Visual aids, such as food photographs, are widely used in estimating food quantities in dietary surveys. The present study aimed to assess how accurately Danish adults and children can estimate food portion sizes using 37 series of photographs illustrating four to six different portion sizes under real-life conditions; determine whether adults were more accurate than children; and estimate the error caused by using portion size photographs to estimate weights of foods consumed in macronutrient calculation. Six hundred and twenty-two adults and 109 children were recruited in three workplace canteens and in two schools, respectively, to estimate their lunchtime portions based on photographs. Participants were instructed to keep the foods separated on their plate when taking lunch. Participants thereafter estimated their own portions by looking at the relevant series of photographs. The actual food portions were then weighed. The proportion of correct estimations was 42% overall (range 19-77%). The mean difference (%) between estimated and actual weight was 17% (range 1-111%). Small portion size photographs were more often used correctly compared to larger portion photographs. Children had as many correct estimations as adults, although they overestimated portions more. Participants using fractions of (or more than) one photograph to estimate the portion of a food had significantly larger errors. When calculating the macronutrient content of a weekly menu using the estimated portion sizes, protein had the largest error (29%). When used in a real-life situation, the portion size photographs validated in the present study showed a certain inaccuracy compared to the actual weights.