Palmitate oxidation rate and action on glycogen synthase in myoblasts from insulin-resistant subjects

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Received 8 October 1999; accepted in final form 6 April 2000.

Elevated plasma lipid and nonesterified fatty acid concentrations reduce insulin-mediated glucose disposal in skeletal muscle. Cultured myoblasts from 21 subjects were studied for rates of palmitate oxidation and the effect of palmitate on glycogen synthase activity at the end of an 18-h incubation in serum- and glucose-free media. Oxidation rates of 40 μM palmitate in cultured myoblasts correlated with the fasting glucose \((r = 0.71, P = 0.001)\), log fasting insulin \((r = 0.52, P = 0.03)\), and insulin-mediated glucose storage rate \((r = -0.50, P = 0.04)\) of the muscle donors. Myoblast glycogen synthase activity can be regulated by 240 μM palmitate, but the changes are associated with the basal respiratory quotient and not with the insulin resistance of the muscle donor. These results indicate that myoblasts producing elevated palmitate oxidation rates in vitro can be used to identify skeletal muscle abnormalities which are primary contributors to insulin resistance in vivo. Effects of 240 μM palmitate on myoblast glycogen synthase activity appear to be mechanistically different from the relationship between myoblast palmitate oxidation rates and insulin resistance of the muscle donor.

Reduced insulin-mediated glucose disposal has been identified as a risk factor for the development of type 2 diabetes mellitus (2). Abnormal glucose storage in skeletal muscle is the principal site for reduced insulin action on glucose disposal (17). Muscle biopsies obtained at the end of a euglycemic hyperinsulinemic clamp on insulin-resistant subjects have reduced insulin-stimulated glycogen synthase activity and reduced uptake of \(^{3}H\)glucose in skeletal muscle glycolysis (33).

A primary mechanism for reduced insulin-stimulated glycogen synthase activity has been difficult to identify in biopsied muscle, which reflects the hormonal and nutritional state of the biopsy donor. Increased concentrations of plasma lipids and nonesterified fatty acids reduce insulin-stimulated glucose storage rates (15, 13) and glycogen synthase activity (1).

Studies of the glucose-fatty acid cycle in skeletal muscle have identified mechanisms that coordinate cellular substrate oxidation and can provide feedback inhibition by cellular lipids on glucose metabolism (24). Products of fatty acid oxidation inhibit phosphofructokinase and pyruvate dehydrogenase, leading to reduced carbohydrate oxidation rates. The inhibition of glycolysis at steps beyond fructose 6-phosphate has been proposed as a mechanism for increased production of hexosamines (8) that result from a transamination from glutamine to fructose 6-phosphate, producing glucosamine 6-phosphate (20). This metabolite can directly inhibit hexokinase (31), and further metabolism produces uridine-5′-diphosphate (UDP)-N-acetyl glucosamine that has been associated with reduced glucose uptake (8). This hypothesis implies initiation of abnormal glucose metabolism in skeletal muscle, which has no cellular abnormality before exposure to elevated extracellular lipid.

This hypothesis does not appear to agree with reports on cultured muscle cells with abnormalities of insulin-stimulated glucose metabolism similar to those measured on the insulin-resistant and diabetic muscle donors in vivo. These cultured muscle cells have changes in glucose transport rates (9), glycogen synthase activity (10, 21), and glycogen synthesis rates (30) that appear to reflect the in vivo glucose metabolism of the insulin-resistant muscle donor. The muscle cells from subjects in each study are grown in an identical culture environment, suggesting that significant differences between cultured cells from control and insulin-resistant subjects reflect intrinsic or genetic alterations in metabolic regulation. If abnormal glucose metabolism in cultured muscle cells from insulin-resistant subjects involves effects of free fatty acids, then these cells may have intrinsic differences in the response of glucose metabolism to fatty acids, including possible differences in fatty acid oxidation rates.

In this study, we treated cultured human myoblasts with palmitic acid to measure possible effects of fatty acids.
acids and fatty acid oxidation on glycogen synthase activity. Results from palmitate oxidation rates and effects on cultured muscle glycogen synthase activity are compared with clinical parameters of obesity and insulin resistance in muscle donors.

