Effect of Physical Exercise or Apelin on Metabolic Syndrome in Rats

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Abstract

Background: Apelin is an adipokine and also a myokine likely to be involved in whole body metabolic adaptive changes that occur in response to regular exercise.

Aim: Is to study the effect of exercise induced apelin release or exogenous apelin on high fat diet/sucrose (HFD/sucrose) induced Metabolic Syndrome (MS).

Material and Methods: 40 male wistar albino rats of local strain weighing 130-180gm were subdivided into 4 equal groups: Control, HFD/sucrose, HFD/sucrose + exercise and HFD/sucrose + apelin-13. At the end of the experiment (after 12 weeks), Body Mass Index (BMI), systolic Blood Pressure (sBP), serum [fasting glucose, glycosylated hemoglobin (HbA1c), fasting insulin, homeostatic model assessment of insulin resistance and sensitivity (HOMA-IR and HOMA-S), Free Fatty Acids (FFA) and total nitrite/nitrate (NOx)] and total thiol (T-SH) in muscle tissue and plasma [tumor necrosis factor alpha (TNF-a) and apelin level]. Pearson correlation coefficient between plasma apelin and (BMI, fasting insulin, HOMA-S and TG) were also done along all groups.

Results: 12 week HFD/sucrose produced typical picture of MS. Combination of HFD sucrose with either exercise or exogenous administration of apelin produced significant improvement of all parameters in comparison to untreated HFD/sucrose group. Interestingly, no significant difference in all parameters between exercise and apelin-treated groups with the exception of a significant reduction in BMI in exercise group compared to apelin-treated group. The increase of plasma apelin by either exercise or apelin treated groups was about 3 folds in comparison to untreated HFD/sucrose group. Significant negative correlation was found between apelin and both (fasting insulin and TG) and significant positive correlation with (HOMA-S) along all groups and significant negative correlation with BMI only in exercise treated group.

Conclusion: Apelin could be regarded as an exercise-induced endocrine activator with multiple beneficial effects through adipocytes and may serve as a novel therapeutic target for metabolic syndrome (obesity and/or type 2 diabetes).

Key Words: Exercise – Apelin – High fat diet – Metabolic syndrome.

Introduction

EXERCISE training may be considered as the most effective non pharmacological tool for Metabolic Syndrome (MS) treatment [1]. Exercise training in animals decreases fat deposition, improves the glucose-stimulated insulin response, increases the glucose transporter concentration and enhances insulin sensitivity [2]. Metabolic Syndrome (MS) has become pandemic and generally defined as three or more of the following: Visceral adiposity, hypertriglyceridemia (>150 mg/dl), low High-Density Lipoprotein (HDL) levels (<50 mg/dl), blood pressure >130/85mmHg, and fasting glucose >100gm/dl [3]. It is highly related to increasing obesity incidence, sedentary life style and excessive caloric intake [3]. Apelin is a bioactive peptide identified as the endogenous ligand of APJ, a G protein, coupled receptors. It is initially synthesized as preproapelin, which consists of 77 amino acid residents. Following enzymatic cleavage, the C-terminus is released into the circulation as the biologically active molecular forms such as apelin-36, 13 and 12 in different tissues and blood steam [4]. Apelin is an adipokine and also a myokine, has pleiotropic effects on glucose and lipid homeostasis and may contribute to the link between increased adipose tissue mass and obesity related metabolic disorders [5]. It was shown that both apelin and its APJ receptors are often colocalized in the same tissues and display similar variations of expression [6]. This apelin/receptor complex has been seen to increase by different forms of exercise [7].

The aim of this work is to study the effect of exercise induced apelin release or exogenous apelin on high fat diet/sucrose (HFD/sucrose) induced Metabolic Syndrome (MS).
Material and Methods

Animals:
This study was carried out on 40 male wistar albino rats of local strain obtained from Animal House of Faculty of Science, Tanta University, during 2015 weighing about 150-180gm. All procedures were approved by Ethical Committee of Tanta University. The rats were housed in isolated animal cages at room temperature. They were maintained on a constant 12 hour dark/light cycle with free access to their experimental diet and water all over the time of the experiment (12 weeks).

