Indinavir alters regulators of protein anabolism and catabolism in skeletal muscle

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Submitted 13 December 2004; accepted in final form 5 April 2005

Hong-Brown, Ly Q., Anne M. Pruznak, Robert A. Frost, Thomas C. Vary, and Charles H. Lang. Indinavir alters regulators of protein anabolism and catabolism in skeletal muscle. Am J Physiol Endocrinol Metab 289: E382–E390, 2005. First published April 12, 2005; doi:10.1152/ajpendo.00591.2004.—The HIV protease inhibitor indinavir acutely impairs basal protein synthesis and translation initiation in skeletal muscle but, in contrast to muscle glucose uptake, does not impair insulin-stimulated signaling of protein synthetic pathways. Indinavir-induced changes were associated with a marked reduction in the phosphorylation of 4E-BP1, S6K1, or mTOR in muscle, but not S6K1 or the ribosomal protein S6. In contrast, the ability of a maximally stimulating dose of insulin to increase the phosphorylation of PKB, 4E-BP1, S6K1, or mTOR was not altered 20 min after intravenous injection. Indinavir increased mRNA expression of the ubiquitin ligase MuRF1, but the plasma concentration of 3-methylhistidine remained unaltered. These indinavir-induced changes were associated with a marked reduction in the plasma testosterone concentration but were independent of changes in plasma levels of IGF-I, corticosterone, TNF-α, or IL-6. In conclusion, indinavir acutely impairs basal protein synthesis and translation initiation in skeletal muscle but, in contrast to muscle glucose uptake, does not impair insulin-stimulated signaling of protein synthetic pathways.

U N T I L A CURE FOR HUMAN IMMUNODEFICIENCY VIRUS (HIV) infection is found, antiretroviral therapy remains the first-line option for the treatment of this patient population. Highly active antiretroviral therapy (HAART) includes treatment with two kinds of reverse transcriptase inhibitors and an HIV protease inhibitor (11). This therapy decreases viral load and stimulates immune function, thereby prolonging survival of patients with AIDS. However, an increasing number of studies report that patients receiving HAART develop a variety of metabolic abnormalities (19). In particular, a majority of patients treated with the protease inhibitor indinavir develop insulin resistance (23, 44–47, 51) characterized by diminished insulin-stimulated glucose uptake in adipose tissue and muscle (51). Protease inhibitors also adversely affect lipid metabolism, as exemplified by the elevated plasma concentrations of triglycerides and cholesterol as well as peripheral lipodystrophy and increased central adiposity (3, 5, 25). Recently, various HIV protease inhibitors have been reported to impair protein synthesis in cultured myocytes (22). However, the cellular mechanisms underlying this decrease in protein synthesis have not been assessed under in vivo conditions.

Altered rates of muscle protein synthesis are often caused by proportional changes in translation efficiency (8, 31, 53). Translation of mRNA into protein is divided into three stages: initiation, elongation, and termination. In many catabolic conditions, decreased muscle protein synthesis impairs mRNA translation initiation, the first rate-determining phase of protein synthesis (8, 28, 31, 35–37). Translation initiation is regulated by a large number of protein factors termed eukaryotic initiation factors (eIFs). A critical point of translational regulation involves binding of the 5′ end of cellular mRNA to the 43S preinitiation complex, which is mediated by the cap-binding protein complex eIF4F (47). The purpose of the present study was to determine whether the acute administration of the antiretroviral drug indinavir would decrease in vivo skeletal muscle protein synthesis under basal conditions and, if so, to determine whether this response was associated with defects in signaling pathways known to regulate translation initiation. Furthermore, because indinavir impairs insulin-stimulated glucose uptake, the ability of insulin to increase translation initiation was also investigated. Finally, catabolic states are often associated with elevations in stress hormones, inflammatory cytokines, and other negative effectors of muscle protein balance. Therefore, we also assessed whether indinavir increased the circulating concentration of corticosterone, tumor necrosis factor (TNF)-α, interleukin (IL)-6, and insulin-like growth factor-binding protein (IGFBP)-1, as well as the tissue level of several ubiquitin ligases that are associated with increased rates of muscle proteolysis.