**METHODS**

Twenty-one Pima Indians were admitted to the metabolic ward of the Clinical Diabetes and Nutrition Section of the National Institutes of Health in Phoenix, Arizona. They gave informed consent for the studies, which were approved by the Ethics Committee of the National Institutes of Health and the Tribal Council of the Gila River Indian Community. Fitness for the study was determined by medical history, physical examination, electrocardiography, and routine biochemical, hematological, and urine testing. None of the subjects was taking any medication, and no subject had any clinically significant abnormalities on these examinations. The subjects' characteristics are shown in Table 1. Cultured muscle from group 1 subjects was studied for palmitate oxidation rate, and cultured muscle from group 2 subjects was studied for palmitate effects on glycogen synthase activity. Cultured muscle cells from groups 1 and 2 that were studied both for palmitate oxidation and palmitate effects on glycogen synthase activity are identified as group 3. Clinical studies were conducted after 5–7 days on a weight-maintaining diet (20% protein, 50% carbohydrate and 30% fat). Body composition was determined by dual-energy X-ray absorptiometry (29), and fat-free mass and percent body fat were calculated. Diabetics were excluded after an oral glucose tolerance test. After an overnight fast, in vivo glucose disposal and substrate oxidation rates were measured by use of ventilated hood indirect calorimetry before (basal) and during a hyperinsulinemic euglycemic clamp (18). Two insulin infusion rates of 40 and 400 μU·min⁻¹·kg⁻¹ were used to reach steady-state plasma insulin concentrations of 1,004 ± 60 (low dose) and 17,200 ± 1,400 (high dose) pM. Nonoxidative glucose disposal is referred to as glucose storage. Substrate disposal and oxidation rates are normalized to kilograms of metabolic body size (KGMBS) (17). Postabsorptive respiratory quotient is referred to as basal RQ.

After an overnight fast, percutaneous muscle biopsies were obtained from the quadriceps femoris muscle after local anesthesia of skin and fascia with lidocaine (14). Primary myoblast cultures were established from human muscle using a previously reported method (30) from this laboratory. Up to 300 mg of skeletal muscle were collected in cold DMEM and finely minced. The cells were dissociated by incubation with 0.25% (wt/vol) trypsin, 0.05% EDTA, 0.1% (wt/vol) type IV collagenase, and 0.1% (wt/vol) BSA at 37°C for 30 min with agitation. The cells were collected by centrifugation at 350 g and were plated in uncoated 60-mm dishes for 1 h at 37°C to remove fibroblasts. The residual cellular material was transferred to 25-cm² rat tail collagen-coated flasks (Bioscat, Becton-Dickinson, Bedford, MA). All subsequent culture ware was collagen coated. Myoblasts were allowed to grow until 100% confluent in DMEM supplemented with 25 mM HEPES, 17% fetal calf serum, 2 mM glutamine, 0.5% chick embryo extract, 100 U/ml penicillin, 100 μg/ml streptomycin, and 0.2% Fungizone ( Gibco BRL, Gaithersburg, MD). The immunoreactivity of skeletal muscle proteins myosin and actinin and the creatinine kinase activity in these muscle cultures were previously reported from this laboratory (30). Primary cultures were trypsinized and subcultured in the above media with 10% fetal calf serum. Cells between 3- and 5-cell population doublings (primary cell monolayers are designated as 1-cell population doubling) were plated at 0.5 × 10⁶ cells per 60-mm dish and were allowed to reach 80–90% confluence. These cells were used to measure palmitate oxidation and the effects of palmitate on glycogen synthase activity after preincubation conditions modified from previously described methods (21, 12). Monolayers were washed twice with Dulbecco's PBS (DPBS) and were cultured at 37°C in DMEM (no glucose) supplemented with 25 mM HEPES, 100 U/ml penicillin, 10 μg/ml streptomycin, 0.2% Fungizone, and 0.5% BSA (fatty acid free, Sigma A7511, St. Louis, MO), plus or minus the indicated concentrations of sodium palmitate (Sigma P9767) containing 0.4 μCi/ml [9,10-3H]palmitic acid (Du Pont NEN, Boston, MA). Total counts for palmitate were determined in 50-μl aliquots obtained at the beginning and end of the glucose-free incubation period. In ten 18-h experiments, a mean of 9.8 ± 1.7 (% ± SE) of 40 μM palmitate was consumed. At the end of the incubation period, the radiolabeled media were saved at 4°C for analysis of palmitate oxidation. The cells at this point remained preconfluent and contained <2.0% multinucleated myocytes. Cell monolayers for assay of glycogen synthase activity were washed at 4°C once each with 0.2 mM phloridzin in and with DPBS. Cell monolayers were covered with 200 μl/dish 30% glycerol, 10 mM EDTA, and 50 mM KF, pH 7.0, and were frozen by floating the 60-mm dish on liquid N₂. Frozen monolayers were stored at −70°C.

