Regulation of Adiponectin Secretion in Human Subcutaneous and Omental Adipose Tissue: Effects of Pioglitazone and Endothelin-1: A Pilot Study

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Abstract

Objective: The study was designed to test the effect of anti-diabetic agent pioglitazone and Endothelin-1 (ET-1) on adiponectin secretion from human adipose tissue in depot dependent manner.

Methods: Subcutaneous adipose tissue (SAT) and omental adipose tissues (OAT) were obtained from 19 subjects, including 6 non-obese controls, 7 obese and 6 obese T2DM patients. Adipose tissue was treated with pioglitazone and ET1. Adiponectin secreted into the culture medium after treatment at different time interval (0, 24, 48, 96 hours) was determined by ELISA and normalized for cellular DNA content.

Results: Basal adiponectin secretion from both the depots significantly associated with serum adiponectin, BMI, waist and HOMA-IR. Though no depot-specific difference was found in adiponectin secretion from SAT and OAT in our population, significant reduction in adiponectin secretion was observed in SAT of obese and T2DM patients compared to controls. Responsiveness to pioglitazone treatment was more in SAT, while ET1 inhibits adiponectin secretion in OAT.

Conclusion: These data suggest that, SAT, appears to be major contributor to regulation of adiponectin in circulation. Pioglitazone stimulate adiponectin secretion in SAT compared to OAT in diabetic patients while ET-1 inhibiting adiponectin secretion in OAT of diabetic patients. We need to focus on mechanism underlying these regulatory agents mediated stimulation or inhibition of adiponectin secretion in human adipose tissue.
Table 1: Characteristics of study subjects (Mean ± SE)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=6)</th>
<th>Obese (n=7)</th>
<th>T2DM (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M : F</td>
<td>2 : 4</td>
<td>3 : 4</td>
<td>2 : 4</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>21.4 ± 1.01</td>
<td>36.4 ± 5.5*</td>
<td>37.8 ± 5.32**</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>82.3 ± 2.86</td>
<td>106.3 ± 7.8**</td>
<td>109.8 ± 9.9**</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>120.0 ± 2.5</td>
<td>122.8 ± 3.59</td>
<td>128.3 ± 1.66*</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>78.3 ± 1.66</td>
<td>77.14 ± 1.8</td>
<td>88.3 ± 1.66**</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>81.67 ± 2.4</td>
<td>91.8 ± 2.8**</td>
<td>165.0 ± 4.8***</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.8 ± 0.21</td>
<td>6.1 ± 1.8*</td>
<td>2.0 ± 0.53</td>
</tr>
<tr>
<td>Serum adiponectin level</td>
<td>13.25 ± 0.25</td>
<td>4.95 ± 0.95***</td>
<td>6.8 ± 1.34*</td>
</tr>
</tbody>
</table>

*p < 0.001, †p < 0.01, ‡p < 0.05, §p < 0.02 for obese and T2DM compared to controls.

Adiponectin secretion from human adipose tissue. Hence the second regulatory agent selected in the present study is ET-1.

To gain insight into metabolic role of adiponectin, this pilot study aims to evaluate differential regulation of adiponectin secretion from subcutaneous and omental adipose tissue depots by regulatory agents TZD and ET-1.

Materials and Methods

Subjects

The study population consisted of a total 19 subjects, consisting of 6 non-obese controls, 7 obese and 6 T2DM patients. Diabetes was defined based on history (for patients taking oral hypoglycemic drugs) or according to WHO criteria of fasting glucose ≥7.0 mmol/l or 2 hr glucose ≥11.1 mmol/l for subjects without a clinical history of diabetes. Most of the diabetic patients were receiving anti-diabetic agent sulphonylurea, metformin or combination of both. Obesity was defined if their BMI ≥25 kg/m² according to cut-off suggested for Asian Indians. Controls were classified as having normal glucose tolerance (Fasting plasma glucose < 6.1 mmol/l and 2 hrs glucose < 7.8 mmol/l).

These subjects were surgical patients from H. N. Hospital and St George’s Hospital, Mumbai, who underwent abdominal surgery. All subjects were fasted overnight before tissue removal. Subcutaneous adipose tissue (SAT) and omental adipose tissues (OAT) (~1-5g) were collected during the surgical procedure and were immediately sent to the laboratory. SAT was collected at the site of transverse lower abdominal incision and OAT was collected from greater omentum.

All the subjects gave their informed consent after the procedure was explained to them. The ethics committee of Sir Hurkisondas Narrotumdas Hospital and Medical Research Society approved the project.