M A T E R I A L S AND METHODS

Animals. Male Sprague-Dawley rats weighing 200–225 g were purchased from Charles River Breeding Laboratories (Cambridge, MA). Rats were acclimated for 1 wk in a light-controlled room (12:12-h light-dark cycle) under constant temperature. Water and standard rat chow were provided ad libitum. All experiments were approved by the Institutional Animal Care and Use Committee of The Pennsylvania State University College of Medicine, Hershey, PA. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
The following morning, rats were administered a primed constant intravenous infusion of indinavir (20 mg/kg + 0.25 mg·kg⁻¹·min⁻¹; Merck, Rahway, NJ) or an equal volume of saline (1.5 ml/h) for a period of 4 h. A primed constant infusion of indinavir was used to maintain a stable plasma concentration of the drug, which is cleared rapidly in rats (23). A comparable dosing regimen impairs insulin-stimulated glucose uptake in muscle (23). At the conclusion of the 4-h protocol, this infusion protocol produced a plasma indinavir concentration modestly higher than peak levels seen in human subjects taking the drug orally (15).

Muscle protein synthesis and RNA and high-energy phosphate content. In the first study, the in vivo rate of muscle protein synthesis was determined using the flooding-dose technique (26, 37–39). Ten minutes before the conclusion of the indinavir or saline infusion, rats were injected intravenously with L-[2,3,4,5,6-³H]phenylalanine (Phe; 150 mM, 30 µCi/ml; 1 ml/100 g body wt). Rats were then anesthetized with pentobarbital sodium, and 10 min after injection of Phe an arterial blood sample was collected in a heparinized syringe from the abdominal aorta for measurement of plasma cytokines and hormones as well as plasma Phe specific radioactivity. Gastrocnemius muscle was freeze-clamped in vivo, and tissues were powdered under liquid nitrogen. Blood was centrifuged and plasma collected. All tissue and plasma samples were stored at −70°C until analyzed. A portion of the powdered muscle was used to estimate the rate of incorporation of [³H]Phe into protein (26, 37–39). To determine whether a change in the number of ribosomes or the efficiency of mRNA translation was responsible for the indinavir-induced changes in muscle protein synthesis, the RNA content of muscle was quantitated. In striated muscle, ~85% of the RNA is ribosomal RNA, and, therefore, alterations in total RNA content reflect changes in the number of ribosomes. Total RNA content was measured from muscle homogenates (26). Translational efficiency was subsequently calculated by dividing the rate of protein synthesis by the RNA content.

An aliquot of powdered gastrocnemius from control and indinavir-treated rats was extracted in cold perchloric acid, neutralized, and used for the determination of adenosine triphosphate (ATP) and creatine phosphate (CP) by standard fluorometric methods.

Indinavir-induced alterations in basal and insulin-stimulated signal transduction. In the second study, rats were infused with indinavir and then challenged with intravenous insulin to determine whether indinavir altered signal transduction pathways central to the regulation of protein synthesis. Animals were treated as described above, except that radiolabeled Phe was not injected. At the conclusion of the infusion, rats were anesthetized with pentobarbital sodium and injected intravenously with either insulin (5 U/kg) or an equal volume (0.5 ml/rat) of saline. The gastrocnemius was collected 20 min later and homogenized for measurement of protein factors important in the control of mRNA translation. This insulin dose maximally stimulates insulin receptor phosphorylation in skeletal muscle with nominal activation of the IGF-1 receptor (31, 35). The 20-min time point was selected because it is the peak of the phosphorylation response for essentially all of the protein factors determined in this study (31, 35).