The release of tritiated water from cell monolayers was measured in 100-μl aliquots of media and was used for the determination of palmitate oxidation (16). Each aliquot at 4°C was brought to 1% BSA and 5% trichloroacetic acid and centrifuged at 10,000 g. After the precipitation procedure on the supernatant was repeated, duplicate 150-μl aliquots were placed in scintillation counting vials, and the tritiated water was removed under vacuum (Savant Speed Vac, Farmingdale, NY) from one vial before measurement of the radioactivity in both vials. The palmitate oxidized in each

<table>
<thead>
<tr>
<th>Table 1. Subject characteristics</th>
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<tr>
<td><strong>Group 1</strong></td>
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<tr>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Male/female</td>
</tr>
<tr>
<td>Age, yr</td>
</tr>
<tr>
<td>Fasting insulin, pM</td>
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<tr>
<td>Fasting glucose, mM</td>
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<tr>
<td>2-h Glucose, mM</td>
</tr>
<tr>
<td>Body fat, %</td>
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<td>Body mass index, kg/m²</td>
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Group 3, subjects common to groups 1 and 2.
sample was calculated as the product of the fraction of total counts evaporated and the moles of palmitate added to each 60-mm plate. Cells per plate and cell viability (>98%) by trypan blue uptake were determined at the end of the glucose-free incubation period with the use of 60-mm dishes set up under parallel conditions with the plates for analysis of palmitate oxidation and glycogen synthase activity.

Frozen cell monolayers for analysis of glycogen synthase activity were collected by scraping the dish at 4°C, homogenizing (Omni International, Waterbury, CT) and then diluting the 4°C 10,000-g supernatant 2.3-fold with 50 mM Tris, 130 mM KF, and 20 mM EDTA, pH 7.8, for enzyme assay, as previously described (14). The active forms of glycogen synthase were assayed at 0.17 mM glucose 6-phosphate (low G-6-P), and maximum glycogen synthase activity (MGS) was assayed at 7.2 mM G-6-P (high G-6-P). Fractional activity is expressed as the activity ratio of active form to maximum glycogen synthase measured at 0.13 mM UDP-glucose. The inter- and intra-assay coefficients of variation for this glycogen synthase assay have been previously described (21).

RESULTS

Palmitic acid oxidation in cultured myoblasts increased between 6- and 18-h incubation in glucose- and serum-free media (Fig. 1A). The dose response of palmitate oxidation to palmitic acid between 8 and 240 μM is shown in Fig. 2. Palmitate oxidation rates were measured at 40 and 240 μM palmitate in cultured muscle cells from 18 subjects over a range of obesity. Palmitate oxidation rates at 40 μM palmitate (Fig. 3) were increased in cultured muscle from subjects with elevated fasting plasma insulin concentrations \( r = 0.52, P = 0.03 \), fasting plasma glucose concentrations \( r = 0.71, P = 0.001 \), and body mass indexes \( r = 0.58, P = 0.01 \). Palmitate oxidation rate at 40 μM palmitate was not significantly related to percent body fat \( r = 0.43, P = 0.08 \); data not shown). Low-dose insulin-mediated glucose storage rates measured in vivo were inversely correlated with palmitate oxidation rates \( r = -0.50, P = 0.04 \); Fig. 3D). Palmitate oxidation rates using 240 μM palmitate varied from 3.0 to 5.6 fmol ⋅ h\(^{-1}\) ⋅ cell\(^{-1}\) and are not significantly correlated with the clinical parameters shown in Fig. 3 (data not shown). Insulin-mediated glucose disposal rates were not significantly correlated with cultured muscle cell palmitate oxidation rates. Basal and insulin-mediated carbohydrate and lipid oxidation rates in vivo were not correlated with cultured muscle palmitate oxidation rates.

