Determination of regional bone blood flow by means of fluorescent microspheres using an automated sample-processing procedure.

The determination of regional blood flow utilizing fluorescent microspheres (FMs) is an established method for numerous organs. Recent progress, in particular the automation of sample processing, has further improved this method. However, the FM method (reference sample technique), which allows repetitive measurement of regional organ blood flow, has so far not been used for the determination of blood flow in bone. The aim of the present study was to establish FM for the quantification of regional bone blood flow (RBBF). Female, anesthetized New Zealand rabbits (n = 6) received left ventricular injections of different amounts of FM at six subsequent time points. In order to examine the precision of RBBF determination, two different FM species were injected simultaneously at the sixth injection. At the end of the experiments the femoral and tibial condyles of each hind limb were removed and the fluorescence intensity in the tissue samples was measured by an automated procedure. In an in vitro study we have shown that acid digestion of the crystalline matrix has no effect on the fluorescence characteristics of FM. The determination of the number of spheres per tissue sample revealed that depending on the tissue sample size up to $3 \times 10^6$ spheres/injection were necessary to obtain about 400 microspheres in the individual bone samples. RBBF values of the tibial and femoral condyles did not differ at
various injection intervals. The tibial blood flow values varied between 6.6 +/- 1.1 and 8.5 +/- 1.4 ml/min/100 g and were significantly higher than those of the femur (4.3 +/- 1.1 to 6.0 +/- 1.8 ml/min/100 g). The bone blood flow values obtained by simultaneous injection of two FM species correlated significantly (r = 0.96, slope = 1.06, intercept = 0.05), the mean difference was 0.39 +/- 1.11 ml/min/100 g. Our data demonstrate that the measurement of RBBF by means of FM allows a valid determination of RBBF.