Palmitate action to inhibit glycogen synthase and stimulate protein phosphatase 2A increases with risk factors for type 2 diabetes

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Mott DM, Stone K, Gessel MC, Bunt JC, Bogardus C. Palmitate action to inhibit glycogen synthase and stimulate protein phosphatase 2A increases with risk factors for type 2 diabetes. Am J Physiol Endocrinol Metab 294:E444–E450, 2008. First published December 4, 2007; doi:10.1152/ajpendo.00386.2007.—Recent studies have suggested that abnormal regulation of protein phosphatase 2A (PP2A) is associated with Type 2 diabetes in rodent and human tissues. Results with cultured mouse myotubes support a mechanism for palmitate activation of PP2A, leading to activation of glycogen synthase kinase 3. Phosphorylation and inactivation of glycogen synthase by glycogen synthase kinase 3 could be the mechanism for long-chain fatty acid inhibition of insulin-mediated carbohydrate storage in insulin-resistant subjects. Here, we test the effects of palmitic acid on cultured muscle glycogen synthase and PP2A activities. Palmitate inhibition of glycogen synthase fractional activity is increased in subjects with high body mass index compared with subjects with lower body mass index (r = −0.43, P = 0.03). Palmitate action on PP2A varies from inhibition in subjects with decreased 2-h plasma glucose concentration to activation in subjects with increased 2-h plasma glucose concentration (r = 0.45, P < 0.03) during oral glucose tolerance tests. The results do not show an association between palmitate effects on PP2A and glycogen synthase fractional activity. We conclude that subjects at risk for Type 2 diabetes have intrinsic differences in palmitate regulation of at least two enzymes (PP2A and glycogen synthase), contributing to abnormal insulin regulation of glucose metabolism.

Both obesity and insulin resistance have been reported as risk factors for development of Type 2 diabetes (7, 25). Resistance to insulin-mediated glucose disposal primarily involves reduced glucose storage in skeletal muscle (28). Glycogen synthase is a rate-determining enzyme for glucose storage (45). Skeletal muscle biopsies obtained during a hyperinsulinemic-euglycemic clamp show reduced glycogen synthase activity in insulin-resistant subjects with reduced glucose storage rates (8). Reduced glucose storage rates and glycogen synthase activity have also been observed during insulin infusion after 2 wk of overnutrition with an average weight gain of 3 kg for nondiabetic subjects with a mean body mass index (BMI) of 25 kg/m² (32). These results indicate that increased insulin resistance and a significant reduction in glycogen synthase activity do not require long-term exposure to increased body mass and could apparently occur secondary to the short-term effects of increased nutrients. Elevated concentrations of plasma lipid and nonesterified fatty acids have been observed to reduce insulin-mediated glucose storage rates (20, 24). Lipid infusion for 2–4 h during a euglycemic-hyperinsulinemic clamp produces insulin resistance, which correlates with reduced skeletal muscle glycogen synthase activity (5). These observations may indicate that lipids or nonesterified fatty acids are functional components of the overnutrition-induced insulin resistance noted above (32).

Cultured human myotubes from individuals with Type 2 diabetes have reduced basal and insulin-stimulated glycogen synthase activity (14). Our group (33) has reported reduced glycogen synthase activity in the presence or absence of insulin using cultured myoblasts from subjects with reduced in vivo insulin-stimulated carbohydrate storage rates. These observations in insulin-resistant subjects indicate that muscle cells isolated in culture contain the same mechanisms for reduced insulin action that produce abnormal glycogen synthase activity in noncultured skeletal muscle biopsies. The positive correlation between in vivo insulin-stimulated carbohydrate storage rates and glycogen synthase activity in myoblasts incubated in the absence of mitogens, including insulin for 42 h (33), suggest that abnormally low glycogen synthase fractional activity (GSFA) could be produced without modulation of the insulin signal transduction pathway. On the basis of the response of skeletal muscle glycogen synthase to lipid infusion in vivo (5), our group (31) has tested the effects of an 18-h incubation of cultured myoblasts with palmitic acid. This study demonstrated a dose-dependent palmitate inhibition of glycogen synthase activity in cultured muscle cells tested in the absence of insulin.

The activity and phosphorylation state of glycogen synthase are regulated in part by glycogen synthase kinase 3 (GSK3) (39). Studies in mouse C2C12 muscle cells suggest that palmitate conversion to ceramide activates protein phosphatase 2A (PP2A), leading to the activation of GSK3 and an inhibition of glycogen synthesis (10, 38). In this study, we compare the effects of palmitate on the activity of glycogen synthase and PP2A in cultured muscle cells from subjects over a range of BMI levels and glucose tolerance.