Palmitate oxidation rates were measured in the absence of glucose. Incubation of cultured muscle cells in the absence of glucose led to a time-dependent increase in glycogen synthase fractional activity (GSFA) that was generally reduced by 40 μM palmitic acid at each time point (Fig. 1B). MGS remained constant between 6 and 18 h of glucose-free incubation (Fig. 1C). In the presence of 40 μM palmitate, MGS was significantly increased at 12 h. After an 18-h incubation in glucose-free media, both 40 and 240 μM palmitate inhibited the mean GSFA in myoblasts from 13 subjects, and MGS was significantly increased with 240 μM palmitate (Fig. 4).

In the absence of exogenous palmitate, etomoxir had no significant effect on glycogen synthase fractional or maximum activity (Table 2). In the presence of palmi-
etomoxir inhibited palmitate oxidation by ~30% and blocked the effects of palmitate to decrease GSFA and increase MGS.

Glycogen synthase activity at low G-6-P (GSA) was determined on myoblasts incubated for 18 h in the presence or absence of palmitate. The ratio of GSA in the presence and absence of palmitate is a measure of the effect of the fatty acid to stimulate or inhibit glycogen synthase assayed at low, physiological G-6-P. Using 240 μM palmitate, changes in GSA range from 40% inhibition to 20% activation (Fig. 5A). The GSA ratio with 240 μM palmitate was negatively correlated with basal RQ of the muscle donor (r = −0.53, P = 0.03). The 240 μM palmitate stimulation of MGS (r = −0.60, P = 0.01), but not the inhibition of GSFA, was correlated with basal RQ (Fig. 5, B and C). The GSA with 40 μM palmitate was not correlated with basal RQ (data not shown).

The results in Table 3 compare myoblasts for GSA activation (GSA ratio > 1.0) with GSA inhibition (GSA ratio < 1.0) using 40 and 240 μM palmitate. Both concentrations of palmitate produced a larger increase in palmitate oxidation and increased GSA activity.

**Table 2. Effect of 1.7 μM etomoxir on 240 μM palmitate oxidation and glycogen synthase activity**

<table>
<thead>
<tr>
<th>Palmitate</th>
<th>Etomoxir</th>
<th>Fractional</th>
<th>Maximum</th>
<th>Palmitate Oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td>−</td>
<td>−</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>103 ± 6</td>
<td>117 ± 20</td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>76 ± 5*</td>
<td>124 ± 10*</td>
<td>100</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>96 ± 16</td>
<td>116 ± 13</td>
<td>69 ± 9*</td>
</tr>
</tbody>
</table>

Data are shown as the mean percentage of control ± SE for 3 subjects. Control values are: glycogen synthase fractional activity, 0.65 ± 0.09; maximum glycogen synthase, 0.59 ± 0.12 fmol · min⁻¹ · cell⁻¹; palmitate oxidation, 11 ± 3 fmol · h⁻¹ · cell⁻¹. *P < 0.05 in paired t-test vs. control.
Reduced insulin-mediated glucose disposal and reduced glycogen synthase activity in skeletal muscle

Table 3. Comparison of GSA between myoblasts with palmitate activation or inhibition of GSA