Immunoprecipitation and Western blotting. Fresh gastrocnemius was homogenized in a 1:4 ratio of ice-cold buffer (20 mM HEPES, pH 7.4, 2 mM EGTA, 50 mM NaF, 100 mM KCl, 0.2 mM α-glycerophosphate, 1 mM DTT, 0.1 mM PMSF, 1 mM benzamidine, 0.5 mM sodium vanadate) (31, 35–39). The homogenates were centrifuged and the supernatants diluted 1:1 with Laemmli sample buffer (50 mM Tris, pH 6.8, 100 mM DTT, 2% SDS, 10% glycerol, 0.1% bromophenol blue). The samples were boiled, run on SDS-PAGE gels, and transferred to PVDF membranes (Pall, Pensacola, FL). The blots were blocked with 5% nonfat dry milk and incubated with polyclonal and monoclonal antibodies. All antibodies were purchased from Cell Signaling Technology (Beverly, MA) unless otherwise indicated. These include antibodies that recognize the phosphorylated (p-) form of PKB (Thr308), p-tuberlin (Thr1462), p-S6 kinase (S6K1; Thr389, Thr412), p-eukaryotic initiation factor (eIF4E-binding protein-1 (4E-BP1; Thr37/46), p-mamalian target of rapamycin (mTOR; Ser2448), and p-S6 ribosomal protein (Ser235/236). Antibodies to total PKB, mTOR, and the ribosomal protein (rp)S6 were obtained from the same source, whereas the tuberin and S6K1 antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Unbound primary antibodies were removed by washing with TBS containing 0.05% Tween 20 and incubated with anti-rabbit or anti-mouse immunoglobulin conjugated with horseradish peroxidase. Blots were incubated with an enhanced chemiluminescence detection system (Amersham, Buckinghamshire, UK) and exposed to Kodak X-ray film (Rochester, NY). The film was scanned (Microtek; ScanMaker IV, Los Angeles, CA) and analyzed using NIH Image 1.6 software (NIH, Bethesda, MD). For quantification of the eIF4E: eIF4G (eIF4E: eIF4B) complexes, the supernatants were immunoprecipitated overnight with an anti-eIF4E monoclonal antibody (kindly provided by Drs. L. S. Jefferson and S. R. Kimball, Penn State College of Medicine, Hershey, PA). Antibody-antigen complexes were collected using BioMag magnetic beads (Qiagen, Valencia, CA). Beads were then washed in Tris-buffered saline (TBS) and proteins eluted using 5X Laemmli sample buffer. The precipitated material was examined by Western blot analysis using total 4E-BP, eIF4E, and eIF4G (Bethyl Laboratory, Montgomery, TX), as described above (35–39).

Northern blot analysis of ubiquitin ligases. Total RNA was isolated (Tri Reagent; Research Center, Cincinnati, OH) and 20 µg of total RNA were run under denaturing conditions in 1.0% agarose-6% formaldehyde gels using 1X HEPEs running buffer. Northern blotting occurred via capillary transfer to Nytran SuperCharge membranes (Schleicher & Schuell, Keene, NH). Oligonucleotides for rat muscle atrophy F-box (MAFBx; atrogin-1, 5’-CCCAAGCCACGAGGACTTGGCGACTCTTGAGACCGTG-3’) and muscle RING finger 1 (Murf1; 5’-AAGGGAAAAGCCTTGGAGGCAGGACCGTG-3’) were synthesized (IITT, Coralville, IA) and radioactively labeled using TdT (Promega, Madison, WI). For normalization of RNA loading, an 18S oligonucleotide was labeled by the same method. Northern blots were hybridized using ULTRAhyb (Ambion, Austin, TX). All membranes were initially washed twice in 2X SSC-0.1% SDS for 5 min at 42°C and once in 0.2X SSC-0.1% SDS for 15 min at 42°C. Finally, membranes were exposed to a Phosphor-Imager screen (Molecular Dynamics, Sunnyvale, CA) and the resultant data quantified using ImageQuant software from Molecular Dynamics.

