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Modulation of DNA synthesis in porcine adipose tissue by growth hormone, insulin and leptin

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ABSTRACT

Adipose tissue plays critical role in energy homeostasis by responding to nutritional, neural, and hormonal signals and secreting adipokines that control feeding, thermogenesis, immunity, and neuroendocrine function. DNA synthesis takes place in the adipose tissue continuously due to multiplication and differentiation of various cell populations. The present study was undertaken to analyze effect of growth hormone, insulin and leptin on DNA synthesis in porcine adipose tissue under *in vitro* conditions and the differential distribution of thymidine incorporation among adipose and stromal-vascular fractions. Adipose tissue explants were incubated with various hormonal combinations in the media containing ^{3}[H] thymidine and its incorporation into cell/DNA was measured later. Growth hormone, insulin and leptin increased thymidine uptake by 4.95, 2.45 and 1.85 times over the non hormonal control tissue explants. Among the combinations GH + leptin and insulin +leptin incubated groups had the lowest and highest thymidine uptake respectively. Out of the total thymidine incorporated in the adipose tissue, 90.49% of the thymidine was taken up by the stromo-vascular cells in comparison to adipocytes (9.51%) in control group. Based on the present study it can be concluded that GH, insulin and leptin increased cellular uptake of thymidine, indicating that all these hormones promoted DNA synthesis and /or multiplication in the cells and stromal vascular fraction of adipose tissue is actively involved in DNA synthesis.

Key words: Adipose tissue, DNA synthesis, Growth hormone, Insulin, Leptin, Thymidine uptake

Adipose tissue plays critical role in energy homeostasis by responding to nutritional, neural, and hormonal signals and secreting adipokines that control feeding, thermogenesis, immunity, and neuroendocrine function (Ahima 2006). Besides adipocytes, adipose tissue contains connective tissue matrix, nerve tissue, stromal-vascular cells, and immune cells (Frayn *et al.* 2003). Although adipocytes express and secrete several endocrine hormones many secreted proteins are also derived from the nonadipocyte fraction of adipose tissue (Fain *et al.* 2004).

Adipose tissue responds to different hormonal, nutritional signals and secretes several endocrine substances (Kershaw and Flier 2004). DNA synthesis takes place in the adipose tissue continuously due to multiplication and differentiation of various cell populations. Stromal vascular and adipocyte components of adipose tissue respond differently to various hormonal stimuli (Wiederer and Löffler 1987, Hauner 1990). However, the information on differential effect of hormones on proliferation and hence DNA synthesis between adipocyte and stromal-vascular fractions is sparse.

The DNA synthesis in cells is influenced under *in vivo* conditions and results from net effect of various endocrines. Pig has high lipogenic activity and well developed adipose tissue hence its a study which could throw light on development of adipose tissue and hence obesity. In animal science, it will be of particular interest to study DNA synthesis and development of adipose tissue as the emphasis is being laid for development of pigs with increased leanness. The present study was undertaken with an objective of analyzing the effect of growth hormone, insulin and leptin on DNA synthesis in porcine adipose tissue under *in vitro* conditions and the differential distribution of thymidine incorporation among adipose and stromal-vascular fractions. The study was conducted using adipose tissue explants to provide better insight to DNA synthesis that occurs under *in vivo*.

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MATERIALS AND METHODS

Collection of adipose tissue, preparation and incubation of adipose tissue explants

The subcutaneous adipose tissue was collected from 6 pigs slaughtered at AICRP unit/slaughter house, Guwahati, Assam. The subcutaneous adipose tissue collected was brought to the laboratory over ice within 30 min. The adipose tissue was washed using prewarmed (37°C) basal media with Hanks salts. The adipose tissue was cut in to small pieces (0.5–0.8cm^3) and placed in media. All the experiments were undertaken after taking necessary aseptic precautions.

