Insulin activation of phosphatidylinositol 3-kinase in human skeletal muscle in vivo

MATTHEW S. HICKEY, CHARLES J. TANNER, D. SEAN O'NEILL, LYDIA J. MORGAN, G. LYNIS DOHM, AND JOSEPH A. HOUMARD
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Hickey, Matthew S., Charles J. Tanner, D. Sean O'Neill, Lydia J. Morgan, G. Lynis Dohm, and Joseph A. Houmard. Insulin activation of phosphatidylinositol 3-kinase in human skeletal muscle in vivo. J. Appl. Physiol. 83(3): 718–722, 1997.—The purpose of this investigation was to determine whether insulin-stimulated phosphatidylinositol 3-kinase (PI3-kinase) activity is detectable in needle biopsies of human skeletal muscle. Sixteen healthy nonobese males matched for age, percent fat, fasting insulin, and fasting glucose participated in one of two experimental protocols. During an intravenous glucose tolerance test (IVGTT) protocol, insulin-stimulated PI3-kinase activity was determined from percutaneous needle biopsies at 2, 5, and 15 min post-insulin administration (0.025 U/kg). In the second group, a 2-h, 100 mM·m -2 ·min -1 euglycemic hyperinsulinemic clamp was performed, and biopsies were obtained at 35, 60, and 120 min after insulin infusion was begun. Insulin-stimulated PI3-kinase activity by 1.6 ± 0.2-, 2.2 ± 0.3- and 2.2 ± 0.4-fold at 2, 5, and 15 min, respectively, during the IVGTT. During the clamp protocol, PI3-kinase was elevated by 5.3 ± 1.3-, 8.0 ± 2.6-, and 2.7 ± 1.4-fold above basal at 15, 60, and 120 min, respectively. Insulin-stimulated PI3-kinase activity at 15 min post-insulin administration was significantly greater during the clamp protocol vs. the IVGTT (P < 0.05). These observations suggest that insulin-stimulated PI3-kinase activity is detectable in needle biopsies of human skeletal muscle, and furthermore, that the euglycemic, hyperinsulinemic clamp protocol may be a useful tool to assess insulin signaling in vivo.

insulin signaling; glucose; muscle biopsy

The molecular aspects of insulin signaling have been the subject of intense scrutiny for many years (cf. Refs. 7, 8, 21). Although the majority of progress in understanding the steps in the intracellular signaling cascade have come from cell culture studies, several investigations have suggested that insulin signaling is defective in rodent models of obesity/insulin resistance (4, 5, 11, 12, 18). In particular, the dual-function lipid-serine protein kinase phosphatidylinositol 3-kinase (PI3-kinase), which appears to be involved in insulin-mediated glucose transport and glucose transporter isofrom GLUT-4 translocation (17, 23–25, 29), has been shown to be downregulated in skeletal muscle from insulin-resistant rodents (5, 11, 18). Moreover, one member of our present group recently reported that PI3-kinase activation by insulin in vitro is defective in human skeletal muscle that is insulin resistant with respect to glucose transport (15). Because insulin signaling may be modified by alterations in the physiological milieu in intact organisms, this is a question of considerable relevance with regard to establishing the mechanisms that regulate insulin signaling. To our knowledge, only one group has studied insulin-stimulated PI3-kinase activation in vivo (3). The purpose of this investigation was to determine the efficacy of measuring insulin-stimulated PI3-kinase activity from needle biopsies of human skeletal muscle by using either a bolus administration of insulin during an intravenous glucose tolerance test or a euglycemic, hyperinsulinemic clamp, two common methods of measuring insulin action.

METHODS

Subjects. All subjects were young, moderately active males who were nonsmokers, were not currently taking medications known to alter carbohydrate metabolism, and had no family history of heart disease or diabetes. All subjects read and signed informed consents before beginning the investigation. Descriptive characteristics of the subjects are presented in Table 1. Percent body fat was determined from the sum of seven skinfolds (26).

Experimental design. The investigation was divided into two phases to allow for the study of different methodologies commonly used to measure insulin action in vivo. In phase 1, eight subjects completed modified 3-h intravenous glucose tolerance tests (IVGTT) as previously described (19). In phase 2, eight subjects completed 2-h euglycemic, hyperinsulinemic clamps. Different subjects were used in each protocol because of concern regarding the effect of a large number of biopsies on insulin signaling. All subjects were tested between 0700 and 0900 after an overnight fast. Subjects refrained from physical exercise for 48 h before the test and were instructed to consume a diet containing at least 250 g of carbohydrate for 3 days before the test.

IVGTT. An insulin-modified IVGTT was performed according to the method of Bergman et al. (2). Glucose (300 mg/kg) was injected intravenously at time 0, and insulin (0.025 U/kg) was injected intravenously at 20 min. Twenty-five blood samples (4 ml each) were obtained between 0 and 180 min and analyzed spectrophotometrically for glucose (Sigma 16-UV, Sigma Chemical, St. Louis, MO) and by microparticle enzyme immunoassay for insulin (IMx, Abbott Laboratories, Abbott Park, IL). Insulin sensitivity (S) was calculated by using the MINMOD program (R. N. Bergman). This program models plasma glucose dynamics when the measured plasma insulin pattern is supplied. In the process of accounting for the measured plasma glucose response, the model infers S. A more thorough description of this model and the underlying assumptions can be found in Bergman et al. (2).

