat oxidation, protein oxidation, and carbohydrate oxidation were measured at energy balance as previously described in a whole-room calorimeter [13,14]. On days 2, 3, and 4, subjects ate a 50% fat/34% carbohydrate/16% protein diet with an isoenergetic energy clamp procedure. An isoenergetic clamp procedure is used to maintain weight by matching the participant's energy intake (EI) with their 24-hour EE so that they remain in energy balance throughout the study regardless of diet composition changes [13]. Participants were housed in the metabolic chamber for a total of 4 days, but remained at Pennington Biomedical Research Center for the entire duration of the study. Three-day cumulative carbohydrate balances were calculated using the formula: carbohydrate intake (kJ) − carbohydrate oxidation in chamber (kJ), summed over the 3 days. Carbohydrate intake was computed as EI for the day times the proportion of EI attributable to carbohydrate for each day's menu (34% for chamber days 2, 3, and 4). Each meal was served to the subjects and consumed within 20 minutes. Vastus lateralis was chosen based on its composition of both oxidative and glycolytic fiber types. Muscle biopsies were performed on days 2 and 4 after an overnight fast.

2.2. Euglycemic-hyperinsulinemic clamp

Insulin sensitivity was measured only at baseline (day −3) by EH clamp [15] before HF/LCD to be considered as an indicator of adaptability to the HF/LCD. After an overnight fast, glucose and insulin (80 mIU/m^2 bovine serum albumin) were administered. The GDR was adjusted for kilogram of lean body mass.

2.3. Maximal aerobic capacity ($V \cdot o_2$max)

Maximal oxygen uptake was determined by a progressive treadmill test to exhaustion [16]. The volume of oxygen (VO_2) and carbon dioxide (VCO_2) was measured continuously using a metabolic cart (V-Max29 Series, SensorMedics, Yorba Linda, CA).

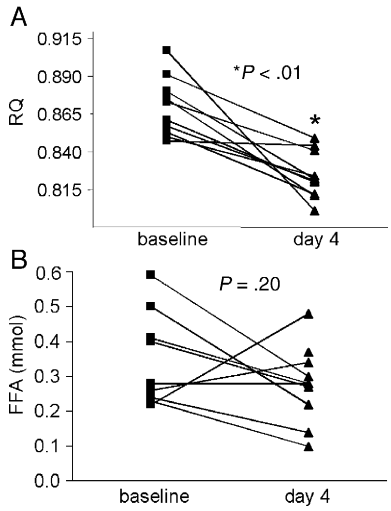

Fig. 2. High-fat/low-carbohydrate diet shifts substrate utilization toward fatty acid oxidation. A, Effect of a 3-day isoenergetic HF/LCD (50%:34% fat) in a cohort of healthy insulin-sensitive males (n = 10) on fatty acid oxidation as measured by RQ during their stay in the metabolic chamber. B, Effect of a 3-day isoenergetic HF/LCD (50%:34% fat) in a cohort of healthy insulin-sensitive males (n = 10) on circulating FFA concentrations during their stay in the metabolic chamber. FFA indicates free fatty acid.

2.4. Body composition

Body fat mass and lean body mass were measured on a Hologic dual-energy x-ray absorptiometer (QDR 4500, Hologic, Waltham, MA).

2.5. Indirect calorimetry

Twenty-four–hour EE and respiratory quotient (RQ) were determined in the whole-room calorimeter, before and during 3 days of isoenergetic HF/LCD to confirm an increase in fat oxidation (Fig. 2A). Energy expenditure was set at 1.4 times the resting metabolic rate measured by metabolic cart and clamped across the 4-day chamber stay.