METHODS

Subjects. Thirty-three Pima Indians and four Caucasians were admitted to the metabolic ward of the Obesity and Diabetes Clinical Research Section of the National Institutes of Health in Phoenix, Arizona. These individuals gave written informed consent for the studies, which were approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases and

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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. Group 1 consists of all 26 of the 37 subjects with data on palmitate effects on myoblast glycogen synthase activity. Group 2 consists of all 25 of the 37 subjects with data on palmitate effects on myoblast PP2A activity. Group 3 consists of 14 of the 37 subjects with data on both enzymes; this group was used to test the relative effects of palmitate on glycogen synthase activity vs. PP2A activity. Percent body fat was calculated from body composition determined by dual-energy X-ray absorptiometry (42). Individuals with diabetes were excluded according to criteria of the World Health Organization after a 75-g oral glucose tolerance test conducted after 3 days on a weight-maintaining diet (20% protein, 50% carbohydrate, and 30% fat).

Subject characteristics
Table 1.

Table 1. Subject characteristics

<table>
<thead>
<tr>
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<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Men/women</td>
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<td></td>
<td>17/8</td>
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<td>Age, yr</td>
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<td>Fasting insulin, pM</td>
<td>220±86</td>
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<td>239±78</td>
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<tr>
<td>Fasting glucose, mM</td>
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<td>4.9±0.6</td>
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<tr>
<td>2 h glucose, mM</td>
<td>7.1±2.0</td>
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<tr>
<td>Body fat, %</td>
<td>31±9</td>
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<td>31±9</td>
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<tr>
<td>Body mass index, kg/m²</td>
<td>32±7</td>
<td>20–46</td>
<td>33±7</td>
</tr>
</tbody>
</table>

Group 3 consists of subjects common to groups 1 and 2.
RESULTS

Glycogen synthase. GSFA in the absence of palmitate (control) is not significantly related to the BMI of the muscle donor (Fig. 1A). In the presence of 240 μM palmitate, GSFA is inversely correlated with BMI ($r = -0.43, P = 0.03$; Fig. 1B). The fraction of control GSFA remaining after cells are treated for 18 h with 240 μM palmitate decreases with increasing BMI ($r = -0.50, P = 0.01$; Fig. 1C). After palmitate treatment, glycogen synthase activity per unit protein measured in low G-6-P (GSA) and high G-6-P (MGS) are negatively, but not significantly, related to BMI (data not shown).

GSFA can be calculated as the product of GSFA and MGS. To investigate the contribution of these activity parameters to the negative relationship between BMI and glycogen synthase activity, group 1 was subdivided into subgroups below and above the median BMI of 33 kg/m², removing the youngest subject and the oldest subject from the low and high BMI subgroups, respectively, to match the two subgroups for age. The clinical characteristics for these two subgroups are shown in Table 2. Myoblasts from these BMI subgroups (Fig. 2) have the same GSFA levels in the absence of exogenous palmitate. Palmitate produces a larger decrease in the GSFA of the high BMI subgroup ($P < 0.03$). In the presence and absence of exogenous palmitate, the high BMI subgroup tended to have lower MGS activities that were not statistically different from results for the low BMI subgroup. Within each subgroup, palmitate produced a significant decrease ($P < 0.006$ and $P < 0.02$) in MGS. This decrease in MGS, however, was not significantly different between the two subgroups. The product of MGS and GSFA in the presence of palmitate resulted in a GSA for the high BMI subgroup that was 73% of the GSA in the low BMI subgroup ($P = 0.05$). None of the measures of glycogen synthase activity in the presence or absence of palmitate correlated with 2-h plasma glucose concentrations (2HPG; data not shown).

![Fig. 1](http://ajpendo.physiology.org/)

**Fig. 1.** Relationship in 26 group 1 subjects between glycogen synthase fractional activity (GSFA) in control myoblasts (A) and 240 μM palmitate-treated myoblasts (B) and fraction of control GSFA remaining after myoblasts are treated with 240 μM palmitate vs. the body mass index (BMI) of the muscle donor (C). Diamonds signify men, and triangles signify women, with open symbols representing Caucasian subjects.