Plasma determinations. The plasma concentration of total testosterone (DPC, Los Angeles, CA), insulin (Linco Research, St. Charles, MO), total insulin-like growth factor I (IGF-I) (37, 39), and corticosterone (DSL, Webster, TX) were determined by radioimmunoassay. The 3-methylhistidine (3-MH) concentration in plasma was determined using reverse-phase HPLC after precolumn derivatization of amino acids with phenyl isothiocyanate (37). The plasma concentrations of glucose and lactate were determined using a rapid analyzer (Analox Instruments, Lunenburg, MA). The homeostasis model assessment (HOMA), which is defined as the fasting insulin concentration (µU/ml) × fasting glucose concentration (mmol/l)/22.5, was used as an index of insulin resistance (33). Plasma free fatty acid (FFA) and triglyceride concentrations were also measured using colorimetric kits (Wako Industrial, Osaka, Japan, and Sigma, St. Louis, MO, respectively). The IGFBP-1 content in plasma was determined by Western blot analysis using commercially available antibodies (Upstate Biotechnology, Waltham, MA) (39). The plasma concentrations of TNF-α and IL-6 were measured using a solid-phase sandwich enzyme-linked immunosorbent assay (BioSource International, Cama-
Table 1. Effect of indinavir on plasma concentrations of hormones and substrates

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Indinavir</th>
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<tbody>
<tr>
<td>Insulin, ng/ml</td>
<td>0.95±0.19</td>
<td>1.44±0.25</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>6.1±0.2</td>
<td>7.0±0.3*</td>
</tr>
<tr>
<td>HOMA</td>
<td>7.3±0.9</td>
<td>12.9±2.1*</td>
</tr>
<tr>
<td>Total IGF-I, ng/ml</td>
<td>748±39</td>
<td>722±44</td>
</tr>
<tr>
<td>Testosterone, ng/ml</td>
<td>3.1±1.0</td>
<td>0.3±0.1*</td>
</tr>
<tr>
<td>Lactate, μmol/l</td>
<td>0.78±0.02</td>
<td>0.67±0.06</td>
</tr>
<tr>
<td>Triglyceride, mmol/l</td>
<td>0.48±0.04</td>
<td>0.60±0.09</td>
</tr>
<tr>
<td>FFAs, meq/l</td>
<td>0.65±0.12</td>
<td>0.59±0.08</td>
</tr>
<tr>
<td>Plasma corticosterone, ng/ml</td>
<td>85.6</td>
<td>91.2±4</td>
</tr>
<tr>
<td>Plasma IGFBP-1, AU</td>
<td>1740±349</td>
<td>4726±546*</td>
</tr>
<tr>
<td>3-MH, μmol/ml</td>
<td>4.5±0.2</td>
<td>4.3±0.2</td>
</tr>
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</table>

Values are means ± SE; n = 10–12 per group. FFA, free fatty acids; IGFBP-1, insulin-like growth factor-binding protein-1; 3-MH, 3-methylhistidine; AU, arbitrary units. Homeostasis model assessment (HOMA) for insulin resistance was calculated as reported in Ref. 33. *P < 0.05 compared with mean from time-matched saline-infused control group.

### RESULTS

Indinavir-induced changes in muscle protein synthesis. Figure 1 (top) illustrates that indinavir decreased protein synthesis in the gastrocnemius by 30%. There was no indinavir-induced decrease in the total RNA per muscle protein (Fig. 1, middle), indicating that the observed changes in muscle protein synthesis resulted from a corresponding change in the efficiency of translation (Fig. 1, bottom) and not a change in the number of ribosomes.

Muscle high-energy phosphate content. There was no difference in the ATP concentration in gastrocnemius between control and indinavir-infused rats (7.11 ± 0.12 vs. 7.22 ± 0.24 μmol/g wet wt, respectively). Likewise, there was no difference in CP levels between the two groups (20.6 ± 1.1 vs. 19.9 ± 1.3 μmol/g wet weight).

Plasma hormone, substrate, and indinavir concentrations. At the conclusion of the 4-h infusion protocol the circulating indinavir concentration averaged 19.7 ± 3.7 μmol/l. Table 1 presents data on selected plasma hormone concentrations under basal fasting conditions. Indinavir tended to increase the plasma insulin concentration, but because of the variability this change did not achieve statistical significance. The plasma glucose concentration was significantly increased (+15%). As a result, indinavir increased the HOMA insulin resistance index by 75%. Indinavir did not alter the plasma IGF-I concentration but did reduce the testosterone concentration by 90%. There was no difference in the plasma concentrations for corticosterone, lactate, FFAs, or triglycerides between control and indinavir-treated rats.

