Original Article

Impaired Fat Oxidation After a Single High-Fat Meal in Insulin-Sensitive Nondiabetic Individuals With a Family History of Type 2 Diabetes

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Individuals with insulin resistance and type 2 diabetes have an impaired ability to switch appropriately between carbohydrate and fatty acid oxidation. However, whether this is a cause or consequence of insulin resistance is unclear, and the mechanism(s) involved in this response is not completely elucidated. Whole-body fat oxidation and transcriptional regulation of genes involved in lipid metabolism in skeletal muscle were measured after a prolonged fast and after consumption of either high-fat (76%) or high-carbohydrate (76%) meals in individuals with no family history of type 2 diabetes (control, $n = 8$) and in age- and fatness-matched individuals with a strong family history of type 2 diabetes ($n = 9$). Vastus lateralis muscle biopsies were performed before and 3 h after each meal. Insulin sensitivity and fasting measures of fat oxidation were not different between groups. However, subjects with a family history of type 2 diabetes had an impaired ability to increase fatty acid oxidation in response to the high-fat meal ($P < 0.05$). This was related to impaired activation of genes involved in lipid metabolism, including those for peroxisome proliferator–activated receptor coactivator-1α (PGC1α) and fatty acid translocase (FAT)/CD36 ($P < 0.05$). Of interest, adiponectin receptor-1 expression decreased 23% after the high-fat meal in both groups, but it was not changed after the high-carbohydrate meal. In conclusion, an impaired ability to increase fatty acid oxidation precedes the development of insulin resistance in genetically susceptible individuals. PGC1α and FAT/CD36 are likely candidates in mediating this response. *Diabetes* 56:2046–2053, 2007

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Received for publication 4 December 2006 and accepted in revised form 22 April 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 24 April 2007. DOI: 10.2337/db06-1687.

Additional information for this article can be found in an online appendix at http://dx.doi.org/10.2337/db06-1687.

ACC, acetyl CoA-carboxylase; adipoR, adiponectin receptor; CPT, carnitine palmitoyltransferase; FAT, fatty acid translocase; FFA, free fatty acid; FFM, fat-free mass; PDK, pyruvate dehydrogenase kinase; PGC, peroxisome proliferator–activated receptor coactivator; REE, resting energy expenditure; RQ, respiratory quotient.

Relatives of individuals with type 2 diabetes are an ideal human model to test for mechanisms in the early development of insulin resistance because approximately two-thirds eventually develop diabetes, and they can be tested before the development of other confounding factors, including hyperglycemia, dyslipidemia, and changes in insulin secretion (1). Insulin-resistant relatives will also develop impaired lipid metabolism, including increased circulating free fatty acids (FFAs) (2), accumulation of triglycerides and other lipid moieties within skeletal muscle (3), and postprandial hypertriglyceridemia (4). However, the mechanism(s) responsible for these early defects in lipid metabolism is not completely elucidated, although we and others suggest that intramuscular triglyceride accumulation may occur secondary to the development of insulin resistance (3,5).

In lean individuals, increasing the proportion of dietary fat switches on fatty acid oxidation, whereas the infusion of glucose and insulin promotes carbohydrate oxidation. The cellular capacity to switch from lipid to carbohydrate and vice versa has been termed "metabolic flexibility," and there is large variation in this parameter between individuals (6). Obese and weight-reduced obese individuals have impaired metabolic flexibility on a whole-body level (7). Fatty acid oxidation ex-vivo from skeletal muscle homogenates is also ~50% lower in morbidly obese women compared with lean women (8). However, controversy exists as to whether basal differences are detectable in less obese individuals (9–12). Factors that have been linked to defective lipid metabolism in humans include reduced peroximsome proliferator–activated receptor coactivator-1α (PGC1α); reduced carnitine palmitoyltransferase 1 (CPT1); a proposed rate-limiting step in carnitine transport of long-chain fatty acids across the mitochondrial membrane; decreased translocation of fatty acid translocase (FAT/CD36) to the mitochondrial membrane; increased acetyl CoA-carboxylase 2 (ACC2) activity, which catalyzes the synthesis of malonyl CoA; reduced adiponectin receptor-1 (adipoR1) and adipoR2 (13), which mediates adiponectin-related increases in fatty acid oxidation and insulin sensitivity; and impaired activity of pyruvate dehydrogenase kinase-4 (PDK4) (14). Other genes that are potentially important in lipid metabolism include the nuclear receptors peroxisome proliferator–activated receptor-δ (PPARδ), which regulates expression of *CPT1b* and *FAT/CD36* (15) and is increased in response to fasting (16), and the orphan nuclear receptor NR4A1 (also known

TABLE 1
Baseline characteristics of study participants with no family history of type 2 diabetes and a strong family history of type 2 diabetes after a 10-h overnight fast

	Control subjects	Subjects with a family history of type diabetes
Sex (M/F)	3/5	2/7
Age (years)	41 ± 7	46 ± 6
Weight (kg)	75.7 ± 13.6	70.1 ± 9.0
BMI (kg/m^2)	26.7 ± 5.3	26.6 ± 5.3
Total fat (%)	35 ± 10	38 ± 12
Total cholesterol (mmol/l)	4.7 ± 0.7	4.9 ± 0.7
HDL cholesterol (mmol/l)	1.2 ± 0.2	1.3 ± 0.2
LDL cholesterol (mmol/l)	2.9 ± 0.5	3.0 ± 0.6
Triglycerides (mmol/l)	1.2 ± 0.6	1.3 ± 0.5

Data are the means ± SD. GIR, glucose infusion rate during the hyperinsulinemic-euglycemic clamp.

as Nur77), which regulates the expression of *UCP3*, *adipoR2*, and *FAT/CD36* in vitro (17). Agonists for PPARδ also increase fat oxidation and prevent weight gain in mice fed a high-fat diet (18), and *NR4A1* expression is increased in response to 3 h of endurance exercise in humans (19).