Diets and chemicals:
- Standard chow diet: Is composed of (60% carbohydrates, 26% protein and 14% fat).
- High fat diet (HFD): Is composed of (45% fat, 35% carbohydrates and 20% protein). It consists of (cooked cow fat, casein, bread and green vegetable).
- Diet induced metabolic syndrome is formed of HFD + drinking water containing 10% sucrose by weight for 12 weeks [8].
- Apelin 13 trifluoroacetate: (Sigma-Chemical Co.) in the form of powder and dissolved in distilled autoclaved water frozen at high concentration (1mg/ml) and aliquoted 20 minutes prior to injection.

Experimental protocol:
Rats were classified into 4 equal groups (each of 10 rats):
I- Control group: Were fed standard chow and plain water for 12 weeks.
II- High Fat Diet/Sucrose Group (HFD/sucrose): Were fed HFD and 10% sucrose in the drinking water for 12 weeks.
III- High fat diet/sucrose + exercise group (HFD/sucrose + exercise): Long term swimming program started along with the HFD/sucrose and formed of 60 swimming sessions, each of 60min/day (5 session in 5 days/week for 12 weeks) [9].
IV- High fat diet/sucrose + apelin-13 group (HFD/sucrose + apelin): Apelin was given along with HFD/sucrose in a dose of 2mg/kg/day i.p. for 12 weeks [10].

Body weight and systolic Blood Pressure (sBP) were followed-up until body weight reached 250-300gm and sBP reached 130-150mmHg at the end of 12th week (data not shown).

At the end of the experiment, animals of all groups were fasted over night, sBP was measured by using tail-cuff sphygomanometer after the rats were prewarmed for 15 minutes by blankets, Body Mass Index (BMI) was measured in anaesthetized rat (0.1ml of 1% sodium barbiturate i.p.) by measuring body weight in (gm) and body length (nose-to-anus) in (cm) and calculated using the following formula [11]:

\[ \text{BMI} = \frac{\text{Body weight (gm)}}{\text{Square body length (cm}^2)} \]

The rats were decapitated, blood samples were collected, centrifuged. Plasma and serum samples were kept into clean storage aliquots at –80ºC. Gastrocnemius muscle was dissected, weighed and preserved in foil at –80ºC.

Experimental methods:
- Fasting serum glucose level by enzymatic colorimetric methods [12].
- Glycosylated hemoglobin % (HbA1c%) was measured by spectrophotometer according to the methods of [13].
- Fasting serum insulin by radioimmunoassay procedure using insulin ELISA kit [14].
- HOMA were calculated according to these formulae [15].

\[ \text{HOMA-IR} = \frac{\text{Fasting insulin (µIU/ml) X fasting glucose (mg/dl)}}{405} \]

\[ \text{HOMA-S (％)} = \frac{\log \text{ fasting insulin (µIU/ml) + log fasting glycaemia (mg/dl)}}{1} \]

- Serum Free Fatty Acids (FFAs) were determined by microtest kit (Roche Applied Science) [16].
- Serum Triglycerides (TG) by Glycerol Phosphate Dehydrogenase (GPO) enzymatic method [17].
- Serum malondialdehyde was assayed by measuring the thiobarbituric acid spectrophotometrically at 532nm [18].
- Total thiol group concentration (T-SH) were assessed in gastrocnemius and expressed in nmol/mg muscle [19].
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- Total serum nitrite/nitrate (NOx) by modified Griess reaction [21].
Results

As shown in (Table 1): HFD/sucrose for 12 weeks produced significant increase in BMI, fasting glucose and insulin, HbA1c, HOMA-IR, FFAs, TG, sBP, MDA and TNF-α and significant decrease in HOMA-S, T-SH and NOx (p≤0.05), and insignificant change in apelin serum level when compared to the control group (p>0.05). Both apelin administration or exercise with HFD/sucrose produced significant decrease of BMI, fasting glucose and insulin, HOMA-IR, FFA, TG, sBP, MDA and TNF-α and significant increase in HOMA-S, muscle TSH, NOx and apelin levels when compared to the untreated HFD/sucrose group (p≤0.05). Interestingly, there were insignificant differences in all parameters between exercise and apelin-treated HFD/sucrose groups (p>0.05) except for significant decrease of BMI in exercise-treated group in comparison to apelin treated group. Moreover, the increase in serum apelin was about three folds in either exercise or apelin-treated groups when compared to the untreated HFD/sucrose group.