Euglycemic clamp protocol. On the subject's arrival, a catheter was placed in retrograde fashion in a hand vein for blood sampling. For the duration of the procedure, the subject placed his hand in a heated box maintained at 60°C to allow for the collection of arterialized blood. A second catheter was placed in an antecubital vein for infusion of glucose and insulin. After determination of baseline glucose and insulin levels, a primed continuous infusion of insulin (100 mU·m -2 ·min -1) was initiated.
min−1) was started. Blood for plasma glucose determination was obtained every 5 min throughout the test, and adjustments were made, as necessary, in the rate of infusion of a 20% dextrose solution to maintain euglycemia. Plasma insulin was determined every 10 min unless otherwise noted. M value (glucose disposal rate) was determined in six subjects as described by DeFronzo et al. (10). Data from two subjects were not available because of an error in storing the glucose infusion rate data.

Muscle biopsy. Percutaneous muscle biopsies were obtained from the belly of the vastus lateralis by using Bergstrom needles with suction applied as described previously (19). Biopsies were obtained basally (before IVGTT or clamp) and at the following time points during each test: 2, 5, and 15 min post-insulin administration in the IVGTT and at 15, 60, and 120 min during the clamp. The time course during the IVGTT was chosen to mimic bolus administration protocols reported in rodents (11, 12), in which activation of PI3-kinase has a relatively short time course. The time course of muscle biopsies during the clamp was on the basis of a previous report regarding in vivo insulin-receptor tyrosine kinase activation (13).

Baseline biopsies were obtained ∼30 min before the start of each test. During both procedures, subsequent biopsies were obtained from alternating legs at each time point. Each of the biopsies was ∼3–4 cm apart. During the clamp, the baseline and 60-min biopsies were obtained from the same leg, as were the 15- and 120-min biopsies. Thus during this procedure there was a minimum of 90 min between sampling in the same leg. Because of the condensed time frame of the IVGTT protocol, samples were obtained from the same leg within 13 min (2- and 15-min samples). All biopsies obtained during the IVGTT or clamp were rapidly frozen and stored in liquid nitrogen before analysis for PI3-kinase activity.

PI3-kinase assay. PI3-kinase was assayed according to the procedure of Goodyear et al. (15) with minor modifications. Skeletal muscle tissue to be used for determination of PI3-kinase activity was homogenized in (1:10, wt/vol) ice-cold homogenization buffer containing 1% Nonidet P-40 in a ground-glass mortar and pestle for 30 s. The homogenate was subsequently solubilized for 60 min at 4°C on a rotator and then centrifuged at 30,000 g for 1 h. The supernatant (∼500 µl) was incubated overnight at 4°C with anti-phosphotyrosine conjugated to agarose beads (∼40 µl, Sigma A-1806). Immunoprecipitates were pelleted at 4°C for 30 s at 13,000 g and washed three times with 1% Nonidet P-40 in phosphate-buffered saline (pH 7.4) containing 100 µM Na3VO4, three times in ice-cold 100 mM Tris(hydroxymethyl)amino-

Table 1. Descriptive characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Clamp (n = 8)</th>
<th>IVGTT (n = 8)</th>
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</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>23.8 ± 1.0</td>
<td>25.4 ± 1.9</td>
</tr>
<tr>
<td>%Fat</td>
<td>19.0–28.0</td>
<td>20.0–30.0</td>
</tr>
<tr>
<td>Fasting glucose, mM</td>
<td>5.2 ± 0.3</td>
<td>4.8–5.4</td>
</tr>
<tr>
<td>Fasting insulin, pM</td>
<td>18.5 ± 1.1</td>
<td>18.7 ± 1.0</td>
</tr>
</tbody>
</table>

Values are means ± SE. Range is in parentheses. n, No. of subjects. IVGTT, intravenous glucose tolerance test.
and hyperlipidemia (9), the regulation of cellular insulin action is a topic of considerable relevance.

The results of this study complement a recent report by Bjornholm et al. (3), in which PI3-kinase activity was measured basally and 40 min after the onset of an insulin infusion in six control and six NIDDM subjects. These authors observed a twofold activation of PI3-kinase in the vastus lateralis in control subjects and no activation in the NIDDM patients. Importantly, the subjects studied by Bjornholm et al. were considerably older (55 yr of age) than the subjects in the present study. Moreover, the peak plasma insulin was higher in the present study (1,029 vs. 650 pM). Both of these factors may contribute to the differences in the magnitude of insulin stimulation in these studies. It should also be noted that Laville et al. (22) recently documented that acute hyperinsulinemia (3 h) increases PI3-kinase mRNA in human skeletal muscle. Taken together, these studies provide evidence that selected cellular aspects of insulin signaling in skeletal muscle are amenable to investigation in vivo in humans.