Despite a convincing body of evidence linking metabolic inflexibility with obesity and insulin resistance, it remains uncertain whether these factors represent a contributing factor or an adaptive consequence of the disease. Pilegaard et al. (20) observed large interindividual differences in the change in expression of PDK4 and several other lipid metabolism genes in response to 1 h of refeeding high-fat and -carbohydrate meals in fasted young physically active men and suggested that this may explain individual responsiveness of skeletal muscle to metabolic changes. Consequently, in the current report, we examined whole-body glucose and fat oxidation after a prolonged fast and in response to refeeding a single high-fat or -carbohydrate meal both in nondiabetic subjects who are at high risk of developing type 2 diabetes and in age-, sex-, and adiposity-matched control subjects. We show that the ability to switch on fat oxidation is impaired after a single high-fat meal in subjects with a family history of type diabetes, and we examined genes in skeletal muscle that may underlie this defect, including *CPT1b*, *FAT/CD36*, *ACC2*, *PDK4*, and *PGC1α*. Other genes that have potential roles in regulating lipid metabolism were also examined, including PPARδ, AdipoR1, AdipoR2, and NR4A1.

RESEARCH DESIGN AND METHODS

Sedentary nonsmoking nondiabetic men and women who either reported no family history of type 2 diabetes (control) or at least two first-degree relatives with type 2 diabetes were recruited by public advertisement. Subjects were excluded if weight had changed by >2 kg in the preceding 6 months, if they were taking any medications known to affect insulin sensitivity or blood pressure, or had a personal history of type 2 diabetes or cardiovascular disease. Subject baseline characteristics are given in Table 1. The study protocol was approved by the Human Research Ethics Committee at St Vincent's Hospital (Sydney, Australia), and subjects provided informed written consent.

Procedures. Subjects attended the clinical research facility on three separate visits, and each visit was at least 3 days but no more than 14 days apart. On the first visit, subjects fasted for 10 h overnight, and body weight was measured in a hospital gown and height measured by a stadiometer. Insulin sensitivity was then measured by 2-h hyperinsulinemic-euglycemic clamp (50 mU/m^2 per min), according to previously described methods (21). Briefly, two intravenous cannula were inserted, one for infusion of regular insulin (Novo Nordisk, Baulkham Hills, NSW, Australia) and glucose (Baxter, Old Toongabbie, NSW, Australia), and the other was placed in the contralateral hand for blood withdrawal with the hand placed in a heating pad. A variable infusion of exogenous glucose was given to maintain glucose concentrations at 5.0 mmol/l. After the clamp, body composition was measured by dual X-ray absorptiometry (Lunar DPX; Lunar Radiation, Madison, WI). At visits 2 and 3, subjects attended the clinical research facility at 8 A.M. to measure various factors in response to a meal after a prolonged fast (20 h). These visits were identical except for the type of meal consumed. At each visit, subjects were weighed, and an intravenous cannula was inserted into the antecubital vein. A blood sample was taken (−60), and subjects rested in the supine position for 30 min. Resting metabolic rate and respiratory quotient (RQ) were determined over a 30-min period (Deltatrac; Datex, Helsinki, Finland). A vastus lateralis muscle biopsy was then performed to obtain ~200 mg of tissue, and the samples were immediately blotted for blood, and any visible fat was removed from the sample. Samples were snap-frozen at −80°C. One female subject from the group of subjects with a family history of type diabetes refused muscle biopsy. After the muscle biopsy, a 0–time point blood sample was taken, and subjects were fed a standard 1,000-kcal meal that was high in either fat (76%) or carbohydrate (76%). The meals were held constant for protein (15%) and were randomly assigned to visit 2 or 3. Detailed meal composition is given in Appendix 1, which can be found in an online appendix (available at http://dx.doi.org/10.2337/db06-1687). Additional blood samples were taken at 30, 60, 120, 180, and 240 min after the meal was completed. A second muscle biopsy was taken at 180 min after the meal, and the metabolic rate and RQ were assessed for 30 min from 210 to 240 min.

Biochemical analytes. Radioimmunoassays were performed for serum insulin and C-peptide (Linco Research, St. Charles, MO). Fasting serum was assayed for total and HDL cholesterol and triglycerides (all by enzymatic colorimetry; Roche, Indianapolis, IN), and LDL was calculated by the Friedewald equation. FFAs were measured by enzymatic colorimetry assay (Wako, Osaka, Japan).

