The association between adipocytes and growth is mediated by growth and differentiation factor (GDF) 5

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All authors have nothing to disclose.

Background

Growth without growth hormone is associated in most cases with obesity, suggesting that adipocytes might have a role in regulating linear growth

The adipocyte tissue is the main site for energy storage and is an exceptionally active secretory tissue, releasing many endocrine and paracrine factors, commonly referred to as adipokines, which affect both peripheral tissues and the central nervous system

The aim of our study was to determine the relationship between adipocytes and bone linear growth (elongation)

Conditioned medium (CM) of 3T3L1 cells induced to differentiate into adipocytes was used for this purpose

Materials and Methods

Metatarsal bone rudiments were dissected from Sprague-Dawley rat fetuses on embryonic day 20-21 and cultured separately in 24/48-well culture dishes. The bones were photographed and measured with Image Pro Plus software

3T3-L1 cells were grown in DMEM supplemented with 10% adult bovine serum. Two days after reaching confluence, the conditioned medium of non differentiated cell was collected (CMF). To induce adipocyte differentiation, cells were treated with 10 µM dexamethasone, 0.25 IU/ml insulin, and 0.5 mM 1-methyl-3-isobutyl-methylxanthine (IBMX) for 48 hours followed by 0.25 IU/ml insulin alone for an additional 48 hours. The conditioned medium was collected after additional 6 days (CMA), centrifuged, filtered and stored at –70°C until use

Oil Red O staining of the 3T3L1 cells confirmed their differentiation into adipocytes (Fig.1 A)

Total RNA was extracted and analyzed with the Mouse Growth Factor RT² Profiler™ PCR Array

GDF5 was measured in the different conditioned media using a commercial ELISA kit

The presence of GDF5 receptor (BMPR1B) was confirmed by immunohistochemistry (Fig.4)

Morphological staining was performed on 6 µm paraffin sections. The hypertrophic zone height, the number of hypertrophic cells per column, the height of hypertrophic cells and the length of the ossification zone were measured using the Image ProPlus software (Fig.5)

Results

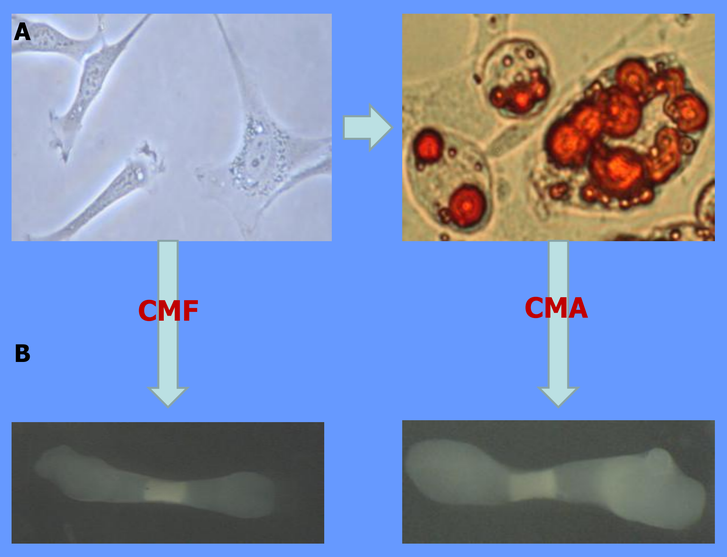

Fig.1: Study design.
A Non- and differentiated 3T3L1 cells in culture. Oil Red O staining show the lipid droplets in differentiated cells (right, CMA) compared to non differentiated cells (left, CMF);
B CMA or CMF medium was added to the culture media of metatarsal bones

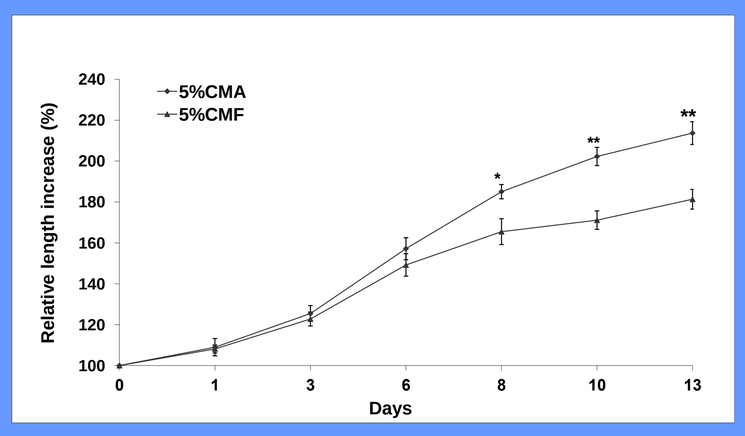

Fig.2: Effect of conditioned medium on metatarsal growth.
***p<0.05; **p<0.005 CMA vs. CMF**
Relative length increase was calculated as % of day "0", the first day of the experiment

Using Mouse Growth Factor RT² Profiler™ PCR Array we found increased expression of 19 growth factors n the adipocytes compared to the non-differentiated 3T3L1 cells
Table 1. Growth factors which expression was significantly increased by adipocyte differentiation (the 9 most affected growth factors are presented)

Gene Name	Fold Amplification
GDF5	727
LEPTIN	277
EGF	194
IL6	39
FGF1	24
GDF10	24
IGF2	13
Nodal	7
VEGF-A	5

GDF5 was previously shown to be involved in bone and cartilage development, maintenance and repair and is a marker for early joint formation. When we added it to the culture medium, GDF5 clearly increased metatarsals length in a dose dependent manner (Fig.3)