PI3-kinase is a dual-function lipid/serine protein kinase that is activated in response to insulin binding in several tissues, including skeletal muscle (5, 11, 12, 14–16). Studies on the role of PI3-kinase in insulin signaling have provided considerable evidence that the activation of this enzyme is an integral component of the cascade involved in GLUT-4 translocation and glucose transport (17, 23–25, 29). Thus the study of insulin-stimulated PI3-kinase activation in the context of measurement of whole body insulin action provides the opportunity to examine the integrity of a proximal step in the insulin-signaling cascade with respect to a functional end point, i.e., glucose uptake.

In the euglycemic clamp protocol, M value is assumed to primarily reflect skeletal muscle glucose disposal (10). In this context, it is noteworthy that M value was positively associated with peak PI3-kinase activation ($r = 0.73, P = 0.06$). Although the small number of subjects precludes any definitive statement, this relationship suggests that the assessment of PI3-kinase as described represents a physiologically relevant measurement. Further support for the use of the clamp comes from prior observations by Freidenberg et al. (13) that activation of the insulin receptor tyrosine kinase during a euglycemic clamp does not occur until ~60 min after the start of the insulin infusion. Our observation that PI3-kinase activation peaks at 60 min agrees closely with this observation. The reduction in activity at 120 min may relate to a downregulation of enzymatic activity. This phenomenon has been observed during short-term incubation of mouse solei (18), although Freidenberg et al. (13) observed a maintenance of insulin receptor tyrosine kinase activity in human skeletal muscle for up to 240 min during a clamp. The nature of the cellular events that are responsible for mediating insulin-stimulated glucose uptake, as well as the factors that regulate PI3-kinase activity, is just beginning to be understood.

In this context, we cannot exclude the possibility that PI3-kinase activity may have peaked at some time...
other than when biopsies were obtained. The ethical issues related to multiple biopsies in humans preclude an exhaustive time-course study. Moreover, there is some concern that the trauma associated with repeated biopsies may artifically elevate PI3-kinase activity. Although we are not aware of any studies that have carefully examined the effect of tissue sampling on PI3-kinase activation in either rodents or humans, it should be noted that the process of obtaining rectus abdominus strips for muscle incubation, which involves the surgical removal of tissue similar to a needle biopsy, does not prevent a substantial insulin-stimulated activation of PI3-kinase (15). We took care during the clamp protocol to leave ~90 min between same-leg biopsies to avoid any confounding effects of the previous sample. Moreover, if a “biopsy effect” were evident, it would be expected to have a greater effect during the IVGTT protocol, when the time course of muscle sampling was condensed. In fact, PI3-kinase activation during this procedure was substantially lower than during the clamp.

The IVGTT protocol is, in many ways, a corollary of the bolus procedure used for several in vivo studies in rodents (5, 11, 12), with the obvious exception that the administered insulin dose is substantially lower in the human model (0.025 vs. 10 U/kg). In this respect, the relatively minor activation of PI3-kinase during this procedure is not surprising. After a bolus intravenous insulin administration, the peak interstitial concentration of insulin has been reported to be temporally delayed relative to the plasma by 13 min (1). Thus, whereas peak plasma concentration occurred at 2 min postbolus in the present study, interstitial concentration should have peaked at or near the time of the 15-min biopsy.

In both euglycemic clamp (20) and IVGTT protocols (1), interstitial insulin concentration has been reported to be ~50% of the plasma concentration at any given time point during each procedure. If we assume the same relationship applies to the present data, interstitial insulin would have been in the range of 500 pM at 60 min of the clamp protocol and ~50 pM at 15 min of the IVGTT. This difference can obviously be considered to contribute to the differences in the magnitude of PI3-kinase activation in each protocol in the present study. The physiological relevance of PI3-kinase activation in each protocol in the present study is a point of emphasis for future studies. The plasma insulin levels during the clamp protocol are considerably higher than typical postprandial values. The extent to which PI3-kinase is meaningful with respect to insulin action is clearly dependent on the ability of physiological alterations in systemic insulin to activate the enzyme. Nevertheless, the limited data available in humans suggest that activation may be physiologically meaningful. Thus Bjornholm et al. (3) have documented a blunted activation in skeletal muscle of NIDDM vs. control patients, whereas we have observed a significant correlation between PI3-kinase activation and M value during the clamp. Because both studies involved small numbers of subjects, more work must be done to confirm and expand on these preliminary observations.

In conclusion, we have reported that PI3-kinase activity is detectable in needle biopsy samples of human vastus lateralis during a euglycemic, hyperinsulinemic clamp, and, to a lesser extent, during an IVGTT. The data suggest that peak activation of PI3-kinase occurs at ~60 min during the clamp, which is in good agreement with prior reports of the time course of insulin receptor tyrosine kinase activation in vivo (13). These preliminary observations are intended to provide a framework within which the cellular mechanisms of insulin signaling in human skeletal muscle can be investigated.

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**REFERENCES**