The subjects had an age range of 30-70 years. Anthropometric measurement including height, weight and waist circumference (abdominal circumference at the level of iliac crest) and clinical details including blood pressure measurement were obtained from the case report. BMI was calculated from the ratio of body weight in kg to height in square meters and expressed as kg/m² units. Fasting blood samples were also obtained after surgery for the determination of serum adiponectin, insulin and plasma glucose levels.

Insulin resistance measured as Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) using following formula: Fasting insulin (μU/ml) x Fasting glucose (mmol/l)/22.5.

Materials

General reagents were of the highest available grade and obtained from Sigma (St.Louis, MO). DMEM:Ham F12 culture medium, Endothelin 1 were from Sigma (St.Louis, MO), HBSS Buffer from (Invitrogen, USA). Pioglitazone was generously provided by Franco-Indian (Mumbai, India). Fasting plasma glucose was measured by the glucose peroxidase method (Randox, San Francisco, Calif., USA). Elisa Kits for human serum adiponectin, insulin were obtained by linco Res Inc (St.Charls, MO, USA). DNA was extracted using the Hipur mammalian genomic DNA extraction kit (HiMedia, Mumbai, India).

Adipose Tissue Organ Culture

Adipose tissue contains many cell types, including adipocytes, endothelial cells, pre-adipocytes, and fibroblasts. Thus, organ culture preserves paracrine interactions among cells that can influence adipocyte metabolism. The major strength of this method is the good maintenance of gene expression and adipocyte function within adipose tissue placed in organ culture for up to 2 weeks.

The subcutaneous and omental adipose tissue collected during abdominal surgery is processed under sterile conditions. The tissue was washed several times with HBSS, to remove the majority of connective tissue and blood clots. The adipose tissue (~100 mg) was cut into fragments and then placed into 35mm Petri dishes with 1.5ml of DMEM:Ham F12 culture medium without Fetal Bovine Serum (FBS) incubated under an atmosphere of 5% CO₂ at 37°C. The adipose tissue was preincubated for 24 hours, and then the medium was replaced with fresh medium containing the indicated substances. The incubation continued for the time indicated (maximum 96 hour).

Pioglitazone was added to achieve a final concentration of 10μM. Endothelin1 was added to achieve a final concentration of 100 nm. The above concentration of regulatory agents was chosen, since these concentrations are suggested to elicit maximal biological effects as found in adipose tissue or other in-vitro cell system. Samples of conditioned medium were taken every 0, 24, 48 and 96 hours and immediately frozen and maintained at -80°C until use. Medium with untreated cells was used as a blank control (Basal level).

Measurement of Adiponectin in Culture Medium

ELISA was used to measure the secretion of adiponectin into the medium of the cultured human adipose tissue explants. According to the kit manufacturer’s directions (Linco Research, Inc.) samples of culture medium were analyzed and human adiponectin standard provided in the kit was used as standard. To normalize for cell number in the assay, DNA was isolated from aliquots of adipose tissue explants and quantified using spectrophotometer. Adiponectin secretion was reported as nanogram per ml of culture medium per microgram of cell DNA.

Statistical Analysis

Quantitative data is expressed as Mean ± SE. Group means were compared using one way ANOVA or unpaired ‘t’ test. To determine the relationship between adiponectin secretion and different parameters of metabolic syndrome, a Bi-variate correlation analysis was used. A ‘p’ value of less than 0.05 was considered as statistically significant. All analysis was performed using SPSS (Version 16).

Results

The clinical characteristics of study subjects including 6 non-obese controls, 7 obese and 6 T2DM patients are shown in Table 1. Mean levels of BMI, waist size, plasma glucose and HOMA-IR were significantly increased and serum adiponectin levels significantly reduced in obese subjects compared to control subjects. In case of diabetic patient mean levels of BMI, waist size, systolic and diastolic blood pressure, plasma glucose were significantly increased and serum adiponectin levels significantly reduced compared to control subjects. No difference was found in HOMA-IR levels may be due to less number of patients or...
Basal level adiponectin secretion significantly associated with HOMA-IR (r = -0.706, p< 0.05) and waist size (r = -0.4, p< 0.05). It also exhibits correlation with serum adiponectin (r = 0.861, p< 0.01), BMI (r = -0.543, p< 0.05) and waist size (r = -0.46, p< 0.05). In case of OAT, adiponectin secretion is significantly with HOMA-IR, but not able to reach statistical significance due to effect of anti-diabetic treatment.

Metabolic disease condition based comparison:
Comparison of adiponectin secretion of obese and diabetic patients with non-obese control from SAT was demonstrated in Figure 2a, where adiponectin secretion significantly reduced in obese and T2DM patients compared to control in SAT (*p < 0.05). Figure 2b presents comparison of adiponectin secretion of obese and diabetic patients with non-obese control from OAT. Where, adiponectin secretion was reduced in obese and T2DM patients compared to control but not able to reach statistical significance in OAT.