### Alterations in eIF4E distribution.

The mechanistic interactions between indinavir and insulin were investigated by analyzing known regulatory steps in the control of translation initiation (18, 47). Neither indinavir nor insulin altered the total amount of eIF4E in gastrocnemius (Fig. 2). In contrast, indinavir increased the amount of eIF4E bound to 4E-BP1 (94%; Fig. 2). Because the hyperphosphorylated γ-isofrom of 4E-BP1 cannot bind to eIF4E, the eIF4E immunoprecipitate contains the two nonphosphorylated α- and β-isofroms of 4E-BP1 that are resolved as a doublet on Western blot analysis. In saline-infused control rats, insulin decreased the inactive eIF4E-4E-BP1 complex by 65%. Insulin also decreased eIF4E association with 4E-BP1 in indinavir-treated rats, and the value did not differ from that of the control group administered insulin.

Indinavir decreased the amount of eIF4E bound to eIF4G by 80% under basal (e.g., nonstimulated) conditions (Fig. 3). In contrast, insulin increased the amount of active eIF4E:eIF4G complex a comparable extent in both control and indinavir-treated rats. To define the mechanism through which indinavir modulates eIF4E availability the phosphorylation state of 4E-BP1 was examined. Mitogen activation and amino acids stimulate multisite phosphorylation of 4E-BP1, leading to the dissociation of 4E-BP1 from eIF4E and permitting the binding of free eIF4E to eIF4G. Under basal conditions, indinavir decreased the phosphorylation of 4E-BP1 by 70% (Fig. 4). Conversely, the extent of 4E-BP1 phosphorylation was more than doubled in muscle from control rats stimulated with insulin. The incremental increase in the phosphorylation of 4E-BP1 induced by insulin in muscle from indinavir-treated rats was comparable to that seen in control animals (Fig. 4).
The interaction between eIF4E and eIF4G can also be regulated, in part, by the phosphorylation of eIF4G, which is enhanced by mitogen stimulation and inhibited by rapamycin (43). Indinavir decreased the content of S1108-phosphorylated eIF4G by 90% compared with values from control muscle (Fig. 5). The extent of eIF4G phosphorylation in muscle increased almost twofold in control rats administered insulin. The increment in eIF4G phosphorylation in response to insulin was comparable in control and indinavir-treated rats [1.1 vs. 0.9 arbitrary units (AU), respectively]; however, because the basal value for the indinavir-treated rats was decreased relative to control values, the value for the indinavir/insulin group remained lower than that of the control/insulin group. The changes in eIF4G phosphorylation were not due to a change in the content of total eIF4G in muscle (Fig. 5).

**S6K1 and S6 phosphorylation.** The Ser/Thr protein kinase referred to as the 70-kDa ribosomal protein S6 kinase 1 (p70S6K1 or S6K1) is also phosphorylated in response to mitogens (12). There was little constitutive S6K1 phosphorylation in muscle from rats in the control/saline group (Fig. 5).
and S6K1 produced by amino acids and growth factors (18). Indinavir decreased the constitutive phosphorylation of mTOR (Ser2448) in muscle by 45% (Fig. 7). Insulin increased mTOR Ser2448 phosphorylation to the same extent in muscle from control and indinavir-treated rats. None of the treatments altered the total amount of mTOR (Fig. 7).

One potentially important mechanism by which mTOR might mediate the phosphorylation of S6K1 and 4E-BP1 in response to nutrients and growth factors is by altering the activity of the tuberous sclerosis complex (TSC), consisting of the TSC1 (hamartin) and TSC2 (tuberin) proteins. Figure 8, A and B, indicates that indinavir did not alter either the total amount or the extent of Thr1462 phosphorylation of TSC2 in skeletal muscle at the time point assessed. Insulin did not acutely alter either total or phosphorylated TSC2 under in vivo conditions. We cannot exclude the possibility that a transient phosphorylation event was missed because only a single time point was assessed for TSC2 phosphorylation. However, this possibility appears unlikely, because phosphorylation of PKB (Thr388), the upstream kinase responsible for TSC2 phosphorylation, was increased at the time point assessed (Fig. 8C). Indinavir did not alter the ability of insulin to increase PKB phosphorylation but did decrease constitutive PKB phosphorylation by 40% under basal conditions (control = 1.00 ± 0.08 AU, indinavir = 0.59 ± 0.09 AU, P < 0.05).