Gene expression. RNA was isolated from ~30 mg of tissue using the acid phenol method (22). Most primers were designed using MacVector (Sigma Aldrich, Castle Hill, NSW, Australia) (Appendix 2, which can be found in the online supplement), and premade primer-probe sets for PGC1α and NR4A1 were purchased from Applied Biosystems (Sydney, NSW, Australia). cDNA was prepared from RNA by use of Superscript II and oligo dT primers (Invitrogen, Mulgrave, VIC, Australia). Real-time quantitative PCR was performed with a 7900HT Fast real-time PCR system (Applied Biosystems) with Power SYBR Green PCR Master Mix (Applied Biosystems) in accordance with the manufacturer's instructions. Samples were run with internal positive and negative controls, and gene product was quantified by comparing samples to known standard concentrations of pure gene product. The C_t value for every sample was measured in duplicate and was normalized to B-actin expression, which was not different between groups at baseline and was not altered in response to either meal.

Statistical analysis. Data are the means ± SE unless otherwise stated. Statistics were analyzed with StatView 5.0 (SAS Institute, Cary, NC). Baseline samples from visits 2 and 3 were averaged and group differences were tested by one-way ANOVA. To determine whether there was any difference between groups in resting metabolic rate adjusted for fat-free mass (FFM), we regressed resting metabolic rate against FFM at baseline to generate residuals. Group differences were then tested by one-way ANOVA. Repeated-measures ANOVA was used to determine differences in response to the meal by group and time. Correlations were performed using Pearson's correlation coefficient. Significance was set at $P < 0.05$.

RESULTS

Baseline characteristics. Baseline characteristics of subjects by group are given in Table 1. Importantly, groups were closely matched for age and BMI. Furthermore, groups were similar for percent body fat, fasting plasma lipids, insulin, and glucose and were within reference ranges for glucose and lipids. In contrast to our previous studies, insulin sensitivity, as measured by the euglycemic-hyperinsulinemic clamp, was not significantly different between groups (Fig. 1) and may reflect that relatives presented and were studied at an earlier time point in the evolution of the disease process compared with previous volunteers (21).

Serum factors. The insulin, C-peptide, glucose, triglyceride, and FFA response to high-carbohydrate and -fat meals are shown in Fig. 2. In response to a high-carbohydrate

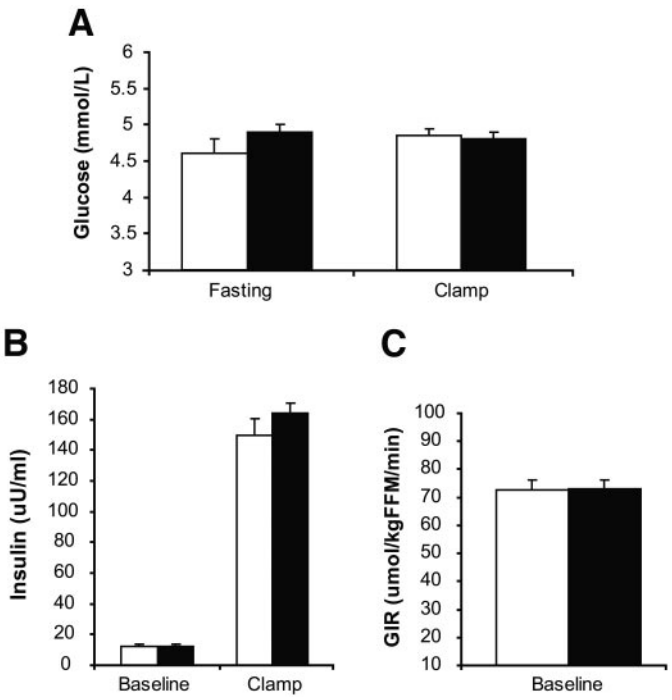

FIG. 1. Basal and steady-state glucose and insulin concentrations during the euglycemic-hyperinsulinemic clamp. No differences were observed at fasting or steady state between relatives and controls. □, control; ■, subjects with a family history of type 2 diabetes.

meal, the serum insulin response tended to be higher in subjects with a family history of type diabetes, as assessed by repeated-measures analysis ($P = 0.06$) and by incremental area under the curve ($P = 0.07$, data not shown). Similarly, C-peptide tended to be higher in those subjects by repeated-measures and incremental area under the curve (both $P = 0.09$), but serum glucose response was not different between groups ($P > 0.5$). In response to the high-fat meal, the insulin and C-peptide response was similar between groups, but the glucose response to the high-fat meal was significantly greater in subjects with a family history of type diabetes ($P < 0.05$), with the mean peak, however, being only 5.3 mmol/l. Serum triglycerides were not changed in response to the high-carbohydrate meal and were significantly increased in response to the high-fat meal ($P < 0.001$), with no differences between groups. Serum FFAs were suppressed in response to the high-carbohydrate meal in both groups at all time points, with no difference between groups. FFA was suppressed at 60 and 120 min after the high-fat meal but had returned to basal levels at 180 and 240 min, with no differences between groups.