Effect of regulatory agent on adiponectin secretion:
Depot-specific comparison
The effect of two regulatory agents, pioglitazone and endothelin-1 on adiponectin secretion were observed in present study and results were expressed as percentage (%) increase or decrease in adiponectin secretion at 96 hr after treatment with regulatory agent compared to untreated cells.

In present study the effect of Pioglitazone and ET-1 on adiponectin secretion on the basis of metabolic disease condition exhibited no difference in adiponectin secretion from SAT of both obese and diabetic patients compared to control subjects. In OAT, no difference in adiponectin secretion was found as effect of pioglitazone but interestingly, ET-1 shows significant decrease in adiponectin secretion of diabetic patient compared to control but not able to reach statistical significance in OAT.

Discussion
To gain insight into metabolic role of adiponectin, in this pilot study we demonstrated differential regulation of adiponectin secretion from subcutaneous and omental adipose tissue depots. The effect of regulatory agents TZD and ET-1 on adiponectin secretion was also examined.

Importance of adipose tissue secretion of adiponectin for regulating amount of circulating adiponectin was reported in few studies. Findings from these studies were inconsistent, since few studies reported that adiponectin release from omental adipocyte accounts for the reduced serum adiponectin observed in obesity. While others found that SAT is important for regulation of adiponectin levels than OAT. This could be due to visceral (omental) adipose tissue is only small fraction of total adipose tissue, even in obese subjects, as stated by the previous literature. In the present study we demonstrated that adiponectin secretion expressed as ng/ml per DNA content. In time course study adiponectin secretion increased with increasing time (from 0, 24, 48, 96 hrs.) in control, obese and diabetic subjects.

Depot-specific comparison
No depot-specific difference was found in adiponectin secretion from SAT and OAT in control, obese and diabetic subjects [Figure 1 (a, b, c)].

Metabolic disease condition based comparison:
Comparison of adiponectin secretion of obese and diabetic patients with non-obese control from SAT was demonstrated in Figure 2a, where adiponectin secretion significantly reduced in obese and T2DM patients compared to control in SAT (*p < 0.05). Figure 2b presents comparison of adiponectin secretion of obese and diabetic patients with non-obese control from OAT. Where, adiponectin secretion was reduced in obese and T2DM patients compared to control but not able to reach statistical significance in OAT.

Effect of regulatory agent on adiponectin secretion:
Depot-specific comparison
The effect of two regulatory agents, pioglitazone and endothelin-1 on adiponectin secretion were observed in present study and results were expressed as percentage (%) increase or decrease in adiponectin secretion at 96 hr after treatment with regulatory agent compared to untreated cells.

When the adipose tissues were treated with pioglitazone, adiponectin secretion was stimulated from both the depots. Though stimulation of adiponectin secretion was higher in SAT than OAT, statistically significant effect was observed only in T2DM patients (Table 2). Interestingly inhibitory action of ET-1 on adiponectin secretion was found from both the depots. Statistically significant inhibition of adiponectin secretion was found in OAT compared to SAT depot of diabetic patients only (Table 2).

Metabolic disease condition based comparison
In present study the effect of Pioglitazone and ET-1 on adiponectin secretion on the basis of metabolic disease condition exhibited no difference in adiponectin secretion from SAT of both obese and diabetic patients compared to control subjects. In OAT, no difference in adiponectin secretion was found as effect of pioglitazone but interestingly, ET-1 shows significant decrease in adiponectin secretion of diabetic patient compared to control. (-9.2 ± 3.78 vs -43.7 ± 15.2, *p < 0.05) (negative value presents the % decrease in adiponectin secretion).

Discussion
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well as at different metabolic condition was examined. The effect of regulatory agent at both depot specific manners as pioglitazone and vasoconstrictor endothelin-1 was determined. Pioglitazone augments adiponectin secretion from cultured adipocyte or adipose tissue.9,13,23,25 These findings are consistent with previous reports on depot dependent study like non-obese control, obese and T2DM. These findings are level irrespective of underlying metabolic disease conditions specific effect in adiponectin secretion was found at basal reduction) than in OAT (approx 30-40 % reduction). In the present study no depot differences in adiponectin secretion from SAT and OAT of individual control obese and diabetic patients were carried out. Depots differences in adiponectin gene expression and secretion have been reported by some but not all investigators and importantly are not always correlated with circulating levels.25,27,29,30 In the present study pioglitazone significantly increased adiponectin secretion from cultured SAT explants as compared to OAT mainly obtained from diabetic patients. These findings are consistent with in vivo and in vitro studies11-14 where they reported TZD’s enhance the expression of adiponectin mRNA and protein from SAT.