<table>
<thead>
<tr>
<th>Palmitate, µM</th>
<th>40</th>
<th>240</th>
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<tr>
<td></td>
<td>GSA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Activation</td>
<td>Inhibition</td>
</tr>
<tr>
<td>Subjects, n</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSFA</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Ratio ±</td>
<td>0.71 ± 0.06</td>
<td>0.79 ± 0.03</td>
</tr>
<tr>
<td>NS (not significant)</td>
<td></td>
<td></td>
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<tr>
<td>MGS</td>
<td>0.95 ± 0.04</td>
<td>0.92 ± 0.04</td>
</tr>
<tr>
<td>Ratio ±</td>
<td>0.47 ± 0.07</td>
<td>0.48 ± 0.04</td>
</tr>
<tr>
<td>Ratio ±</td>
<td>1.24 ± 0.07</td>
<td>1.92 ± 0.03</td>
</tr>
<tr>
<td>GSA</td>
<td>0.33 ± 0.04</td>
<td>0.37 ± 0.03</td>
</tr>
<tr>
<td>Ratio ±</td>
<td>1.18 ± 0.06</td>
<td>0.85 ± 0.04</td>
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Values are means ± SE. GSFA, glycogen synthase fractional activity; GSA, glycogen synthase activity; MGS, maximum glycogen synthase activity. Group 2 subjects were divided into those with palmitate activation of GSA (ratio ± >1.00) and inhibition of GSA (ratio ± <1.00). Ratio ± palmitate is used to show magnitude of palmitate effect. MGS and GSA are expressed in fmol · min⁻¹ · cell⁻¹. *Non-paired t-test between GSA activation and GSA inhibition subgroups. NS, nonsignificant.
have been demonstrated to be secondary to elevation of plasma lipids (1, 13, 15). Triglyceride content is elevated in skeletal muscle from insulin-resistant subjects (23). These observations indicate that altered lipid metabolism, presumably in skeletal muscle secondary to elevated plasma lipids, could contribute to the abnormal glucose uptake of insulin-resistant subjects. The concept of abnormal glucose metabolism in skeletal muscle occurring only as a secondary effect of elevated plasma lipids seems to conflict with results showing abnormal glucose transport and glycogen synthase activity in muscle cells from insulin-resistant subjects compared with muscle cells from normal subjects cultured in identical media (9, 10, 21). The purpose of this study was to test myoblasts from insulin-resistant subjects for the existence of intrinsic abnormalities in free fatty acid metabolism, which, if also unique to fully differentiated skeletal muscle of insulin-resistant subjects, could contribute to abnormal glucose metabolism in vivo. Myoblasts from subjects over a range of insulin resistance and obesity were tested for palmitate oxidation rates and for effects of palmitate on glycogen synthase activity.

The importance of the observations obtained in this study is based in part on the fact that differences in metabolic characteristics between cultured cells from different subjects are not the result of in vivo hormone and nutrient variations experienced by muscle cells in vivo. The cultured muscle cells from each subject were grown in an identical culture environment and were the product of a large number of cell divisions over at least a 1-mo period of time, with media changed on a weekly basis. Despite this normalized environment, these cultured myoblasts had characteristics of palmitate oxidation rates and palmitate-induced changes in glycogen synthase activity that reflect the muscle donors’ clinical characteristics for insulin resistance and regulation of substrate oxidation (basal RQ), respectively. A variety of mechanisms could explain this link between metabolic regulation in the whole body and in cultured muscle cells. Cultured muscle cells may retain cytoplasmic metabolic regulators from the in vivo muscle environment of the muscle donor. This explanation is unlikely, considering the large number of cell population doublings occurring over 1 mo or more of culture and considering that each doubling diluted the original cell cytoplasm by one-half. It is also possible that each muscle donor’s muscle cell metabolic characteristics are retained in the replicating nucleic acids that control early steps in myoblast differentiation. These metabolic characteristics could be the result of the prebiopsy environment of the myoblast or the genotype of each individual’s muscle tissue. All of these explanations share the concept that the cause (for example) of elevated palmitate oxidation in cell culture and insulin resistance in vivo has a common mechanism in the subjects studied here.

Recent studies have shown a direct relationship between plasma free fatty acid concentrations and total body lipid oxidation rates. This relationship has been observed between subjects in the basal state (18) and for subjects after an increase or decrease in plasma free fatty acid concentrations (15). These studies suggest that regulation of liver and muscle cell lipid oxidation has an extracellular origin, presumably relating to the subject’s plasma lipid concentrations. The elevated oxidation rate of palmitate in cultured muscle from insulin-resistant/obese subjects suggests that skeletal muscle from these subjects has an inherent increase in palmitate oxidation rate that is independent of in vivo plasma free fatty acid concentrations. The fact that the relationship between myoblast palmitate oxidation rates and in vivo measures of insulin resistance were observed only with 40 μM palmitate suggests that 240 μM palmitate saturates a specific site that has an increased affinity for palmitate regulation of long-
chain fatty acid (LCFA) oxidation rates in insulin-resistant compared with -sensitive subjects. A correlation between myoblast palmitate oxidation rates and total body lipid oxidation rates is probably unlikely, considering that palmitate represents only one type of fatty acid, and muscle tissue accounts for only 20–30% of total resting oxygen uptake in a nonobese subject (34).