Table (2) showed significant negative correlation between plasma apelin level and either fasting insulin and TG levels along all group (p<0.001), and positive correlation between plasma apelin and HOMA-S along all groups (p<0.001). However, significant negative correlation between plasma apelin and BMI only in exercise treated group (p<0.001).

Table (1): Mean ± standard deviation and statistical significance of all studied parameters among studied groups.

<table>
<thead>
<tr>
<th>Groups parameter</th>
<th>I- Control No. (10)</th>
<th>II- HFD/sucrose No. (10)</th>
<th>III- HFD/sucrose + exercise No. (10)</th>
<th>IV- HFD/sucrose + apelin No. (10)</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI gm/cm²</td>
<td>0.49±0.07</td>
<td>0.90±0.07a</td>
<td>0.62±0.06b,c</td>
<td>0.72±0.06b</td>
<td>70.48</td>
</tr>
<tr>
<td>Fasting glucose mg/dl</td>
<td>81.60±5.10</td>
<td>129.10±18.16a</td>
<td>88.70±9.03b</td>
<td>93.70±9.03b</td>
<td>34.46</td>
</tr>
<tr>
<td>HbA1c %</td>
<td>4.61±0.87</td>
<td>6.28±0.82a</td>
<td>5.87±0.53</td>
<td>6.06±0.47</td>
<td>11.52</td>
</tr>
<tr>
<td>Fasting insulin µU/ml</td>
<td>9.14±1.19</td>
<td>23.89±2.1a</td>
<td>13.29±2.31b</td>
<td>13.82±2.36b</td>
<td>30.84</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.41±0.87</td>
<td>6.70±1.30a</td>
<td>2.92±0.86b</td>
<td>3.11±0.84b</td>
<td>39.68</td>
</tr>
<tr>
<td>HOMA-S %</td>
<td>88.60±10.59</td>
<td>33.80±7.13a</td>
<td>63.30±10.12b</td>
<td>61.10±9.15b</td>
<td>57.50</td>
</tr>
<tr>
<td>FFAs mmol/L</td>
<td>0.87±0.07</td>
<td>1.92±0.11a</td>
<td>1.76±0.09b</td>
<td>1.72±0.10b</td>
<td>246.69</td>
</tr>
<tr>
<td>TG mg/dl</td>
<td>143.20±7.50</td>
<td>240.30±15.32a</td>
<td>187.70±11.23b</td>
<td>190.90±9.53b</td>
<td>124.11</td>
</tr>
<tr>
<td>sBP mmHg</td>
<td>112.00±10.33</td>
<td>132.00±10.33a</td>
<td>1.17±0.33b</td>
<td>1.19±0.35b</td>
<td>6.43</td>
</tr>
<tr>
<td>MDA µmol/mg tissue</td>
<td>2.66±0.91</td>
<td>10.00±2.81a</td>
<td>6.23±1.84b</td>
<td>5.63±1.40b</td>
<td>25.84</td>
</tr>
<tr>
<td>T-SH nmol/mg tissue</td>
<td>50.22±14.34</td>
<td>20.90±7.06a</td>
<td>71.60±13.56b</td>
<td>61.90±14.02b</td>
<td>30.39</td>
</tr>
<tr>
<td>TNF-α ng/ml</td>
<td>25.40±7.55</td>
<td>85.10±9.66a</td>
<td>56.50±9.93b</td>
<td>64.80±10.25b</td>
<td>69.57</td>
</tr>
<tr>
<td>NOx µmol/L</td>
<td>173.00±16.02</td>
<td>102.50±15.14a</td>
<td>192.00±19.38b</td>
<td>182.50±15.14b</td>
<td>72.05</td>
</tr>
<tr>
<td>Plasma apelin ng/ml</td>
<td>3.58±1.34</td>
<td>4.44±1.39</td>
<td>13.12±2.62b</td>
<td>13.95±2.90b</td>
<td>64.24</td>
</tr>
</tbody>
</table>

a: Significant HFD/sucrose versus control Group (II vs. I).
b: Significant HFD/sucrose + exercise versus HFD/sucrose Group (III vs IV vs. II).
c: Significant HFD/sucrose + exercise versus HFD/sucrose + apelin (III vs. IV).