![Fig. 2](http://ajpendo.physiology.org/)

**Fig. 2.** Effect of 240 μM palmitate on group 1 subgroups with BMI less than (open bars, $n = 12$) or greater than (solid bars, $n = 11$) 33 kg/m². Shown are GSFA results (top), maximum glycogen synthase activity results measured at high glucose-6-phosphate (MGS; middle), and glycogen synthase activity results measured at physiological glucose-6-phosphate (GSA; bottom). Activity is expressed as nmol·min⁻¹·mg protein⁻¹. #P < 0.02 for palmitate action within the BMI subgroup; *P < 0.03 comparing palmitate effects (0–240 μM) between BMI subgroups; &P = 0.05 vs. same variable for BMI <33 kg/m².

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### Table 2. Clinical characteristics of group 1 subgroups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Body Mass Index &lt;33 kg/m²</th>
<th>Body Mass Index ≥33 kg/m²</th>
<th>P</th>
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<tbody>
<tr>
<td>Men/women</td>
<td>7/5</td>
<td>6/5</td>
<td>NS</td>
</tr>
<tr>
<td>Age, yr</td>
<td>28±8</td>
<td>29±7</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting insulin, pM</td>
<td>175±25</td>
<td>270±97</td>
<td>&lt;0.009</td>
</tr>
<tr>
<td>Fasting glucose, mM</td>
<td>4.7±0.5</td>
<td>5.2±0.4</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>2 h glucose, mM</td>
<td>7.2±1.8</td>
<td>7.0±2.1</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27±3</td>
<td>39±4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>28±7</td>
<td>37±6</td>
<td>&lt;0.003</td>
</tr>
</tbody>
</table>

Values are means ± SD. NS, not significant. *One subject was omitted because no protein data were available.
Protein phosphatase. Myoblast PP2A activity in the presence or absence of palmitate did not show any relationship with BMI of the group 2 muscle donors (data not shown). In the absence of palmitate, no relationship was observed between myoblast PP2A activity and 2HPG (data not shown). Myoblast PP2A in the presence of palmitate was significantly correlated with 2HPG ($r = 0.44$, $P = 0.03$; Fig. 3A). When 2HPG increases for the myoblast donors, palmitate action on myoblast PP2A activity changed from inhibition to stimulation ($r = 0.45$, $P < 0.03$; Fig. 3B).

Group 3. All myoblasts from subjects in groups 1 and 2 with assays of both glycogen synthase and protein phosphatase were used for group 3 to compare palmitate action on the activities of glycogen synthase and type 2A protein phosphatase. The relationships of palmitate action on GSFA (Fig. 4A) or on PP2A (Fig. 4B) with BMI and 2HPG, respectively, were shown to be similar to those observed in the larger groups but were not significant in the 14 group 3 subjects. No correlation was observed for palmitate action on GSFA compared with palmitate action on PP2A (Fig. 4C). Palmitate action on GSA or MGS was also not correlated with palmitate effects on PP2A (data not shown).

DISCUSSION

Reduced insulin-mediated glucose disposal measured during a euglycemic-hyperinsulinemic clamp has been identified as a risk factor for Type 2 diabetes mellitus (7). Glycogen synthase regulates a rate-limiting step for glucose storage in skeletal muscle. Glucose storage has been identified as a principal site in vivo for reduced insulin action on glucose disposal (28). In insulin-resistant subjects, reduced glycogen synthase activity has been observed in muscle biopsies obtained during a euglycemic-hyperinsulinemic clamp (8). In cultured muscle cells from obese, insulin-resistant subjects, glycogen synthase activity is reduced in the presence or absence of insulin (14, 33). The mechanism for reduced glycogen storage and glycogen synthase activity in these subjects has not been identified; however, several studies have demonstrated a potential role for PP2A activity (2, 10, 17).

Free fatty acids have been described as an important link between obesity and Type 2 diabetes (3). They have been identified as agents causing reduced insulin secretion by the pancreas (49), elevated hepatic glucose production during a euglycemic-hyperinsulinemic clamp (6), and reduced insulin-mediated glucose disposal in peripheral tissues (4). The in-
creased effects of free fatty acids in the tissues of obese and insulin-resistant subjects are generally assumed to result from increased cellular exposure to plasma free fatty acids secondary to the increased fat cell mass of obesity. Previous in vivo studies have demonstrated that lipid infusion produces inhibition of skeletal muscle glycogen synthase activity and reduced insulin-mediated glucose uptake (5, 20, 24).