Potential negative regulators of muscle mass. The plasma concentration of the inflammatory cytokines TNF-α and IL-6 were below the level of detection (<15 pg/ml) for both control and indinavir-treated rats (data not shown). Table 1 shows that indinavir increased the plasma concentration of IGFBP-1 almost threefold. A similar indinavir-induced increase was seen for muscle MuRF1 mRNA content (control = 17 ± 2 AU/18S vs. indinavir = 69 ± 7 AU/18S, P < 0.05), but there was no detectable change in the expression of muscle MAFBx-1 (control = 96 ± 8 vs. indinavir = 109 ± 11 AU/18S, P = not 6A). Indinavir did not appreciably change electrophoretic mobility of the bands, suggesting that the relative phosphorylation of S6K1 was unaltered under basal conditions. Insulin decreased band mobility in control rats, and a comparable stimulation of S6K1 phosphorylation was observed after indinavir treatment. Hierarchical multisite phosphorylation of residues in the linker region (e.g., Thr389) leads to full and complete activation of the kinase (20). There was a relative low level of constitutive Thr389 phosphorylation under basal fasting conditions in control muscle (Fig. 6B). There was a marked increase in S6K1 phosphorylation at the Thr389 site in muscle from control animals administered insulin. A comparable increase in Thr389 phosphorylation was observed in muscle from indinavir-treated rats injected with insulin. Insulin also stimulated the phosphorylation of S6K1 at residue Thr2421/Ser2424 (e.g., residues in the autoinhibitory domain) to the same extent in both control and indinavir-treated rats (data not shown).

The phosphorylation state of rpS6, a component of the 40S ribosome and a physiologically relevant S6K1 substrate, exhibited a constitutive level of phosphorylation in muscle from control rats (Fig. 6C). Basal phosphorylation of rpS6 was not altered in muscle from indinavir-treated rats compared with time-matched control rats. In response to insulin, S6 phosphorylation increased sixfold in both control and indinavir-treated rats (Fig. 6C).

mTOR, tuberous sclerosis complex 2, and PKB phosphorylation. The proline-directed Ser/Thr protein kinase mTOR is a common intermediate in the phosphorylation of both 4E-BP1 and S6K1 produced by amino acids and growth factors (18). Indinavir decreased the constitutive phosphorylation of mTOR (Ser2448) in muscle by 45% (Fig. 7). Insulin increased mTOR Ser2448 phosphorylation to the same extent in muscle from control and indinavir-treated rats. None of the treatments altered the total amount of mTOR (Fig. 7).

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and is critical to maintaining normal rates of protein synthesis. During translation initiation, the eIF4E-mRNA complex binds to eIF4G and eIF4A to form the active eIF4F complex. One mechanism for modulating the formation of the eIF4F complex is by altering the distribution of eIF4E between inactive and active protein complexes. The assembly of the functional eIF4F complex is controlled in part by 4E-BP1, which functions as a cap-dependent translational repressor (27, 32, 48). This binding protein obstructs the interaction of eIF4G with eIF4E and limits assembly of the active eIF4F complex (20). Increased phosphorylation of 4E-BP1 in response to mitogens results in the release of eIF4E, its binding to eIF4G, and stimulation of mRNA translation (32, 48). Indinavir decreased the phosphorylation of the translational repressor molecule 4E-BP1 under basal (e.g., no exogenous hormone stimulation) conditions. This change was associated with an increased amount of the inactive 4E-BP1·eIF4E complex and a reciprocal decrease in the amount of the active eIF4G·eIF4E complex. The association of eIF4E with eIF4G may be rate limiting for muscle protein synthesis (28, 35–39). Our results are consistent with the observed decrement in basal muscle protein synthesis. A decrease in the amount of eIF4E·eIF4G complex has also been reported in cultured myocytes treated with indinavir, but, in contrast to our in vivo findings, the response was independent of a change in 4E-BP1 phosphorylation (22).