Fatty acid oxidation and diet-induced thermogenesis. There was no difference between groups at baseline for energy expenditure adjusted for FFM ($P = 0.25$). As expected, energy expenditure was significantly increased by the high-fat and -carbohydrate meals, but this was not different between groups ($P > 0.4$) or by meal type ($P = 0.16$) (Fig. 3). Meal size, expressed as a percentage of resting energy expenditure (REE), was also not different between groups (80 and 87% of REE for control subjects and those with a family history of type diabetes, respectively; range 62–112%), and no relationships were observed between meal size, expressed as a percentage of REE, and any variables examined (data not shown). The fasting RQ was not different between control subjects and those with a family history of type diabetes. RQ was significantly increased in response to the high-carbohydrate meal, indicating increased glucose oxidation, and there was no difference detected between groups. After the high-fat meal, we observed that the subjects with a family history of type diabetes had an impaired ability to reduce RQ compared with control subjects ($P < 0.05$), indicating an impaired ability to switch on fatty acid oxidation (Fig. 3).

Gene expression. To determine the mechanisms involved in the impaired ability to upregulate fat oxidation, we examined genes putatively involved in fatty acid regulation at baseline and at 3 h in response to a meal. Genes measured were *PGC1α*, *ACC2*, *PPARδ*, CPT1b, FAT/CD36, AdipoR1, AdipoR2, and *NR4A1*. Variation in the basal expression of genes in response to the 20-h fast was low for most genes investigated, and no statistical differences were detected between the two basal biopsies (Fig. 4), and so basal levels were averaged. There was no difference in basal expression of lipid metabolism genes between control subjects and those with a family history of type diabetes (data not shown). The basal expression of *PGC1α* and *FAT/CD36* was positively related to insulin sensitivity, as measured by the clamp ($r = 0.6$, $P < 0.01$) (Fig. 5).

The high-fat meal increased expression of *PPARδ* ($P = 0.002$), *CPT1b* ($P = 0.05$), *ACC2* ($P = 0.04$), and *NR4A1* ($P = 0.02$) and decreased expression of *AdipoR1* ($P = 0.009$) and *AdipoR2* ($P = 0.01$) (Fig. 6*A*). The response for *ACC2* to the high-fat meal was significantly different between groups, with subjects with a family history of type diabetes having a significantly lower increase in ACC2. Furthermore, control subjects tended to increase PGC1α and FAT/CD36, whereas subjects with a family history of type diabetes had a nonsignificant lowering of PGC1α and FATCD36, leading to statistical differences between groups in the response of these genes to a high-fat meal ($P < 0.04$) (Fig. 6*B*). The nuclear receptors PPARδ and NR4A1 were increased in response to the high-carbohydrate meal in both groups ($P = 0.002$ and $P = 0.04$, respectively). No other lipid metabolism genes were changed in response to the high-carbohydrate meal.

DISCUSSION

Metabolically healthy skeletal muscle is characterized by its ability to switch rapidly between glucose and fat oxidation in response to homeostatic signals. Obese insulin-resistant individuals and individuals with type 2 diabetes are metabolically inflexible (23). However, it is not clear whether metabolic inflexibility is a cause or a consequence of insulin resistance and type 2 diabetes. Therefore, we examined a human model where insulin resistance and diabetes had not yet developed and challenged this system by a prolonged fast followed by either high-fat or -carbohydrate meals. First, we showed that the insulin-sensitive relatives of individuals with type 2 diabetes had an impaired ability to increase fatty acid oxidation 4 h after a single high-fat meal. We also showed general differences in the activation of genes involved in lipid metabolism in response to either high-carbohydrate or -fat meals, and in particular we showed reduced expression of adipoR1 and -2 in response to high-fat meal, suggesting nutrient-specific regulation of these receptors. Third, we showed differential changes in the expression of *PGC1α*, *FAT/CD36*, and *ACC2* between control subjects and those with a family history of type diabetes in response to the high-fat meal. This study suggests that metabolic flexibility may be involved in the