Increased palmitate oxidation rates in cultured muscle from insulin-resistant subjects may be the result of reduced carbohydrate oxidation rates in the cultured muscle cells. Reduced carbohydrate oxidation could occur secondarily to the reduced glucose transport or phosphorylation that has been reported in cultured muscle (9) and has been suggested by nuclear magnetic resonance measures of G-6-P in skeletal muscle in vivo (26) for non-insulin-dependent diabetic subjects. Considering the 18-h glucose starvation used for the data presented here, reduced glucose transport is not a likely cause of a putative decreased carbohydrate oxidation in myoblasts from insulin-resistant/obese subjects. Therefore, elevated lipid oxidation in the insulin-resistant/obese subjects is likely to develop according to a different mechanism.

Lipid infusion activates the hexosamine biosynthetic pathway in human skeletal muscle (8), and glucosamine stimulates leptin production in skeletal muscle from Zucker rats (32). Leptin activates fatty acid oxidation in cultured islets and appears to reduce the triglyceride content of skeletal muscle by the same mechanism (27). These observations suggest that elevated palmitate oxidation rates in cultured muscle from insulin-resistant/obese subjects could involve abnormalities in the ability of palmitate to control fatty acid metabolism through hexosamine and leptin action.

The effect of palmitate and palmitate oxidation on myoblast glycogen synthase activity was studied during an 18-h glucose starvation, because this condition enhances glycogen synthase fractional activity (11) and palmitate oxidation rates (preliminary studies, data not shown). The time-dependent increase in glycogen synthase fractional activity is probably caused by glycogen depletion (5), which can reduce inhibition of type 1 protein phosphatase (22). The results in Fig. 4 indicate that palmitate can alter glycogen synthase activity by increasing the maximum glycogen synthase activity and by reducing the fractional activity. These two changes in synthase activity are generally associated with increases in the enzyme concentration and in the enzyme phosphorylation state, respectively. Both of these actions by palmitate are blocked by a 31% decrease in palmitate oxidation rate (Table 2).

Studies on the glucose-fatty acid cycle support a mechanism for inhibition of glucose metabolism secondary to the release of products of fatty acid oxidation that can inhibit pyruvate dehydrogenase and phosphofructokinase (24). The resulting increase in hexosamines can lead to inhibition of glycogen synthase activity (4). Similarly to several clinical studies of the effects of lipids on skeletal muscle glycogen synthase activity (1, 13), we can demonstrate an inhibition of glycogen synthase fractional activity in the presence of free fatty acid. The reversal of palmitate action with the carnitine palmitoyl transferase I inhibitor etomoxir (6) supports the importance of fatty acid transport into mitochondria and, potentially, fatty acid oxidation in the action of palmitate on glycogen synthase activity. As an energy source, palmitate oxidation could limit glycogen depletion by preserving cellular glucose in glucose-free media. In the perfused rat hindquarter, oleate protected against depletion of glycogen in fast- and slow-twitch red muscle (25). In a study of corticosterone-treated rats, several muscle groups had a significant decrease in muscle glycogen and an increase in glucose utilization when fatty acid oxidation was inhibited during etomoxir treatment (7). These results suggest that lipid oxidation could spare glycogen, which, as an inhibitor of glycogen synthase activity, could inhibit glucose storage. This mechanism would require the maintenance of elevated glycogen by fatty acids in muscle cells of obese/insulin-resistant subjects. Elevated glycogens have not been reported as a characteristic of obese/insulin-resistant subjects, suggesting that this mechanism may contribute to abnormal glucose uptake by small elevations in muscle glycogen that would be difficult to detect. The concept of one mechanism controlling glycogen synthase fractional and maximum activity is supported by the ability of etomoxir to prevent palmitate effects on both measures of synthase activity.