2.6. Animal study

A similar experiment was conducted in mice with a 3-week intervention using a control group and an HF/LCD group to offset the lack of a control group within the human cohort. Male C57Bl/6J mice were housed at room temperature with a 12-hour light-dark cycle for 5 weeks. Six mice consumed control diet ad libitum (Research Diets, D12450B, New Brunswick, NJ: 10% of energy from fat, 70% of energy from carbohydrate, and 20% of energy from protein) and 7 mice consumed an HF/LCD ad libitum (Research Diets, D12451: 45% of energy from fat, 35% of energy from carbohydrate, and 20% of energy from protein). All animals ate the control diet ad libitum for 2 weeks, and 7 were switched to HF/LCD ad libitum for 3 additional weeks. Food intake was not measured. Gastrocnemius muscles were chosen based on similarity in fiber type composition compared with human vastus lateralis and was then dissected and snap-frozen in liquid nitrogen.

2.7. RNA extraction

Human and mouse total RNA from 50 to 100 mg vastus lateralis and gastrocnemius muscles, respectively, was isolated with Trizol reagent (Invitrogen, Carlsbad, CA). The quantity and the integrity of the RNA were confirmed by Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA).

2.8. Oligonucleotide microarrays

Microarrays were performed as previously described [10]. A further analysis was applied with a more conservative P value of greater than .01 and less than .05.

2.9. Real-time quantitative reverse transcriptase–polymerase chain reaction for RNA

RNA sample pairs (1 μg) were reverse transcribed using iScript cDNA synthesis kit (BioRad, Hercules, CA). All primers and probes were designed using Primer Express version 2.1 (Applied Biosystems, Roche, Branchburg, NJ). Sequences of primers and probes are shown in Supplemental Table 1. Real-time quantitative reverse transcriptase–polymerase chain reaction (qRT-PCR) reactions [17] were performed as 1-step reactions in ABI PRISM 7900 (Applied Biosystems) using the following parameters: 1 cycle of 48°C for 30 minutes, then 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds, and 60°C for 1 minute. For all assays performed using Taqman primers and probe, RPLP0, which is the human equivalent of the murine 36B4 [18], was the internal control. For all assays performed using SYBR Green I (Applied Biosystems, Roche), 18S was the internal control. Cyclophilin B was used for all murine assays. All expression data were normalized by dividing target gene by internal control.

2.10. Statistical analysis

Statistical analysis was performed using 2-tailed paired Student t test before vs after HF/LCD (human) and unpaired Student t test for control vs HF/LCD (mouse) to establish

Table 1
Characteristics of the study population before the HF/LCD

	Mean ± SD	Range
Age (y)	23.0 ± 3.1	
Height (cm)	179.7 ± 6.3	
Weight (kg)	78.9 ± 13.2	
BMI (kg/m^2)	24.3 ± 3.0	21.0-30.7
WHR (AU)	0.88 ± 0.1	
RQ (AU)	0.87 ± 0.01	0.85-0.90
$V \cdot O_2$max (mL kg^{-1} min^{-1})	49.4 ± 4.0	45.5-56.1
Fasting glucose (mg/dL)	78.4 ± 4.7	
Fasting insulin (μU/mL)	4.6 ± 1.4	
GDR (mg kg FFM^{-1} min^{-1})	14.7 ± 4.1	9.9-24.5
Cumulative CHO balance (kJ)	139.9 ± 163.9	
FFA (mmol)	0.4 ± 0.1	
Body fat (%)	16.2 ± 3.2	11.5-20.4

Ten male subjects were chosen from the larger study population based on a high aerobic capacity and insulin sensitivity. BMI indicates body mass index; FFM, fat free mass; CHO, carbohydrate; FFA, free fatty acids.

Table 2
Microarray hits in glucose metabolism pathways

Low laser analyses		
Gene	Fold change	*P*
PFKFB3	↑ 3.3	<.01
PDK4	↑ 1.7	<.01
GYS1	↓ 1.7	<.01
GALT	↓ 1.6	<.01
PDHB	↓ 1.7	<.01
MAP2K3	↓ 2.0	<.01
UGP2	↓ 2.0	<.01

MAP2K3 indicates mitogen-activated protein kinase kinase 3; UGP2, UDP-glucose pyrophosphorylase 2.

effects of the intervention. All values are presented in figures and tables as sample (raw) means ± SE. Population characteristics are represented as means ± SD. Type I error rate was set a priori at $P < .05$. Analysis was performed using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA).