Table (2): Correlation between plasma apelin and [Body Mass Index (MBI), fasting insulin, HOMA-S and Triglycerides (TG)] along all studied groups.

<table>
<thead>
<tr>
<th>Serum apelin (mg/ml)</th>
<th>Control</th>
<th>HFD/sucrose</th>
<th>HFD/sucrose + exercise</th>
<th>HFD/sucrose + apelin</th>
</tr>
</thead>
<tbody>
<tr>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>BMI (gm/cm²)</td>
<td>-0.159</td>
<td>0.661</td>
<td>-0.525</td>
<td>0.119</td>
</tr>
<tr>
<td>Fasting insulin µU/ml</td>
<td>-0.247</td>
<td>0.493</td>
<td>-0.971</td>
<td>0.001*</td>
</tr>
<tr>
<td>HOMA-S (%)</td>
<td>0.317</td>
<td>0.371</td>
<td>0.979</td>
<td>0.001*</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>-0.197</td>
<td>0.529</td>
<td>-0.919</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*: Statistically significant at p<0.001.
Discussion

It is clear from the results of the present work that 12 weeks consumption of HFD/sucrose induced Metabolic Syndrome (MS) as evidenced by obesity, hyperglycemia, dyslipidemia and hypertension. HFD causes hyperpermeability of blood and lymphatic vessels and hypertrophy of adipocytes and consequently impaired glucose tolerance and Insulin Resistance (IR) which is the basic pathognomonic feature of MS [2]. Apelin now appears as a player in glucose and lipid metabolism and also modulates insulin secretion. Moreover, different animals and human studies proved the increased apelin levels in obesity and type 2 diabetes and that this increase is a compensatory mechanism to overcome Insulin Resistance (IR) [24]. Apelin/APJ signaling promotes lymphatic and blood vessels integrity and acts as a gate keeper of fatty acid transport in both adipose tissue and muscles [25]. At the same time apelin improved glucose tolerance via increased glucose uptake in skeletal muscle and adipose tissue via increased phosphorylation of endothelial Nitric Oxide Synthase (eNOS), Adenosine Monophosphate Activated Protein Kinase (AMPK) and protein kinase B (Akt) via Gi protein [26]. Apelin also stimulated several components of insulin pathways including phosphatidylinositol triphosphate/Akt (PI3K/Akt), thus, it has direct positive effect on glucose handling. This pathway may be considered as an explanation for apelin's effects on Insulin Sensitivity (IS) [27]. As regard the relation between apelin secretions and insulin, apelin secretion is stimulated by insulin (via PI3K/Akt pathway) as a compensatory mechanism in cases of obesity and type 2DM, however, in cases of severe IR, the level of endogenous apelin might be insufficient and inefficient to counteract IR as found in the results of this study in which the increase of endogenous apelin in response to HFD/sucrose is insignificant. At the same time, apelin inhibits insulin secretion through direct action on beta cells [28]. This is confirmed by the negative correlation of apelin level with fasting insulin levels along all groups. However, Dray et al., showed the relation is tissue dependent and according to the severity of insulin resistance [6].

