Quantitative ultrasound (US) measurements have been suggested for screening of osteoporosis. However, soft tissues overlying bones affect reliability of the measurements. In this in vitro study, a novel dual frequency ultrasound (DFUS) technique is introduced for elimination of the errors induced by soft tissues on bone pulse-echo US measurements. In DFUS, US reflection from soft tissue-bone interface is measured with two different US frequencies. By knowing the frequency specific US attenuation and speed in adipose and lean tissues, the effect of soft tissue can be determined. DFUS, conducted at frequencies of 2.25 MHz and 5.0 MHz, was validated using human trabecular bone samples (n = 25) covered with heterogeneous soft tissues. DFUS, reduced (p < 0.01) the mean error induced by soft tissues from 58.6% to -4.9% and from 127.4% to 23.8% in broadband ultrasound backscattering and integrated reflection coefficients (at 5.0 MHz), respectively. Our results suggest that DFUS is a technique capable to minimize the errors induced by the soft tissue overlying the bone. As no reflection information within soft tissue (adipose-lean tissue interface) is needed in pulse-echo measurements DFUS may enhance the accuracy of ultrasound measurements. Thereby, DFUS shows a significant clinical potential.