3. Results

3.1. High-fat/low-carbohydrate diet regulates expression of genes involved in glucose metabolism

The characteristics of the study population are presented in Table 1. Fasting glucose, insulin, and free fatty acids were unchanged as the diet was switched from "standard" fat-carbohydrate content (35%:49%) to high-fat–low-carbohydrate content (50%:34%; data not shown). Intrasubject changes in skeletal muscle gene expression before vs after 3 days eating the HF/LCD were identified using oligonucleotide microarrays according to low laser analysis previously described [10].

The 3-day isoenergetic HF/LCD significantly changed the expression of 297 genes according to the low laser "hit" list ($P < .01$; Supplemental Table 2). One hundred sixty-three genes were up-regulated, and 135 were down-regulated by the HF/LCD. A further analysis of the low laser data set revealed an additional 72 genes regulated by the intervention (P value >.01 and <.05; Supplemental Table 3). From these analyses, 7 of those genes were known to be involved in glucose metabolism by visual inspection or through gene ontogeny analysis (Table 2). Of the glucose metabolism genes, 5 were down-regulated and 2 were up-regulated. Fructose-2,6-biphosphatase 3 and pyruvate dehydrogenase kinase, isoenzyme 4 increased at the RNA expression level, whereas GYS1, galactose-1-phosphate uridyltransferase (GALT), pyruvate dehydrogenase, β subunit (PDHB), mitogen-activated protein kinase kinase 3, and uridine diphosphate (UDP)-glucose pyrophosphorylase 2 decreased in expression.

All of the genes are involved in the oxidation, storage, or glucose transporter expression [19]. All qRT-PCR results are based on the 7 genes from the initial low laser analysis ($P < .01$).

3.2. High-fat/low-carbohydrate diet shifts substrate utilization toward fatty acid oxidation

Within this cohort of insulin-sensitive males, although circulating free fatty acid concentrations did not increase (Fig. 2B), we were able to demonstrate an increase in fatty acid oxidation on the HF/LCD as measured by a significant decrease in their RQ during their 4-day stay in the metabolic chamber (Fig. 2A) (0.87 ± 0.01 to 0.82 ± 0.01 arbitrary units [AU], $P < .01$).

3.3. Confirmation of microarray expression of glucose metabolism genes in human skeletal muscle by quantitative real-time RT-PCR

Because of phenotypic changes in the subjects as evidenced by a decrease in RQ, as well as displaying a