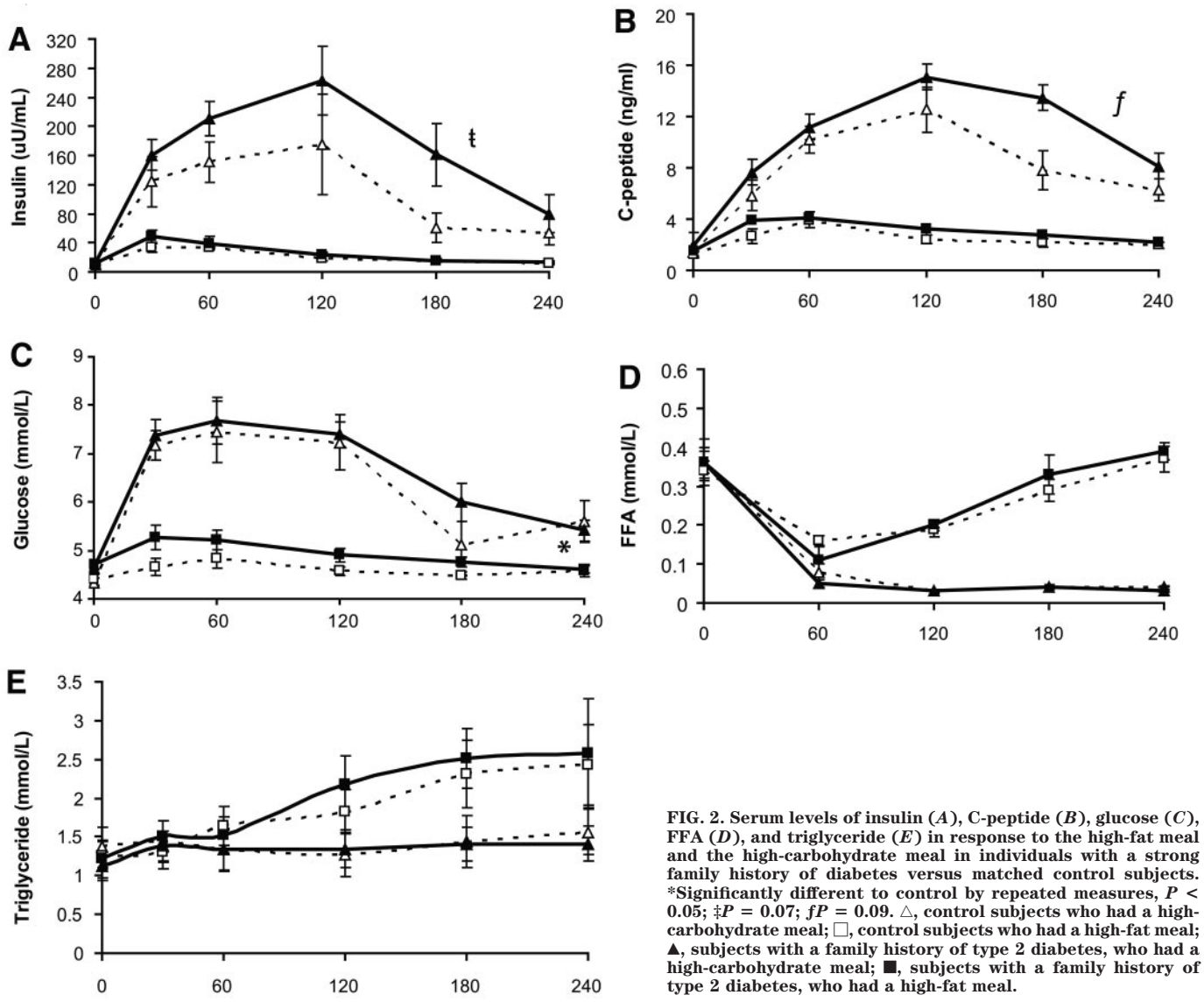

FIG. 2. Serum levels of insulin (*A*), C-peptide (*B*), glucose (*C*), FFA (*D*), and triglyceride (*E*) in response to the high-fat meal and the high-carbohydrate meal in individuals with a strong family history of diabetes versus matched control subjects. *Significantly different to control by repeated measures, $P < 0.05$; ‡$P = 0.07$; *f*$P = 0.09$. △, control subjects who had a high-carbohydrate meal; □, control subjects who had a high-fat meal; ▲, subjects with a family history of type 2 diabetes, who had a high-carbohydrate meal; ■, subjects with a family history of type 2 diabetes, who had a high-fat meal.

development of insulin resistance in genetically susceptible individuals and that defective regulation of *PGC1α* and *FAT/CD36* may be involved in this response.

The ability to switch on fat oxidation in response to an increase in dietary fat is variable between subjects and may translate to a positive fat balance and weight gain over time (24). Furthermore, an impaired ability to oxidize fatty acids is postulated to increase intramyocellular triglycerides and other lipid intermediates in the cell, which interfere with insulin signaling (25,26). In the current study, we observed that basal fat oxidation was similar between control subjects and those with a family history of type diabetes, but the latter had an impaired ability to switch on fat oxidation after a single high-fat meal. This was observed in the absence of detectable differences in insulin sensitivity by the clamp or of differences in postprandial FFAs or triglycerides in response to the meal, which accompany decreased insulin sensitivity (4). Therefore, we speculate that impaired fatty acid oxidation is a primary defect in the development of insulin resistance in individuals with a strong genetic predisposition for developing type 2 diabetes. However, we did not measure insulin resistance by low-dose clamp, and the subjects with a family history of type diabetes did trend toward an increased insulin response after the high-carbohydrate meal, which may be indicative of insulin resistance. On the other hand, other studies have shown that hepatic insulin resistance is not present at this stage in human relatives of individuals with type 2 diabetes (27,28), and all other indicators of insulin resistance, such as fasting insulin, homeostasis model assessment, or metabolic flexibility to carbohydrate ingestion, were not different between groups. Furthermore, it is likely that the 20-h fast was sufficient to "stress the system," potentially allowing an early metabolic defect to become apparent after a high-carbohydrate meal when not apparent by the clamp. Because no differences were observed in serum FFAs, this study also suggests that fatty acid uptake into skeletal muscle was not altered in nondiabetic relatives of individuals with type 2 diabetes. This has previously been reported to be similar or increased in obese rodent models and humans (12,29) and may eventually result in increased triglyceride storage within skeletal muscle (3). Also of note, a small but significantly higher glucose response was observed in relatives of individuals with type 2 diabetes in response to the high-fat meal. However, this was in the normal physiological range, and we suggest that if it were clinically important, it would have invoked an insulin