The increase in Free Fatty Acids (FFA) by HFD is linked to IR through intracellular lipid accumulation with subsequent inactivation of glycolysis and glucose uptake and build up of toxic metabolites like diacyl glycerol and ceramides which lead to IR [27]. Apelin decreases FFA by 2 mechanisms; decreases supply side: apelin inhibits lipolysis in an AMPK, Gi and Gq-dependent fashion [29] and increasing amount of perilipin surrounding lipid vacuoles giving them a greater stability and resistance to lipase [30], and increases demand side: Apelin increases body temperature, oxygen consumption, mitochondrial UCP1 and PGC1α expression, mitochondrial enzyme activity and expression of respiratory chain components leading to increase in mitochondrial biogenesis and FFA oxidation in muscles [31]. Long term peripheral injection of apelin decreases weight and Triglyceride (TG) content of different adipose tissues in chow-fed and obese mice possibly through up regulation of aquaporin 7 (AQ7) in hypertrophic adipocytes via PI3K signaling pathway. AQ7 facilitates glycerol transport from these cells leading to decrease in TG synthesis due lack of substrate [32]. However, no effect of apelin on lipolysis has been suggested by Attane et al., (2011) [33]. The antihypertensive effect of apelin may be due to its potent Vasodilation (VD) effect in an endothelial and Nitric Oxide (NO) dependent way and also through inhibition of Angiotensin II (Ang II) mediated Vasconstriction (VC) as apelin/APJ receptor complex acts in a counter regulatory pathway against the pressor action of Ang II/AT-1 receptor pathway [34]. Wu et al., related the beneficial effects of AngII inhibition, at least in part, to restoration of p38/ERK dependent apelin/APJ expression in diet-induced obesity related hypertension [34].

Moreover, apelin is a second catalytic substrate for Angiotensin Converting Enzyme-2 (ACE-2). The latter is a negative regulator of renin angiotensin aldosterone system and can directly metabolizes AngII to beneficial Ang (1-7) whose actions are apposite to those of AngII and its AT-1 receptor signaling [35]. However, some reports have associated apelin with no change [36] or increase of BP.

Reactive Oxygen Species (ROS) overproduction in obesity is a key contributor to obesity-associated metabolic diseases via vicious positive cycles between inflammation and oxidative stress. On the other hand, excess ROS production initiates activity of apelin/APJ system to suppress ROS production [38]. Apelin also promotes antioxidant enzyme expression via mitogen activated protein kinase/extracellular signal regulated kinase 1/2 (MAPK/ERK1/2) [39] and suppresses the expression of pro-oxidant enzyme (NADPH oxidase) [40]. Furthermore, apelin is able to relieve oxidative stress-induced dysregulation of mitochondrial biogenesis, inhibits release of pro-inflammatory cytokines (TNF-α and IL-6) and stimulates release of anti-inflammatory factors (adiponectin) [39]. The anti-
oxidant properties of apelin in adipocytes suggest its potential role as therapeutic target for obesity and obesity-associated metabolic diseases. However, the significant positive correlation of apelin plasma levels and HOMA-S and insignificant correlation with BMI may suggest the link of apelin to type 2 DM rather than obesity.

In the present study, 12 weeks of exercise along with HFD/sucrose significantly improved all metabolic profile, SBP, oxidative and inflammatory parameters and increased apelin plasma level to about 3 folds. Also, there were insignificant difference in exercise group when compared to apelin treated group except for the reduction of BMI in exercise group as compared to apelin treated group. These 2 observations might suggest that the beneficial role played by exercise may be mediated at least in part through its increasing effect of apelin levels. Kadoglou et al., reported that the increase in apelin levels induced by 12 weeks of aerobic exercise in type 2 DM is mostly via cAMP and Ca^{++} [41]. Other studies agreed the increase of apelin with different forms of exercise [5,7,42].

On the other hand, other studies reported decreased apelin serum levels with exercise [1,43]. Exercise increased phosphorylation and activity of various proteins involved in insulin and leptin signal transduction in hypothalamus of obese individuals leading to improved insulin sensitivity [2]. Physical exercise decreases BP via decreased peripheral vascular resistance, cardiac debit followed by decreased vagal tone [44].

The antioxidant defenses of chronic exercise training are due to up regulation of antioxidant enzyme, and downregulation of NADPH oxidase [48]. The anti-inflammatory mechanisms of chronic moderate intensity exercise may be due to increased release of IL-10 and Interleukin-1 Receptor Antagonist (IL-IRA) and concomitant inhibition of TNF-α production [46]. Exercise also reduced expression of toll-like receptors and nuclear transcription of nuclear factor kappa-B (NF-kB) on monocytes and macrophages [47]. Exercise may improve the capacity to regenerate endothelial cell after injury, increase laminar shear stress and reduce the release of adhesion molecules [48].

Conclusion:

Apelin could be regarded as an exercise induced endocrine activator with multiple beneficial metabolic effects through adipocytes and may serve as therapeutic target for metabolic syndrome (obesity and/or type 2 diabetes).

References


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