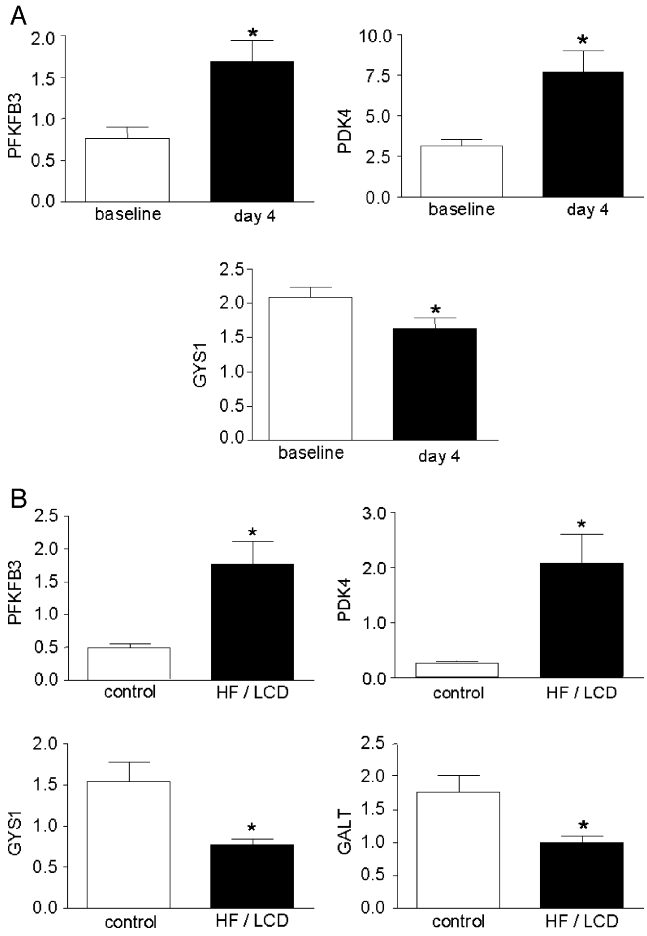

Fig. 3. High-fat/low-carbohydrate diet regulates messenger RNA for genes involved in glucose metabolism in healthy young men and mice. A, Effect of a 3-day isoenergetic HF/LCD (50%:34% fat) in a cohort of healthy insulin-sensitive males (n = 10) at baseline and after HF/LCD intervention on the expression of genes involved in glucose oxidation (PFKFB3 and PDK4) and glucose storage (GYS1). Messenger RNA was quantified by qRT-PCR. Data are shown as means ± SE and corrected for the expression of RPLP0. RPLP0 indicates ribosomal protein, large, P0. B, Effect of a 21-day ad libitum feeding of an HF/LCD (45%:35%) in a cohort of C57Bl/6J mice (control, n = 6; HF/LCD, n = 7) on genes involved in glucose oxidation (PFKFB3 and PDK4) and glucose storage (GYS1 and GALT). Data are shown as means ± SE and corrected for the expression of cyclophilin B.

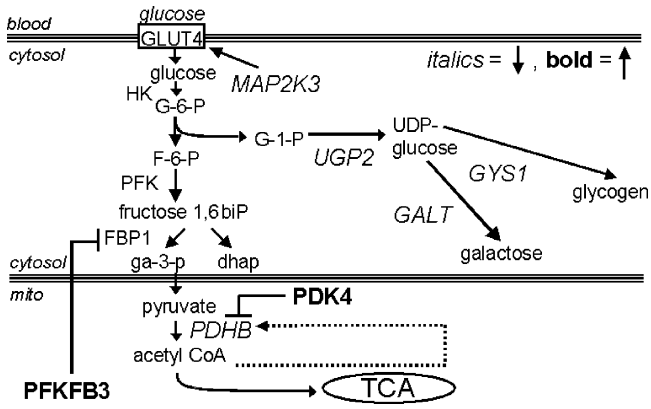

Fig. 4. Transcriptional regulation of glucose flux. Upon entry into the muscle cell, glucose can either be oxidized via glycolysis and enter the tricarboxylic acid (TCA) cycle or it can be stored as glycogen and galactose via nonoxidative glucose metabolism. The 9 genes that were altered by a 3-day HF/LCD in human skeletal muscle according to low laser microarray analysis are marked blue if down-regulated and red if up-regulated. UGP2, UDP-glucose pyrophosphorylase 3; MAP2K3, mitogen-activated protein kinase kinase 3; FBP1, fructose-1,6-biphosphatase; HK, hexokinase; PFK, phosphofructokinase.

positive carbohydrate balance subsequent to intervention, we then sought to confirm the expression of these glucose metabolism genes by real-time qRT-PCR. Of the "hits" from the low laser microarray list ($P < .01$), 3 genes were "confirmed" (Fig. 3A): PDK4 (0.09 ± 0.01 to 0.16 ± 0.03 AU, $P < .01$), PFKFB3 (1.61 ± 0.26 to 3.28 ± 0.52 AU, $P < .01$), and GYS1 (2.17 ± 0.17 to 1.71 ± 0.16 AU, $P < .01$).