We have previously reported palmitate inhibition of glycogen synthase in cultured human myoblasts (31). This observation is compatible with a report of palmitate or ceramide-mediated reduction in mouse C2C12 myotube glycogen synthesis (38). Here, we demonstrate increased palmitate inhibition of GSFA in cultured muscle cells from subjects over a range of BMI. Although the GSFA was decreased by palmitate in the myoblasts from the more obese subjects (Fig. 1), the control cell GSFA did not correlate with increasing BMI. The comparison of age-matched subgroups from group 1 subjects with BMI above or below 33 kg/m² (Fig. 2) indicates that both GSFA and MGS are significantly decreased by palmitate in each subgroup. A decrease in MGS suggests a reduction in total available enzyme. A decrease in GSFA suggests increased phosphorylation and inactivation of available enzyme. These results indicate that palmitate can change both enzyme turnover rate and the phosphorylation state of glycogen synthase as mechanisms for reducing glucose storage rates. The significantly greater palmitate inhibition of GSFA in the high-BMI subgroup compared with the low-BMI subgroup suggests an abnormality in palmitate regulation of glycogen synthase phosphorylation state in the high-BMI subgroup. The results for MGS in Fig. 2 do not support an increased palmitate-mediated reduction in glycogen synthase protein in the high-BMI subgroup.

These observations indicate that equimolar concentrations of extracellular long-chain fatty acids could produce greater reduction of glucose storage in skeletal muscle of obese subjects in vivo because of an intrinsically greater sensitivity or response of GSFA to long-chain fatty acids. The increased palmitate action on glycogen synthase in myoblasts from obese subjects may also be the enzymatic explanation for the previously reported palmitate inhibition of insulin-stimulated glycogen synthesis in myotubes from insulin-resistant subjects (19). The latter study in myotubes and the results reported here are unique in describing increased palmitate action on the glucose storage pathway in cells from subjects at risk for developing Type 2 diabetes.

In an attempt to understand the mechanism for increased palmitate inhibition of GSFA in cultured muscle from obese subjects, we measured palmitate effects on PP2A activity under the same conditions used to measure glycogen synthase activity. Fourteen of these cultures with and without palmitate were simultaneously assayed for glycogen synthase activity and PP2A activity. This phosphatase is specifically identified by inhibition with nanomolar okadaic acid in the absence of divalent cations and represents a potentially large family of phosphatase enzymes that have been characterized as holoenzymes (reviewed in Ref. 18). Examples of this complex contain the catalytic subunit and a scaffolding subunit that interact with regulatory subunits. The number of different PP2A holoenzyme complexes involved in palmitate regulation of PP2A in myoblasts is not known. PP2A activity has been implicated in palmitate inhibition of glycogen synthesis (10). Palmitate is incorporated into ceramide (30), which can activate PP2A activity (11). In C2/C12 mouse myotubes, palmitate activated PP2A, which apparently dephosphorylated and inactivated protein kinase B (10). Palmitate-initiated dephosphorylation of protein kinase B is associated with decreased phosphorylation of GSK3 and reduced glycogen synthesis after insulin stimulation (38). GSK3 phosphorlates several critical sites on glycogen synthase that, along with other kinase activities, progressively inhibit glycogen synthase activity (39, 40).

Abnormal regulation of PP2A activity in relation to diabetes has previously been reported in two different tissues. In the Goto-Kakizaki rat model for diabetes, adipocyte basal cytosolic PP2A activity was elevated compared with levels shown in controls and failed to suppress in the presence of insulin (2). In a study that used human skeletal muscle, PP2A catalytic subunit protein reduced insulin-mediated downregulation in individuals with Type 2 diabetes (17). This result indicates that the synthesis and degradation rates of a specific PP2A catalytic subunit isoform could describe a second mechanism that contributes to or explains elevated PP2A activity in diabetic subjects. In isolated β-cells, ceramide increases PP2A activity (26). In isolated rat islets (21), palmitate-induced increases in ceramide inhibit glucose-stimulated insulin gene expression. Long-term exposure to fatty acids inhibits glucose-induced insulin secretion in rats (49). Together, these studies of pancreatic cells indicate that impaired early insulin secretion, which is a principal risk factor for development of Type 2 diabetes in humans (48), could be secondary to fatty acid-mediated increases in PP2A activity.

In results reported here in human myoblasts from subjects over a range of 2HPG levels, palmitate action on PP2A activity changes from inhibition to activation as oral glucose tolerance deteriorates. Our group (31, 33) has previously rationalized that metabolic characteristics in the cultured myoblast, which relate to the clinical characteristics of the cell donor, are intrinsic to the cells from each research subject, as opposed to a response of each cell’s metabolism to the in vivo nutrient and hormonal differences at the time of tissue biopsy. Adipokines such as TNF-α, which can have large effects on muscle glucose metabolism (1), are therefore unlikely to be responsible for the alterations in palmitate action observed here in cultured muscle cells. The assumption that the stimulation of PP2A activity by palmitate is inherent in the myoblasts from subjects with worsening glucose tolerance suggests that a similar inherent increased palmitate response could exist in other tissues from the same subjects. It is possible, therefore, that a mechanism for increased palmitate stimulation of PP2A could be responsible for the abnormal regulation of glucose metabolism where PP2A has been suggested to regulate pathways associated with the development of diabetes. Examples include the adipocytes in Goto-Kakizaki diabetic rats (2), skeletal muscle glycogen synthesis in mouse and humans (10, 17, 19), and pancreatic cells in rats (21, 26).

