There is a growing interest in using acoustic contrast agents with high-frequency ultrasound (> 15 MHz) in order to better visualize microcirculation. Experiments were performed with polycaprolactone-shelled agents (POINT Biomedical, San Carlos, CA) having mean diameters of 0.56, 1.1, and 3.4 μm. The agents were heavily diluted in filtered water and injected through a 200 μm channel into the focal zone of a 40-MHz transducer that had a focal length of 12 mm and an outer diameter of 6 mm. Backscatter signals from single agents were digitized using tone bursts of 5 to 20 cycles at peak-negative pressures of 0.6 to 6.3 MPa. 1000 valid single-bubble backscatter events at each exposure condition were digitized and then analyzed for 20-MHz subharmonic content. The data showed that the subharmonic response was initiated between 5 and 10 cycles and the likelihood of a subharmonic event increased as the number of cycles increased. A subharmonic backscatter response was most likely at 3.9 MPa for the 3.4 μm agent and 1.7 MPa for the 0.56 and 1.1 μm agents. The increased pressure for subharmonic activity for larger agent was consistent with its larger size.