Supplementary Data

Supplementary Methods

Western blot analysis

A total of 1 μg of each reaction, calculated using the Schaffner and Weissmann method (53), were prepared in NuPAGE® LDS sample buffer (4×) and loaded on to a NuPAGE Novex 4%–12% Bis-Tris 1 mm gel. Proteins were separated with 1× NuPAGE MES SDS running buffer for 1 h at 200 V. The proteins were transferred overnight at 4°C to a hybond nitrocellulose enhanced chemiluminescence membrane in an XCell II™ blot module in 1× NuPAGE transfer buffer adjusted to contain 10% methanol. The membranes were prepared for immunoblotting by blocking with 5% nonfat milk powder in 1× Tris buffer saline with tween (TBST), pH 7.5. Primary antibody (1/5000 for both human branched-chain aminotransferase [hBCAT] proteins) was prepared in 5% nonfat milk powder in 1× TBST and incubated overnight at 4°C before several washes with 1× TBST. Secondary antibody (1/5000) linked with horse radish peroxidase (HRP) was added for 45 min at room temperature and then washed with 1× TBST. The positive bands were visualized using BM chemiluminescent HRP substrate and imaged using Alpha Innotech Fluorchem Q system.

Expression and measurement of hBCAT specific activity

The hBCAT and mutant proteins were overexpressed and purified as previously described (16, 18). The transaminase activity of these proteins was measured as previously described by Conway *et al.* (16, 17). Briefly, BCAT activity in all samples was measured at 37°C in a buffer containing 25 m*M* potassium phosphate (pH 7.8), 5 m*M* DTT, 1 m*M* [1-^{14}C] keto isovalerate, and 0.25 m*M* pyridoxal 5-phosphate. A unit of hBCAT activity was expressed as 1 μmol of ^{14}C-valine formed min^{-1} at 37°C. All assays were performed in triplicate.