3.4. Expression of glucose metabolism genes in mouse skeletal muscle after 21 days of ad libitum HF/LCD

We next tested whether the changes in gene expression that we found in the clinic were present in a murine model of HF/LCD-induced obesity. We fed C57Bl/6J mice either a 10%:70% or a 45%:35% HF/LCD for 3 weeks. We chose 2 murine genes involved in glucose oxidation and 2 genes involved in glucose storage from the human experiments. The changes in gene expression were in the same direction, but of a greater magnitude than those seen in the human experiments. As measured by real-time qRT-PCR the results in rodents matched those in man (Fig. 3B): PDK4 (0.31 ± 0.03 to 1.05 ± 0.07 AU, $P < .01$), PFKFB3 (0.65 ± 0.04 to 1.08 ± 0.17 AU, $P < .01$), GYS1 (3.60 ± 0.66 to 0.66 ± 0.06 AU, $P < .01$), and GALT (4.37 ± 0.89 to 0.81 ± 0.07 AU, $P < .01$).

4. Discussion

During insulin stimulation the skeletal muscle is the major site for glucose disposal [20]; thus, defects in insulin-mediated glucose uptake and in its metabolic fate in this tissue are thought to be responsive to the macronutrient composition of the diet. Since the proposal of Randle et al [9] in 1963 of a glucose–fatty acid cycle that embodies direct competition between substrates for mitochondrial oxidation, a plethora of mechanisms have been put forward to explain how fuel substrates could interfere with glucose disposal in skeletal muscle [21-23]. In vivo, the release and/or intermediate products of lipid fuels (fatty acids and ketone bodies, respectively) released into the circulation (eg, in starvation or diabetes) may inhibit the catabolism of glucose in muscle, as well as its storage [7,24].

Although an increase in free fatty acid concentrations was not seen in this cohort (Fig. 2B), fatty acid flux through the muscle is by necessity increased in these subjects as demonstrated by a decrease in 24-hour RQ (Fig. 2A) to match the fat intake in this experimental paradigm [11]. Therefore, substrate utilization is shifting more toward oxidation of fatty acids by reducing the oxidation of glucose; the uptake and utilization of glucose should consequently be reduced. A negative carbohydrate balance occurs immediately after consumption (1-2 days) of an HF/LCD, indicating a continued glucose oxidation and glycogen depletion as fat oxidation takes days to catch up to fat intake [25,26]. A positive carbohydrate balance was observed at the end of the 3-day HF/LCD in this cohort of healthy young males, thus indicating a decrease in glucose oxidation (Table 1). We also observed a decrease in the messenger RNA levels of a number of genes involved in the oxidative, as well as the nonoxidative, glucose metabolism. Even more striking is the matching murine data showing a greater magnitude of change with a longer exposure to an HF/LCD (3 days vs 3 weeks). The extent of the effects of HF/LCD intake on muscle glucose metabolism reflects the length of each model's exposure to the diet. For example, the human cohort responded with about a 30% change in gene expression after 3 days of HF/LCD, whereas the murine model had about a 70% change in gene expression with 3 weeks of exposure. These results indicate that the effects of the HF/LCD are not transient. Even more compelling is the concurrent change in phenotype, such as decreased RQ (Fig. 2A), positive carbohydrate balance (Table 1), and in the genes controlling glucose uptake and metabolism in response to the perturbation of this diet (Table 2).

A variety of model systems have largely confirmed the validity of Randle's [27] experiments depicting the mechanism of substrate competition through changes in enzymatic activity as a result of product feedback inhibition, at least in terms of the short-term effects of fatty acids on muscle glucose metabolism and storage [27,28]. Our results supplement those of Randle by demonstrating a down-regulation of genes involved in glucose oxidation and storage when fat intake is increased and carbohydrate intake is decreased. Our findings relate to changes in messenger RNA expression only, and we acknowledge that many of the enzymes involved in glycolysis and glycogen synthesis are regulated allosterically, and through covalent modification. Transcriptional regulation (Fig. 4) may enhance the Randle

cycle or changes in the activation of signaling molecules/pathways such as PKC or ceramides [7,22].