GDF5 signaling requires both BMP receptor type II (BMPR-II) and type IB (BMPR-IB). BMPR-II is constitutively active and upon ligand binding, trans-phosphorylates BMPR-IB. The presence of BMPR-IB in metatarsal bone is shown in Fig.4

GDF5 affected the height of the hypertrophic zone (A), the number of cells in each column (B), the height of the hypertrophic cells (C) and the length of the ossification zone (D) (Fig.5)

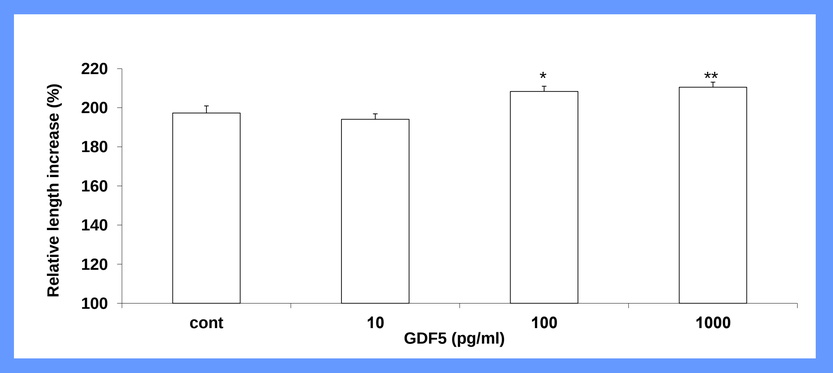

Fig.3: Dose dependent effect of GDF5 on metatarsal growth.
***p<0.05,**p<0.005 vs control**
Relative length increase was calculated as % of day "0", the first day of the experiment

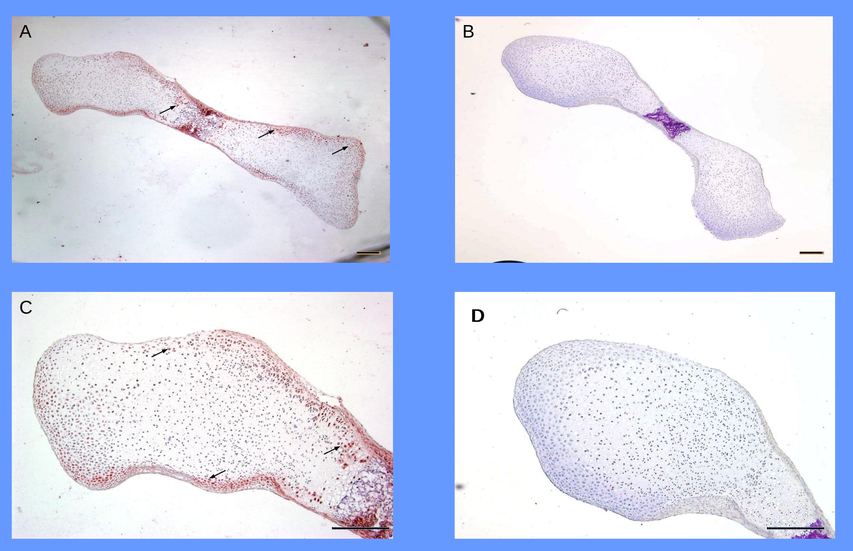

Fig.4: Immunohistochemical localization of BMPR-IB.
A,C-positive staining
B,D-negative control

A, B - magnification X40,
C, D-magnification X100

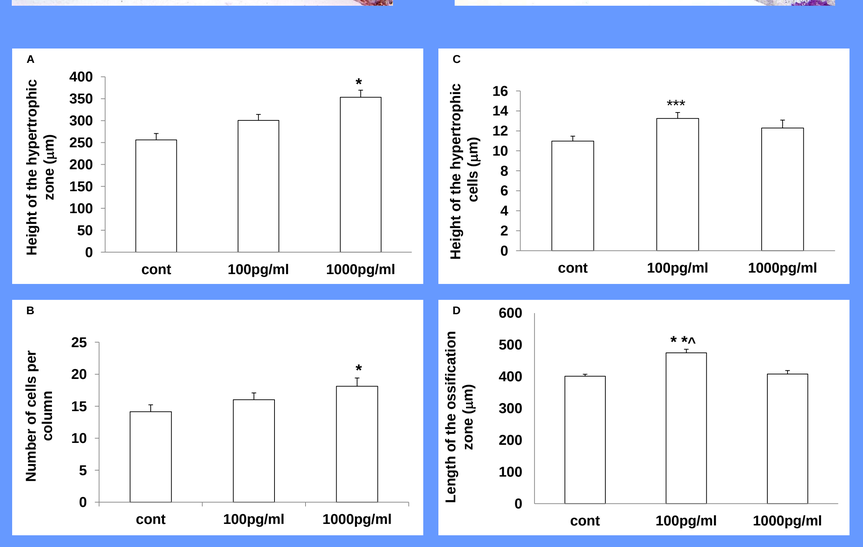

Fig.5: Quantitative histology of bones treated with GDF5 at concentrations of 100 and 1000pg/ml.
***p<0.05, **p<0.01, ***p<0.001 vs control**
^p<0.01 vs GDF5 1000pg/ml

Conclusions

This study shows that GDF5, a skeletal growth factor, is produced and secreted by adipocytes in culture and stimulates the growth of metatarsals *in vitro.* The results add a new potential mediator to the obesity-growth link. Further studies are required to investigate the clinical relevance of our findings: whether adipocytes secrete GDF5 *in vivo* and the manner by which this stimulates growth