Indinavir impaired the basal phosphorylation of protein factors important in the regulation of peptide chain initiation, including mTOR, 4E-BP1, and eIF4G. mTOR represents a bifurcation point for the regulation of 4E-BP1 and S6K1 phosphorylation (18, 53). Therefore, it was unexpected that indinavir decreased both mTOR and 4E-BP1 phosphorylation but did not alter the constitutive phosphorylation of rpS6. However, multiple factors modulate rpS6 phosphorylation, and it is possible that an alternative pathway(s) is stimulated in response to indinavir (55, 56).

It is noteworthy that the indinavir-induced decrease in protein synthesis did not result from a reduction in energy stores (as indexed by muscle ATP and CP content) or oxidative metabolism (as indexed by lactate concentration). These findings are in contrast to the myopathy produced by other classes of antiretroviral agents that result from inhibition of mitochondrial ATP-generating enzymes and produces hyperlactacidemia (9, 42). In addition, the reduced translational efficiency was apparently not due to a generalized stress response, because the plasma corticosterone concentration was not altered.

Our data indicate that impaired translational efficiency, not diminished capacity, is primarily responsible for the indinavir-induced decrease in protein synthesis. Other diseases and drugs that inhibit translational efficiency function primarily by altering various protein factors that regulate peptide chain initiation (28, 36–39, 53). One of the rate-controlling steps in translation initiation is the assembly of the eIF4F protein complex, composed of eIF4A, eIF4E, and eIF4G, that facilitates the binding of mRNA to the 43S preinitiation complex. One of these protein components, eIF4E, binds directly to the mGTP cap structure present at the 5′ end of the large majority of eukaryotic mRNA involving eIF4F and limits assembly of the active eIF4F complex (20). Increased phosphorylation of 4E-BP1 in response to mitogens results in the release of eIF4E, its binding to eIF4G, and stimulation of mRNA translation (32, 48). Indinavir decreased the phosphorylation of the translational repressor molecule 4E-BP1 under basal (e.g., no exogenous hormone stimulation) conditions. This change was associated with an increased amount of the inactive 4E-BP1·eIF4E complex and a reciprocal decrease in the amount of the active eIF4G·eIF4E complex. The association of eIF4E with eIF4G may be rate limiting for muscle protein synthesis (28, 35–39). Our results are consistent with the observed decrement in basal muscle protein synthesis. A decrease in the amount of eIF4E·eIF4G complex has also been reported in cultured myocytes treated with indinavir, but, in contrast to our in vivo findings, the response was independent of a change in 4E-BP1 phosphorylation (22).

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Our data indicate that impaired translational efficiency, not diminished capacity, is primarily responsible for the indinavir-induced decrease in protein synthesis. Other diseases and drugs that inhibit translational efficiency function primarily by altering various protein factors that regulate peptide chain initiation (28, 36–39, 53). One of the rate-controlling steps in translation initiation is the assembly of the eIF4F protein complex, composed of eIF4A, eIF4E, and eIF4G, that facilitates the binding of mRNA to the 43S preinitiation complex. One of these protein components, eIF4E, binds directly to the mGTP cap structure present at the 5′ end of the large majority of eukaryotic mRNA
Insulin increased the amount of eIF4E bound to eIF4G and decreased the amount of eIF4E bound to the translational repressor molecule 4E-BP1. This redistribution of eIF4E was associated with the hyperphosphorylation of 4E-BP1, and these changes in eIF4F complex assembly are consistent with earlier reports (28, 31). Insulin also produced a robust stimulation of S6K1 and rpS6 phosphorylation, as previously reported (28, 31, 35, 59). Indinavir-treated rats had a relatively normal redistribution of eIF4E into the active eIF4F complex as well as an appropriate stimulation of PKB, 4E-BP1, mTOR, S6K1, and rpS6 phosphorylation. In contrast to its inhibition of insulin-mediated glucose uptake, indinavir does not impair insulin signal transduction pathways importantly in the translational control of protein synthesis in muscle.