By use of 240 μM palmitate, the changes in glycogen synthase activity in low G-6-P (GSA is the product of fractional activity and maximum glycogen synthase activity) correlate with the muscle donor’s basal RQ. These results indicate that palmitate can decrease GSA in myoblasts from subjects with a low basal RQ and increase GSA in subjects with a low basal RQ (Fig. 5). These observations suggest that the same regulatory mechanism that balances total body substrate oxidation in vivo is involved with the mechanism for 240 μM palmitate action on GSA in cultured muscle. Cultured myoblasts should provide a useful system to further define regulation of basal substrate oxidation in vivo based on mechanisms for palmitate regulation of GSA in cultured muscle cells.

Dividing the group 2 subjects into palmitate activators of GSA (GSA ratio >1.00) and inhibitors of GSA (GSA ratio <1.00) indicates that GSA inhibition is the result of a greater palmitate-induced decrease in glycogen synthase fractional activity and a smaller palmitate-induced increase in maximum glycogen synthase activity (Table 3, 240 μM palmitate). These results demonstrate that, with the use of 240 μM palmitate, the greater decrease in glycogen synthase fractional activity is not caused by a greater increase in maximum glycogen synthase activity.

Reduction of glycogen synthase activity has been demonstrated for insulin-resistant subjects (3) and is secondary to the infusion of lipid into human subjects (1). The action of palmitate to reduce GSA in myoblasts from some muscle donors could be expected to relate to
the insulin resistance of these subjects. The results in Table 4 show that subjects with inhibition of myoblast GSA at 40 or 240 μM palmitate have insulin-mediated glucose disposal and storage rates that are not reduced compared with subjects where palmitate stimulates GSA. This observation may be the result of the study system where, for example, a reduced concentration of intracellular glucose (cells were in glucose-free media for 18 h) or the absence of insulin could be critical for the association of palmitate suppression of GSA and in vivo measures of insulin resistance. Alternatively, we are not aware of any reports indicating increased LCFA inhibition of glycogen synthase activity in skeletal muscle of insulin-resistant subjects. The absence of a relationship between palmitate suppression of GSA and in vivo insulin resistance may reflect deleterious effects of obesity and free fatty acids on glucose storage (13, 15) that are independent of the primary abnormalities that initiate insulin resistance. These data do not support increased sensitivity or response of glycogen synthase activity to LCFA as a primary characteristic of muscle tissue in insulin-resistant subjects. The correlation of this response with basal RQ, however, could indicate that this clinical predictor of weight gain (28) shares regulatory pathways that are unique to muscle where LCFA are more effective in reducing GSA. These hypotheses do not preclude the existence of primary abnormalities that lead to insulin resistance and also reduce glycogen synthase activity in muscle tissue from insulin-resistant subjects. Previous studies have demonstrated reduced glycogen synthase activity in cultured muscle from insulin-resistant subjects (10, 21).

Cultured muscle cells from insulin-resistant subjects have elevated palmitate oxidation rates after at least 1 mo in culture and extensive numbers of cell population doublings from the original primary myoblasts. These observations suggest that the unique inherent metabolic control that produces elevated palmitate oxidation rates in myoblasts can contribute to insulin resistance in skeletal muscle in vivo. The elevated LCFA oxidation rates could inhibit glucose metabolism directly or may reflect the regulatory status of a common “upstream” site that contributes to insulin resistance by a separate mechanism. The unique metabolic regulation that increases palmitate inhibition of myoblast glycogen synthase activity may be a mechanism for LCFA inhibition of glucose metabolism in obesity that is independent of primary factors for the development of insulin resistance. Cultured myoblasts from human subjects can be used to characterize these metabolic factors that relate to insulin resistance and elevated basal RQ. The abnormalities in cellular metabolism can be studied in the absence of extracellular nutrient and hormone variations that exist between subjects.

We thank the clinical staff for their professional assistance. Most of all, we are grateful to the volunteers for their cooperation during this study.

REFERENCES