Through the use of bioinformatics and microarrays, we expand the view of the glucose–fatty acid cycle beyond enzymatic activity to the level of gene transcription. In our analyses of the low laser microarray data set (Supplemental Table 2), we found more than 300 genes regulated by a 3-day HF/LCD in our human subjects. Four of our glucose metabolism genes (PFKFB3, PDK4, PDHB, and GALT) were found in the analyses, and 2 of the 4 genes were confirmed by qRT-PCR. This is consistent with the noise inherent in current microarray technologies [29,30]. After reporting changes in expression of the genes in the glucose metabolism gene cluster, we are left with the question of how the macronutrient composition of the diet affects other genes. The HF/LCD significantly regulated the expression of many OXPHOS genes, a few genes involved in fatty acid metabolism, and various other genes known and unknown (Supplemental Tables 2 and 3).

Recent studies suggest that both short- and long-term increases in fatty acids down-regulate oxidative phosphorylation in skeletal muscle [10,24]. Cluster analysis suggests that the changes in OXPHOS genes and the carbohydrate metabolism genes described herein are involved in a long-term transcriptional loop; however, the identity of the overall transcriptional control system remains unknown. It has been suggested that peroxisome-proliferator–activated receptor-gamma co-activator 1 alpha (PGC1α) plays a pivotal role as a link between a high-fat diet and oxidative phosphorylation. Furthermore, the implication that both OXPHOS and carbohydrate metabolism genes are regulated at the transcriptional level is important for 2 reasons: (*a*) the unidentified transcriptional control system may be a "master regulator" of energy homeostasis for both glucose and mitochondria, and (*b*) strategies to identify the key regulators of this system should include both subsets of these genes.

Consonant with recent evidence by Pehleman et al [31] demonstrating the enzymatic regulation of glucose disposal in human skeletal muscle after an HF/LCD, our results expand this view to a transcriptional co-regulation of glucose metabolism in this same tissue, as well as murine skeletal muscle after an HF/LCD. Pehleman et al found a dramatic decrease in the activity of PDH with a concomitant increase in the enzymatic activity of PDH kinase in response to a 56-hour eu-energetic HF/LCD (73%:5%) within a healthy human cohort of insulin-sensitive males. Although our results are not as dramatic, probably due to difference in dietary fat/carbohydrate content (50%:34% vs 73%:5%), we do show the same effects on PDH kinase at the level of transcription. Together, our results identify both enzymatic activity and gene expression as targets of the transcriptional regulation.

Several studies over the past decade have shown PDK4 message, protein, and activity decrease as a result of increased free fatty acids, starvation, and diabetes [25,32,33]. More recently, PDK4 has become the target of diabetic drug investigations [34,35]. Pyruvate dehydrogenase kinase, isoenzyme 4 promotes gluconeogenesis and suppresses glucose oxidation and maintains glucose levels in starvation. Although prior studies bolster the credibility of PDK4 as a diabetic drug target, our result argues against this view. We demonstrate that although glucose oxidation is suppressed, the transcription of genes before and after PDK4 is down-regulated by HFD. Thus, the inactivation of PDK4 might not be effective in the setting of HFD/"lipotoxicity."

The original studies that formed the basis of the glucose–fatty acid cycle [9,23,26,36] elucidated (1) fatty acid–induced desensitization of insulin-mediated glucose transport and (2) inhibitory effects of increased fatty acid oxidation on glucose metabolism. According to the Randle hypothesis [9,27], an increase in lipid oxidation in muscle will decrease glucose oxidation by suppression of the mitochondrial pyruvate dehydrogenase complex, with a consequential reduction of glycolytic flux resulting in an increase in glucose-6-phosphate, inhibition of hexokinase activity, and ultimately leading to decreased glucose uptake. Nonoxidative metabolism (storage) of glucose is therefore also predicted to be decreased. This mechanism has been reexamined and evolved over the past few decades [37], but the cellular effect is consistently observed. Differences in glucose-6-phosphate levels and mechanisms aside, a consistently observed effect of increased fat intake is the reduction of nonoxidative glucose metabolism [38]. These data provide a basis for a transcriptional switch to regulate substrate utilization within the skeletal muscle in response to changes in dietary macronutrient content [39]. Overall, the novel contribution of this article is its identification of multiple regulated genes within the same pathway by HF/LCD.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.metabol.2006.07.003.

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