The TSC1/2 heterodimer functions primarily in an mTOR-dependent manner to regulate the phosphorylation of S6K1 and 4E-BP1. Alterations in the amount of TSC1/2 are often inversely proportional to the extent of phosphorylation of these downstream signaling intermediates (17, 57). However, our data indicate that such a mechanism is not operational after in vivo administration of indinavir, because the observed decrease in the constitutive phosphorylation of mTOR, eIF4G, and 4E-BP1 was not associated with a detectable alteration in the total amount of TSC2. Moreover, indinavir did not alter the Thr1462 phosphorylation of TSC2, which, if decreased, might be expected to activate TSC1/2 activity and inhibit mTOR signaling (24). We cannot exclude the possibility that indinavir or insulin altered TSC2 phosphorylation at a site other than Thr1462 or at a time point not assessed. Both insulin and IGF-I stimulate TSC2 phosphorylation in HeLa cells by a phosphatidylinositol 3-kinase-dependent mechanism (10). We were unable to confirm a similar response to insulin in skeletal muscle in vivo. A transient response may have been missed because only a single time point was assessed, but this scenario is unlikely, because insulin increased PKB phosphorylation, which is thought to be the primary upstream mediator of TSC2 phosphorylation. It remains to be determined whether indinavir alters other mTOR-interacting proteins such as raptor, rictor, and GβL.

Potential negative physiological regulators of muscle protein balance were also assessed in the present study. Indinavir did not alter the plasma concentration of corticosterone or the inflammatory cytokines TNF-α and IL-6. Therefore, although exogenous administration of steroids or cytokines can impair the translational control of muscle protein synthesis (37, 54), the endogenous upregulation of these mediators does not appear to be operational in response to the acute infusion of indinavir. In contrast, indinavir increased the circulating concentration of IGFBP-1. This response may have a regulatory role in altering protein balance, as increases in IGFBP-1 are reported to decrease basal protein synthesis under in vivo conditions and in cultured myocytes (16, 39). The relative importance of the increased IGFBP-1 in regulating initiation in response to indinavir remains to be determined.

Finally, muscle protein content represents a balance between protein synthesis and degradation. A number of catabolic stimuli that produce muscle atrophy, including immobilization, denervation, glucocorticoids, and endotoxin, increase the mRNA expression of MuRF1 and MAFBx/atrogin-1 (4). These muscle-specific ubiquitin ligases are important in muscle remodeling (52). Our data indicate that indinavir rapidly increases the mRNA content of MuRF1, but not atrogin-1, in gastrocnemius muscle. Because of differences in the temporal activation of these genes, it is possible that atrogin-1 would also have been increased if the indinavir infusion had been of longer duration. Despite the increase in MuRF1 mRNA, we did not observe an elevation in the 3-MH concentration in blood. Although it is recognized that measurement of protein breakdown based on 3-MH concentration and excretion has limitations (13), these data are consistent with the conclusion that indinavir did not increase myofibrillar degradation at this early time point. The effect of longer-term indinavir treatment on protein balance, both synthesis and degradation, will need to be assessed in future studies.

Our results demonstrate that indinavir depresses basal muscle protein synthesis, and this impairment appears mediated by defects in mTOR and the formation of a functional eIF4F complex but not a diminished activity of S6K1. In contrast to the previously reported indinavir-induced insulin resistance pertaining to glucose uptake (23, 44–47, 51), the ability of insulin to stimulate translation initiation in muscle was not altered by indinavir. The observed changes in the translational control of protein synthesis were independent of defects in energy production, as well as excess secretion of glucocorticoids and inflammatory cytokines, but were associated with a reduction in testosterone. Hence, it is tempting to speculate that therapeutic measures designed to enhance peripheral insulin action in HIV-infected patients might also have the beneficial consequence of improving muscle protein balance.

ACKNOWLEDGMENTS

We thank Drs. Leonard S. Jefferson and Scot R. Kimball (Pennsylvania State College of Medicine) for the eIF4E antibody. Additionally, we thank Danuta Huber and Nobuko Deshpande for expert technical assistance.

GRANTS

This work was supported in part by National Institutes of Health Grants AA-11290 and GM-39277.

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