The adipose tissues explants were incubated in cell culture media containing different hormone combinations as follows:

Group	Hormone (s) (Concentration in media)
1	Insulin (100nM)
2	Growth hormone (50nM)
3	Leptin (20nM)
4	Growth hormone (50nM) + insulin (100nM)
5	Insulin (100nM) + leptin (20nM)
6	Leptin (20nM) + growth hormone (50nM)

Measurement of ^{3}[H] thymidine uptake by adipose tissue explants

Incorporation of ^{3}[H] thymidine into DNA was measured in cultured adipose tissue slices as per May *et al.* (1994). Fresh explants of subcutaneous adipose tissue were transferred to 6 well culture plates, 5 ml culture media was added, and plates were incubated for 12 h at 37°C. The culture media consisted of Eagles Basal Media with Hanks salts, 25 mmol/l glucose, 10% fetal bovine serum and 1 µCi/ml ^{3}[H] thymidine. After ^{3}[H] thymidine incubation, the tissues were collagenase treated and was rinsed with 150 mmol/l NaCl. Samples were incubated for 1 h in a shaking water bath at 37°C. At the end of the incubation period, vial contents were centrifuged at 12,000×g for 10 min. The top layers containing the fat cells were transferred to new tubes, leaving the stromal-vascular fraction behind. To lyse the cells, 0.5 ml of 20% TCA was added to both fractions, the samples centrifuged at 14 000×g for 15 min, and the top layer aspirated. The resulting pellet was dissolved in 1 ml of 0.5 mol/l NaOH and 0.25 ml was transferred to a clean scintillation vial. To this, 10ml of scintillation cocktail was added and radioactivity counted on a liquid scintillation counter. The thymidine uptake was calculated by deducting the residual radioactivity in the media from preincubation radioactivity and specific activity of ^{3}[H] thymidine (6500 mci/mmole).

Data analysis

The data was analyzed using ANOVA followed by post hoc Bonferroni comparisons test as per standard statistical methods (Snedecor and Cochran 1989).

RESULTS AND DISCUSSION

Adipose tissue explants were incubated with various hormonal combinations for 12 h in the media containing ^{3}[H] thymidine and counts per min were measured to assess ^{3}[H] thymidine incorporation into cell/DNA. The results of the study are presented in Table 1. All the hormones studied alone or in combination increased the thymidine uptake significantly (P<0.01). GH, insulin and leptin increased thymidine uptake by 4.95, 2.45 and 1.85 times over the non hormonal control tissue explants. Among the combinations GH + leptin and insulin +leptin incubated groups had the lowest and highest thymidine uptake, respectively.

When the relative distribution of radioactivity was assessed it was observed that 9.51% of the overall thymidine uptake was by adipocytes and rest of thymidine uptake (90.49%) was taken up by stromo-vascular cells present in the adipose tissue in control group. There was significant differences among the various hormonal incubations on differential thymidine incorporation between adipocytes and stromal-vascular fraction. The differential distribution of thymidine incorporation among adipocytes and stromal-vascular fraction of the adipose tissue (Fig. 1) was insignificant between groups (P>0.05).

The increase thymidine uptake in the adipose tissue indicates increased DNA synthesis in adipose tissues following hormonal stimulation. GH increased DNA synthesis and proliferation in several cells (Nielsen 1982). Our results falls in line with earlier observations (Nielsen 1982, Wabitsch *et al.* 1996). Increased DNA synthesis requires the thymidine and supports the observations of the present study. Wabitsch *et al.* (1996) also reported that thymidine incorporation by 2.6-fold over basal values. Thus, the increased uptake of thymidine in GH treated groups may due to increased mitogenic activity caused by GH.

In the present study, insulin increased the DNA synthesis and thymidine uptake by the cells, as observed in previous studies (Lockwood *et al.* 1967, Hsueh and Stockdale 1974). The values observed in the present study are similar to those

Table 1. ^{3}[H] Thymidine incorporation into DNA in adipose tissue incubated with hormones

Hormone (s)	^{3}[H] Thymidine incorporation (pmoles/mg adipose tissue/ min, ×10^{-4})	Times of the control value
Insulin	4.9±0.58^{ad*}	2.45
GH	9.9±0.32^{b}	4.95
Leptin	3.7±0.56^{cad}	1.85
Insulin+GH	5.9±0.58^{dac}	2.95
Insulin+leptin	7.2±0.58^{bcd}	3.60
GH+leptin	3.5±0.54^{cd}	1.75
Control	2.0±0.23^{cd}	1.00

*The values with same superscript within a column did not differ significantly (P<0.05).