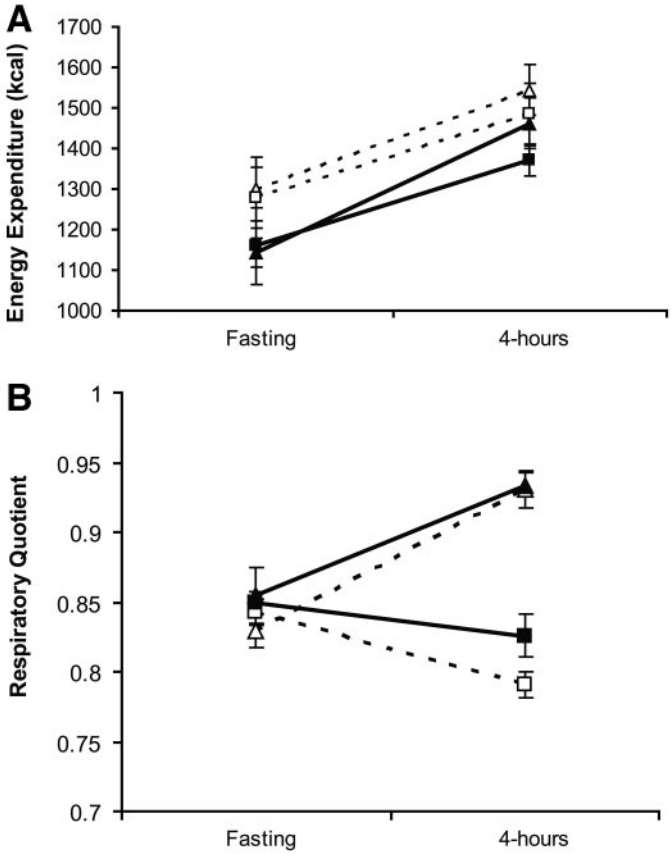

FIG. 3. Impaired fatty acid oxidation in response to a high-fat meal in individuals with a strong family history of diabetes versus matched control subjects. *$P < 0.05$. △, control subjects who had a high-carbohydrate meal; □, control subjects who had a high-fat meal; ▲, subjects with a family history of type 2 diabetes, who had a high-carbohydrate meal; ■, subjects with a family history of type 2 diabetes, who had a high-fat meal.

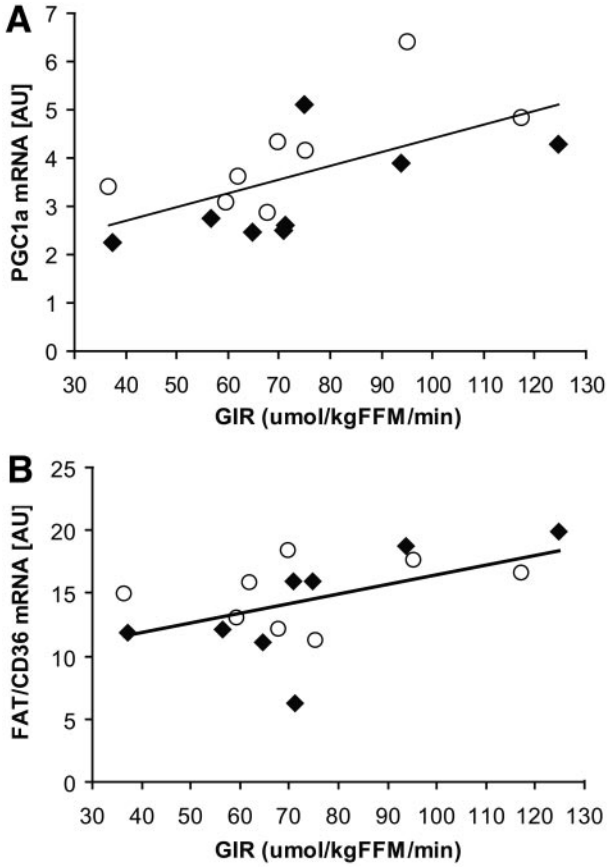

FIG. 5. Positive relationship between insulin sensitivity and the expression levels of PGC1α and FAT/CD36 across the whole group. Both $r = 0.6$, $P < 0.01$. ○, control; ▲, subjects with a family history of type 2 diabetes. GIR, glucose infusion rate.

response, but that it may indicate a higher "gluconeogenesis set" to fat input in this population.

We then examined potential mechanisms in skeletal muscle that may account for the impairments in fatty acid oxidation that were observed after the prolonged fast and refeed. The prolonged fast was designed to represent a metabolic challenge to skeletal muscle, requiring a sustained increase in the reliance on lipid and protein metabolism. Previous studies of refeeding after prolonged fasting in lean healthy subjects have found marked heterogeneity in the transcriptional response in *PDK4* and lipoprotein lipase (*LPL*), suggesting that individual differences in genetic profile may play an important role in adaptive molecular responses (20). In the current study, *PDK4* expression was not different between groups. However, the change in *PGC1α*, *FAT/CD36*, and *ACC2* expression in response to the high-fat meal was significantly lower in relatives of individuals with type 2 diabetes

