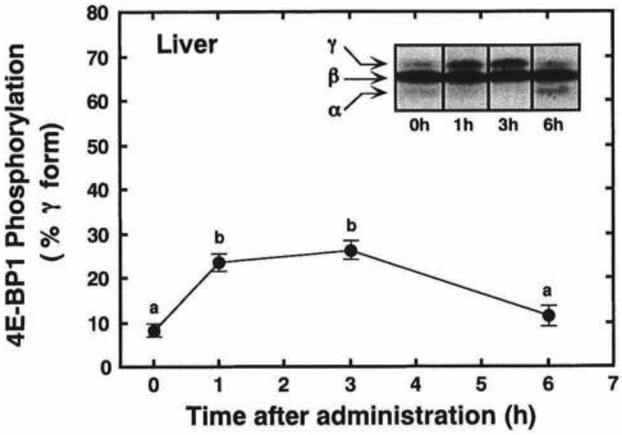

Fig. 2. Changes in the phosphorylation of 4E-BP1 in the liver of rats after leucine administration. 4E-BP1 is resolved into three bands on SDS-polyacrylamide gels, with the top band (γ-band) corresponding to the most highly phosphorylated species. A line graph displays the amount of 4E-BP1 in the γ-phosphorylated form, expressed as a proportion of the total 4E-BP1. The inset shows a representative immunoblot with the position of α-, β-, and γ-forms of 4E-BP1 noted to the left. Each value is the mean±SE for 5 rats. Means not sharing a common superscript are significantly different ($p<0.05$) by the Tukey-Kramer multiple-comparison test.

concentrations of isoleucine and valine in rats (*24*). The mechanism of this effect is unknown, but it could involve specific stimulation of branched-chain amino acid oxidation by the consumption of excess leucine (*24*).

The phosphorylation state of 4E-BP1 can conveniently be examined by resolution of the phosphorylated forms of the protein during SDS-polyacrylamide gel electrophoresis. During SDS-polyacrylamide gel electrophoresis, 4E-BP1 is resolved into multiple electrophoretic forms, termed α, β, and γ, representing differentially phosphorylated forms of the protein. The most highly phosphorylated form, the γ form, exhibits the slowest electrophoretic mobility and is the only one of the three that does not bind to eIF4E. In this study, therefore, the phosphorylation of 4E-BP1 was expressed as the percentage of the protein in the γ form. Leucine administration stimulated phosphorylation of 4E-BP1 in both the liver (Fig. 2) and skeletal muscle (Fig. 3). In the liver (Fig. 2), maximal stimulation was observed between 1 and 3 h with a decrease in the phosphorylation of 4E-BP1 at 6 h. In the skeletal muscle (Fig. 3), the amount of 4E-BP1 in the γ form reached a maximal level within 1 h, then decreased at 3 h, but the values at 3 and 6 h remained elevated.

Upon activation, S6K1 is typically resolved into multiple electrophoretic forms after its separation by electrophoresis on SDS-polyacrylamide gel, with increased phosphorylation being associated with decreased electrophoretic mobility (*25*). In the present study, therefore, the effect of leucine administration on the phosphorylation of S6K1 was investigated in skeletal muscle and the liver by a protein immunoblot analysis. For S6K1, we quantified the ratio of the more heavily phos-

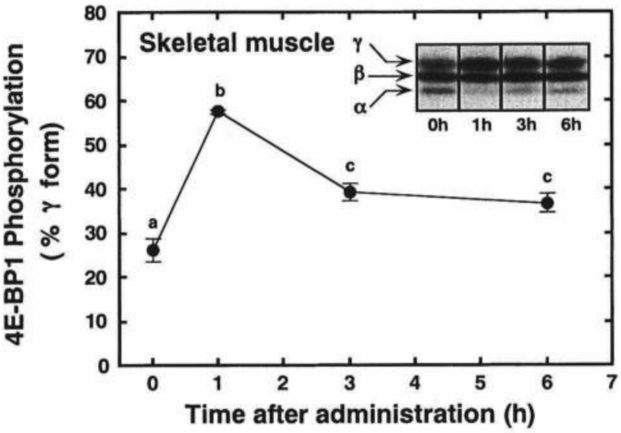

Fig. 3. Changes in the phosphorylation of 4E-BP1 in the skeletal muscle of rats after leucine administration.

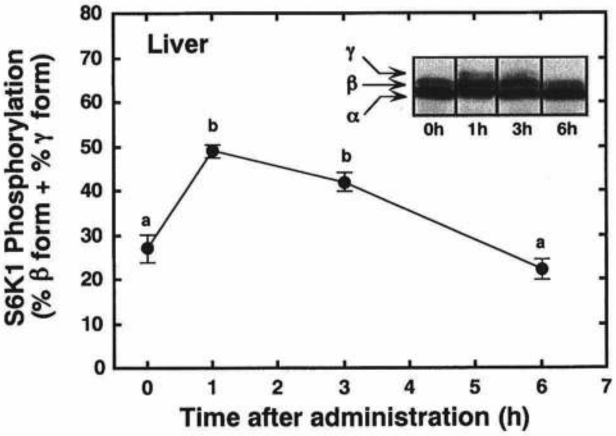

Fig. 4. Changes in the phosphorylation of S6K1 in the liver of rats after leucine administration. S6K1 is resolved into multiple electrophoretic forms on SDS-polyacrylamide gels. The rapidly migrating band is arbitrarily designated α, and the more slowly migrating bands β and γ. A line graph displays the amount of S6K1 in the β and γ forms, expressed as a proportion of the total S6K1. The inset shows a representative immunoblot with the position of α-, β-, and γ-forms of S6K1 noted to the left. Each value is the mean±SE for 5 rats. Means not sharing a common superscript are significantly different ($p<0.05$) by the Tukey-Kramer multiple-comparison test.

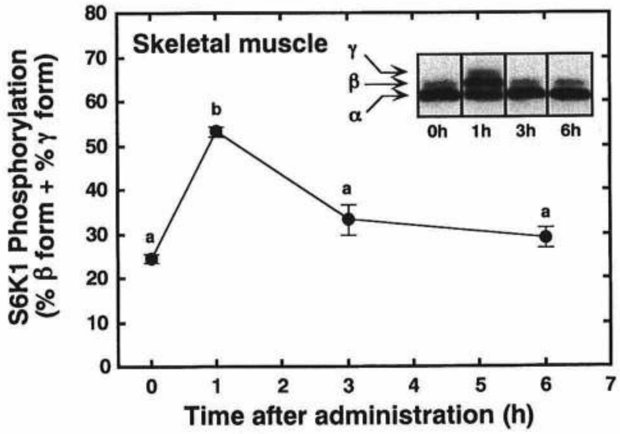

Fig. 5. Changes in the phosphorylation of S6K1 in the skeletal muscle of rats after leucine administration.