OBJECTIVE: The study of human preadipocytes is hampered by the limited availability of adipose tissue and low yield of cell preparation. Proliferation of preadipocytes using common protocols, including fetal bovine serum (FBS), results in a markedly reduced differentiation capacity. Therefore, we were interested in developing an improved culture system that allows the proliferation of human preadipocytes without loss of differentiation capacity.

RESEARCH METHODS AND PROCEDURES: Adipose tissue samples were taken from subjects undergoing elective abdominal surgery. Cells were seeded at various densities and cultured using different formulations of proliferation media including factors such as fibroblast growth factor-2 (basic fibroblast growth factor), epidermal growth factor, insulin, and FBS either alone or in combination. Cells were counted and induced to differentiate after confluence. After complete differentiation, cells were harvested, and glycerol-3-phosphate dehydrogenase activity was measured. Cells were subcultured for up to five passages.

RESULTS: The proliferation medium with 4 growth factors (PM4), consisting of 2.5% FBS, 10 ng/mL epidermal growth factor, 1 ng/mL basic fibroblast growth factor, and 8.7 μM insulin, resulted in lower doubling times at all seeding densities tested (0.05 x 10(4) to 1.5 x 10(4)) compared with medium supplemented with 10% FBS. In contrast to cells in FBS medium, cells
grown with PM4 medium retained full differentiation rate (glycerol-3-phosphate dehydrogenase activity, 493 +/- 215 vs. 41 +/- 17 mU/mg, p< 0.01). Differentiation capacity was fully retained at least for up to three passages in PM4 medium. DISCUSSION: The use of PM4 medium results in substantial proliferation of human preadipocytes with preserved differentiation capacity. This novel technique represents a valuable tool for the study of human adipose tissue development and function starting from small samples.