Table 2 : Effect of Pioglitazone and ET-1 on Adiponectin secretion from SAT compared to OAT at different metabolic states

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Obese</th>
<th>T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pioglitazone SAT</td>
<td>67.12 ± 28.09</td>
<td>74.84 ± 38.03</td>
<td>59.14 ± 16.87 *</td>
</tr>
<tr>
<td>OAT</td>
<td>42.44 ± 19.06</td>
<td>37.0 ± 10.27</td>
<td>20.5 ± 5.6</td>
</tr>
<tr>
<td>Endothelin-1 SAT</td>
<td>-13.24 ± 5.97</td>
<td>-31.67 ± 7.17</td>
<td>-16.6 ± 6.27</td>
</tr>
<tr>
<td>OAT</td>
<td>-9.2 ± 3.78</td>
<td>-15.68 ± 7.28</td>
<td>-43.7 ± 15.2 *</td>
</tr>
</tbody>
</table>

*p < 0.05 for SAT compared to OAT.

Results were expressed as percentage increase or decrease in adiponectin secretion at 96 hr after treatment with regulatory agent compared to untreated cells. (Negative value indicates the % decrease in adiponectin secretion)

adiponectin secretion from SAT is significantly lowered in T2DM compared to non-obese control subjects. Thus may be contributing to decreased serum adiponectin levels. In OAT, the adiponectin secretion was decreased in both the metabolic disorder condition (obesity, T2DM) but not able to reach statistical significance may be due to wide distribution of adiponectin secretion among unrelated human subjects. Interestingly, reduction in adiponectin secretion in obese and T2DM compared to non-obese control subjects was found more in SAT (approx 50-60 % reduction) than in OAT (approx 30-40 % reduction).

To better understand the impact of regional fat distribution on adiponectin secretion, comparison of adiponectin secretion from SAT and OAT of individual control obese and diabetic patients were carried out. Depots differences in adiponectin gene expression and secretion have been reported by some but not all investigators and importantly are not always correlated with circulating levels.25,27,29,30 In the present study no depot specific effect in adiponectin secretion was found at basal level irrespective of underlying metabolic disease conditions like non-obese control, obese and T2DM. These findings are consistent with previous reports on depot dependent study of adiponectin secretion from cultured adipocyte or adipose tissue.9,13,23,25

In addition to depot specific effect at basal level, few studies have also demonstrated that adiponectin expression and secretion from adipose tissue are regulated by a wide variety of regulatory agent including insulin, TZDs, TNF α, β adrenergic agonists, IL6 etc.9,31-33 In the present study, the responsiveness of adiponectin secretion to the treatment of antidiabetic agent pioglitazone and vasoconstrictor endothelin-1 was determined. The effect of regulatory agent at both depot specific manners as well as at different metabolic condition was examined.

Though Motoshima et al reported contradictory findings that TZD augment adiponectin secretion from OAT but not SAT.9 Thus response revealed to TZD treatment by SAT in current study might be contributing to increased adiponectin levels in circulation after TZD treatment. We need to focus on mechanism underlying TZD mediated stimulation of adiponectin secretion in human adipose tissue.

Recently ET-1 has also been implicated as a regulatory agent in the secretion of adipocyte derived secretory factor like leptin, resistin and adiponectin from 3T3L1 adipocyte. These studies in 3T3L1 adipocyte demonstrated that ET-1 inhibits basal and insulin stimulated adiponectin secretion through phosphatidylinositol 4, 5 bisphosphate (PIP2) modulation of actin cytoskeleton.18,19

But virtually, no data is available where effect of ET-1 on adiponectin secretion from human adipose tissue was studied. For the first time we report that effect of ET-1 inhibits adiponectin secretion in omental adipose tissue of T2DM patients compared to non-obese control subjects. We need to focus on the mechanism of action responsible for the effects of ET-1 on adiponectin secretion in omental fat of diabetic patients.

Limitation of this study is the limited number of sample in each group. Since it is very difficult to obtain subcutaneous and omental adipose tissue samples at different metabolic condition from surgically operated subjects.

Conclusion

In summary, this pilot study demonstrated that, in diabetic condition decreased adiponectin secretion in SAT may be contributing to regulation of adiponectin in circulation. Further studies are needed with large number of patients to confirm this finding. Interestingly depot specific studies on effect of regulatory agents reveal that pioglitazone stimulates adiponectin secretion in SAT compared to OAT in diabetic patients while ET-1 inhibits adiponectin secretion in OAT of diabetic patients. We also need to focus on mechanism underlying different regulatory agent mediated stimulation or inhibition of adiponectin secretion in human adipose tissue.

Acknowledgement

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Conflict of Interest

There is no conflict of interest.
References