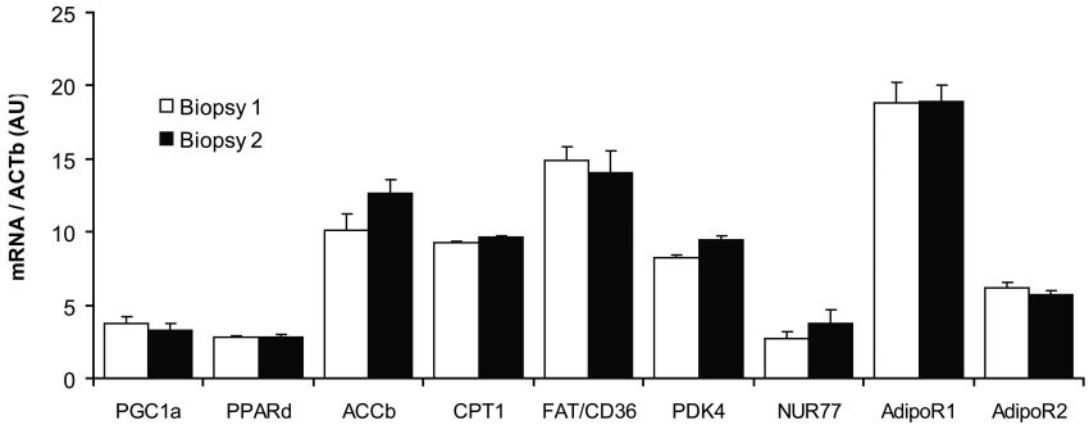

FIG. 4. Low variation in the basal expression of genes involved in fatty acid oxidation between biopsy 1 and biopsy 2. All genes were normalized for β-actin, which was not statistically different between biopsies.

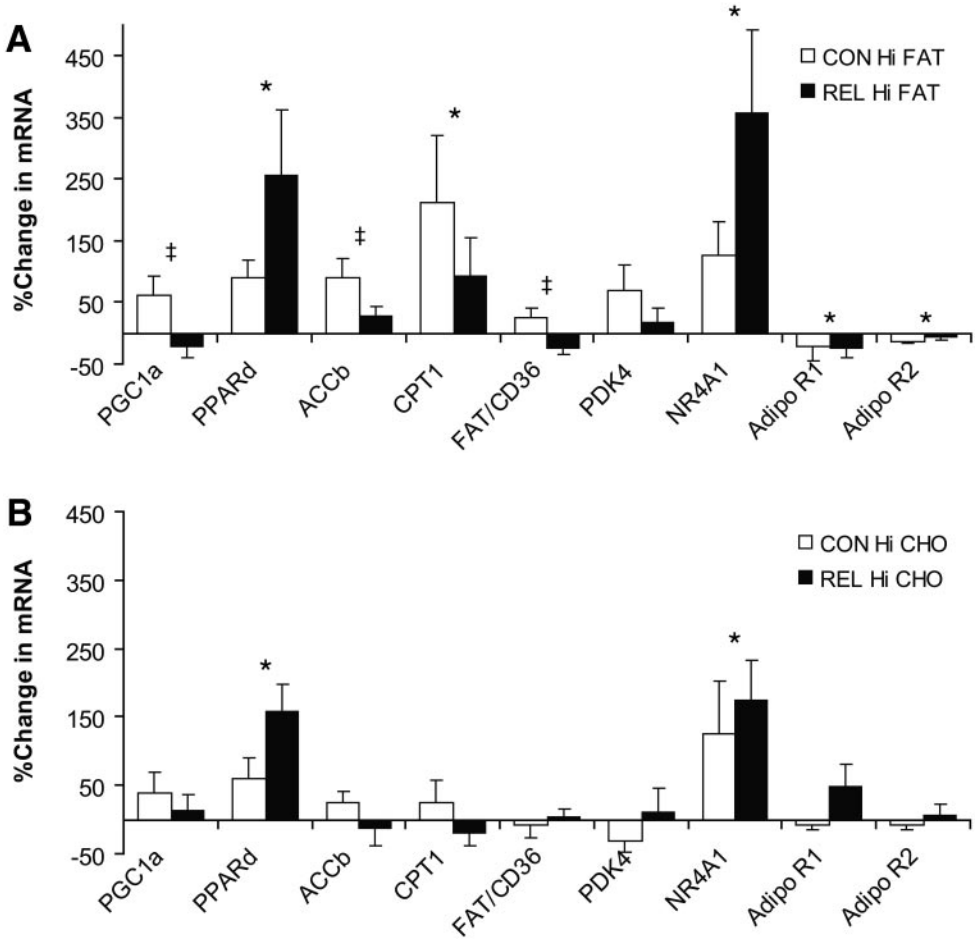

FIG. 6. Percent change from baseline in expression of genes involved in lipid metabolism in skeletal muscle after high-fat meal (*A*) and high-carbohydrate meal (*B*). All genes were normalized for β-actin, which was not statistically different in response to a meal or between groups. *Significantly changed from baseline by the meal, $P < 0.05$; ‡significant difference in response between relative and control groups, $P < 0.05$. □, control subjects; ■, subjects with a family history of type 2 diabetes.

compared with control subjects. PGC1α is a transcriptional coactivator that controls genes involved in oxidative phosphorylation and fatty acid metabolism, and it is lower in insulin-resistant individuals (30–32).