Palmitate or ceramide have previously been described as metabolites capable of desensitizing insulin action in mouse myotubes (10) and in 3T3-L1 adipocytes (41). An increased palmitate-desensitizing action was described above for insulin-stimulated glycogen synthesis in insulin-resistant compared with normal subjects (19). This desensitization may involve ceramide activation of specific kinases, which compete for phosphorylation of insulin receptor substrate-1, reducing insu-
inhibition of glycogen synthase (35) by the hexosamine pathway; could provide different phosphorylation sites on the synthase of the multiple phosphorylation sites on glycogen synthase (37) (16), which based on the reported hierarchical phosphorylation the addition of glucose would also 1) inhibition of GSFA as noted above and reduce GLUT4 translocation to the plasma membrane, reducing glucose uptake (43). The absence of an inverse relationship between palmitate inhibition of GSFA and palmitate stimulation of PP2A activity suggests that the PP2A activity measured here in the postmitochondrial supernatant may not be the primary source of palmitate regulation of the synthase in the same subcellular fraction. Given the complex regulatory schemes and multiple isoforms available for PP2A regulation (reviewed in Ref. 18), the failure to observe a phosphatase-to-synthase relationship does not preclude the possibility for involvement of a PP2A enzyme in the abnormal regulation of GSFA. Further studies are needed to identify the specific isoforms of PP2A regulated by palmitate in human muscle and which complex can regulate glycogen synthase activity.

Additional mechanisms should be considered to explain palmitate action on glycogen synthase activity. Palmitate stimulates the activation of 5′-AMP-activated protein kinase (AMPK) by AMPK kinase. AMPK increases fatty acid oxidation in L6 myotubes (46) and phosphorylates a glycogen synthase peptide in rat skeletal muscle that can inhibit synthase activity (9). If AMPK activity were increased in skeletal muscle of obese subjects, it could explain both the previously reported increase in palmitate oxidation (31) and the increased inhibition of glycogen synthase reported here.

The previous study from this laboratory on palmitate inhibition of glycogen synthase activity in human myoblasts (31) failed to show any relationship between palmitate action on glycogen synthase activity and variations in muscle donor insulin resistance or BMI. To increase palmitate oxidation rates (27) and GSFA (16), the previous study omitted glucose during the 18-h palmitate treatment. The authors (31) speculated that the absence of glucose during palmitate treatment could have blocked an abnormal response of myoblasts from obese insulin-resistant subjects to palmitate effects on glycogen synthase activity. The different conclusion reached here using glucose in the study of the relationship between palmitate action on GSFA and the BMI of the myoblast donors could be based on one or a combination of potential glucose effects on palmitate inhibition of GSFA. The addition of glucose in the present study would 1) likely reduce palmitate oxidation (27), potentially providing more palmitate for increased ceramide production in the higher BMI subjects. The resulting increased inhibition of protein kinase B and activation of GSK3 could increase inhibition of GSFA as noted above and reduce GLUT4 translocation to the plasma membrane, reducing glucose uptake (43). The addition of glucose would also 2) lower control GSFA (16), which based on the reported hierarchical phosphorylation of the multiple phosphorylation sites on glycogen synthase (37) could provide different phosphorylation sites on the synthase with altered specificity for fatty acid stimulated kinase activities; 3) increase glucose available for formation of glucosamine-6-phosphate, leading to increased glycosylation and inhibition of glycogen synthase (35) by the hexosamine pathway (29). Here, palmitate could increase glucose flux through the hexosamine pathway by the inhibition of glycolysis described for the glucose-fatty acid cycle (36) or by increasing glutamine fructose-6-phosphate aminotransferase (47). The results presented here in myoblasts from human subjects demonstrate an increased response to palmitate for 1) inhibition of GSFA as subject BMI increases and 2) activation of PP2A in subjects with more impaired oral glucose tolerance. Both of these observations are made in the insulin-free state, but the underlying mechanism could cause or contribute to previously identified examples of reduced insulin action.

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REFERENCES