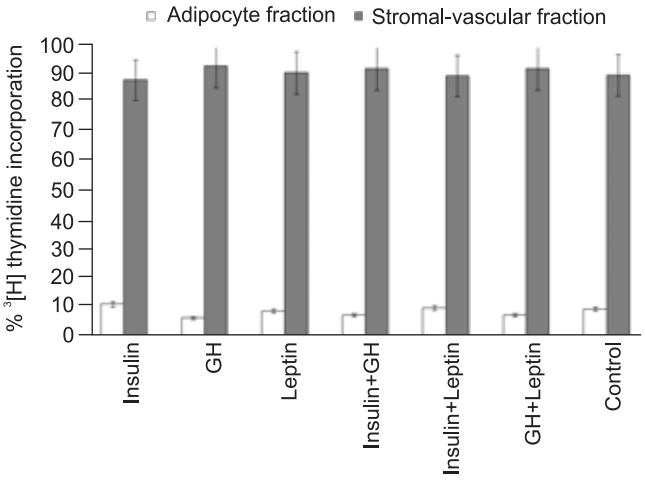

Fig. 1. Differential distribution of [3][H] thymidine incorporation in adipose tissue incubated with hormones.

obtained by Hsueh and Stockdale (1974) and departed from results of Baumrucker and Stemberger (1989), who observed 10-fold increase in thymidine uptake in respect of control. This difference might have resulted from difference in tissues used, physiological state of animal from which tissues were collected, experimental design etc.

Earlier studies (Machinal-Quelin *et al.* 2002, Ramsay 2005, Crespi and Denver 2006, Zhou *et al.* 2008) suggested that the leptin also promotes thymidine intake/DNA synthesis in cells, which is confirmed by the present study.

The hormonal combinations (insulin +GH, GH+leptin and leptin+insulin) altered the thymidine uptake pattern compared to when incubated alone in adipose tissue. The lowering of thymidine incorporation when leptin/insulin was incubated with GH indicates that the hormonal actions may interact with each other, even though the mechanism of interaction is currently not known. Based on the observations of the present study it can be inferred that GH, insulin and leptin increased cellular uptake of thymidine, indicating that all these hormones promoted DNA synthesis and /or multiplication in the cells.

Out of the total thymidine incorporated, more than 90.49% of the thymidine was taken up by the stromo-vascular cells in comparison to adipocytes (9.51%) in control group. The findings of the present study is supported by Adams (2004), who also observed the relative % of thymidine incorporation. Besides adipocytes, adipose tissue contains connective tissue matrix, nerve tissue, stromal-vascular cells, and immune cells (Frayn *et al.* 2003).The adipocytes are mature cells in which the scope of multiplication or DNA synthesis is limited, whereas the stromal-vascular cell fraction contains preadipocytes and mesenchymal, which are in state of mitosis, therefore require more thymidine for DNA synthesis (Adams 2004). Therefore, due to increased mitotic activity present in stromal-vascular cell fraction, the thymidine uptake was highest as observed in the present study. The differential incorporation of thymidine in different hormone incubated groups is suggestive of variation of individual hormone or combination on DNA synthesis and hence proliferation of adipose tissue. GH promoted proliferation of stromal-vascular fraction where as insulin promoted adipose tissue DNA synthesis. It has been observed in earlier studies that GH increased both cell number and [3H]-thymidine incorporation, an effect which was found to be mediated by insulin-like growth factor-I (IGF-I) (Wabitsch *et al.* 1996*).* The stimulant action of thymidine incorporation in adipose tissue explants was between GH and insulin. The leptin induced proliferation acting through MAP kinase pathway in both preadipocyte and adipocyte fractions (Machinal-Quelin *et al.* 2002, Ramsay 2005) which is supported by the present study.

Based on results it can be inferred that GH, insulin and leptin increased cellular uptake of thymidine, indicating that all these hormones promoted DNA synthesis and /or multiplication in the cells. The stromal vascular fraction represents highly proliferative component in the adipose tissue and is influenced by the hormones.

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