In the current study, basal *PGC1α* expression was not different between groups, but a relationship was observed between insulin sensitivity and expression of basal *PGC1α* across the whole group. Previous studies in humans indicate that *PGC1α* expression is decreased in response to isocaloric high-fat feeding for 3 days in a metabolic chamber and in response to prolonged lipid infusion (33,34). These studies suggest that the reduction in PGC1α is secondary to, rather than a primary cause of, insulin resistance. However, the experimental conditions in these studies (reduced physical activity and supraphysiological elevations in FFAs) may affect *PGC1α* expression through alternate mechanisms. In our study in response to a physiological fat bolus, we observed that *PGC1α* expression tended toward an increase in response to a high-fat meal in control subjects, but relatives of individuals with type 2 diabetes tended to decrease *PGC1α* expression. Similarly, we observed that *FAT/CD36* expression tended to increase in control subjects after the high-fat meal, but it was reduced in the group with the strong genetic predisposition to type 2 diabetes. FAT/CD36 probably plays an important role in regulating long-chain fatty acid oxidation in humans because specific blockade of FAT/CD36 by sulfo-*N*-succimidyl-oleate reduces palmitate oxidation by ~90% (35). In fact, whether CPT1b is rate limiting for fatty acid oxidation in humans is controversial (36), and certainly in this study we did not see any difference in expression of *CPT1b* between groups. We also observed a greater increase in *ACC2* mRNA expression in response to the high-fat meal in the control group. This was contrary to our hypothesis because increased expression of *ACC2* may increase ACC2 protein and activity, which increases malonyl CoA and inhibits CPT1b. Interestingly, previous studies of high-fat feeding for 1 week also showed increased *ACC2* mRNA expression (37), but no change in total or phosphorylated ACC2. From this study, we conclude that refeeding a single high-fat meal after a prolonged fast was sufficient to lead to impaired activation of key genes involved in lipid metabolism in insulin-sensitive relatives of individuals with a genetic predisposition to type 2 diabetes and that FAT/CD36 and PGC1α are probably important in this response; however, these changes were not related to changes in FFA, insulin, or glucose, and fitness levels were not assessed and may also have been important in this response.

AdipoR1 and adipoR2 mediate adiponectin-related increases in fatty acid oxidation and glucose uptake (13,38). Previous studies have reported decreased *adipoR1* and

adipoR2 gene expression in muscle in insulin-resistant subjects with a family history of type 2 diabetes (13). In the current study, basal adipoR1 and adipoR2 expression were not different between groups but were significantly decreased in response to a single high-fat meal in both groups. AdipoR1 was decreased 23% and adipoR2 just 10%, indicating that adipoR1 may be more sensitive to dietary fat. Expression of *adipoR1* has previously been reported to increase during conditions of increased fat oxidation and insulin sensitivity, including weight loss (39) and aerobic exercise training (40), and we speculate that the downregulation of adipoR1 may be a mechanism by which high-fat diet induces insulin resistance. A recent study examining the effects of knockout of adipoR supports this because knockout of adipoR1 increased adiposity and insulin resistance, whereas knockout of adipoR2 increased insulin sensitivity and prevented high-fat diet–induced obesity (41). In rodents, both *AdipoR1* and *adipoR2* expression are reduced in response to refeeding (42). Prolonged high-fat feeding also reduced *adipoR1/R2* expression in mice, although this may be species specific because high-fat diets did not alter *AdipoR1/R2* expression in rats (43). Interestingly, we observed that refeeding carbohydrate did not change *adipoR1* or *adipoR2* expression in humans. Other studies in humans have shown that the *adipoR1/R2* expression is not changed after insulin infusion (44) or after insulin addition to primary muscle culture (45). Together, these findings strongly suggest that adipoR expression is not regulated by glucose or insulin in humans.

NR4A1 and PPARδ have putative roles in lipid metabolism and therefore were also expected to increase in response to the high-fat meal. Interestingly, the relatives of individuals with type 2 diabetes tended to increase PPARδ more than the control subjects, although this was not statistically significant, and variability in this response was large. Furthermore, this was not translated downstream to CPT1b or FAT/CD36, and from this we speculate whether PPARδ-mediated activation of lipid metabolism genes is impaired in relatives of individuals with type 2 diabetes. Contrary to our hypothesis, both PPARδ and NR4A1 were also increased in response to the high-carbohydrate meal. *NR4A1* is actually better known as an early response gene that is induced by stress, inflammatory stimuli, and cytokines (46,47). Because of the significant increase in response to both types of meals, we speculate that refeeding may activate NR4A1 via stress and/or inflammatory responses, but further investigation of NR4A1 in response to nutrition and lipid metabolism is clearly warranted.

In conclusion, insulin-sensitive relatives of individuals with type 2 diabetes displayed an impaired ability to increase whole-body fatty acid oxidation in response to a single high-fat meal. This was associated with an impaired ability to upregulate lipid metabolism genes, including *PGC1a* and *FAT/CD36*. This study suggests that an impaired ability to increase fat oxidation in response to high-fat meals precedes the development of insulin resistance in genetically susceptible individuals.

ACKNOWLEDGMENTS

This study was funded in part by a grant from the Rebecca L. Cooper Foundation. L.K.H. is the recipient of a National Health and Medical Research Council biomedical fellowship. S.G. was funded by the Danish Medical Council.

Our thanks go to the staff of the Garvan Clinical Research Facility and, in particular, the study coordinator, Marisa Greco. We also thank the volunteers who participated in the study.

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