Impact of Exercise on Apelin/APJ Expression in Skeletal Muscle and Adipose Tissue in Normal and Obese Male Albino Rats; Possible Interaction with Serum IL-6 and TNF α

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Abstract

Background: Apelin is a recently discovered adipokine that contributes to glucose and obesity related conditions. Until now, its responses to muscle exercise are largely unknown. We aim to investigate the impact of regular swimming exercise on Apelin/APJ expression in Visceral Adipose Tissue (VAT) and skeletal muscle of High Fat Diet (HFD)-induced obesity rats.

Methods: A total of 40 male albino rats (160-180gm) were divided into the following groups each containing 10 rats. Group 1: Control group (fed commercial rat chew diet). Group 2: Obese group (fed HFD containing 20% protein, 35% carbohydrate, and 45% fat for 12 weeks). Each group was randomly subdivided to the following subgroups: Subgroup A: Non exercising group. Subgroup B: Exercising group enrolled in a 4 week regular swimming exercise protocol. Body weight, glycemic parameters, lipid profile, and serum TNF-α & IL-6 levels were assessed at baseline and after 4 weeks of exercise. VAT and skeletal muscle samples were taken for the determination of gene expression of Apelin/APJ receptor.

Results: HFD-induced obese rats showed impairment in lipid profile with rise in insulin resistance together with a significant rise in circulating levels of inflammatory markers, TNFα and IL-6. Swimming exercise improved lipid profile, and increased insulin sensitivity and body weight reduction were observed. Parallel measurements of apelin/APJ expression in skeletal muscle and VAT of control and obese rats showed an increased APJ expression in VAT but not in skeletal muscle of obese rats. Interestingly, at the end of the swimming exercise protocol, an increase of APJ expression was observed in skeletal muscle but not in VAT of control rats. In addition, swimming exercise down-regulated Apelin/APJ receptor expression in VAT, and upregulated it in skeletal muscle.

Conclusion: Our results indicate that Apelin/APJ upregulation in muscle tissue and down-regulation in VAT may be one of the mechanisms by which swimming exercise combat obesity and related disorders.

Key Words: Apelin/APJ – Obesity – Exercise – Adipose tissue – Skeletal muscle – IL-6 – TNF.

Introduction

RECENT changes in human lifestyles, including high-calorie diets and lack of physical exercise, have resulted in increases in the occurrence of obesity [1]. Physical inactivity causes the accumulation of visceral fat and consequently the activation of a network of pro inflammatory adipokines, such as Tumor necrosis factor alpha (TNF-α), Interleukin-6 (IL-6), and others, which promote the development of obesity, insulin resistance, atherosclerosis, hypertension, tumor growth, and thereby the development of the diseases belonging to the “diseasome of physical inactivity” [2].

Regular exercise appears to induce anti-inflammatory effects, suggesting that physical activity per se may suppress systemic low-grade inflammation that accompanies obesity [3]. However, very little is known about mechanisms of cross talk between muscle and other organs that could underlie effects of exercise on systemic health.

In line with the acceptance of Adipose Tissue (AT) as an endocrine organ [4,5], path-breaking work during the last decade demonstrated that skeletal muscle is an active endocrine organ releasing myokines, which might in part be responsible for the beneficial effect of exercise [6,7]. These myokines are described to communicate with cells in an autocrine/paracrine manner, locally within the muscles, or in an endocrine fashion to distant tissues. IL-6 was the first discovered myokine because of the observation that it increases up to 100-fold in the circulation during physical exercise [8,9].

Interestingly, many of the contraction-regulated myokines are additionally known to be secreted
by adipocytes. These proteins are termed adipomyokines [10].

Among the numerous so-called adipomyokines, Apelin, a novel adipocyte-secreted factor acting on APJ receptor, was recently identified as a myokine, being also secreted and expressed in skeletal muscle [11,12]. Apelin was recently presented as a new player in the control of glucose homeostasis being regulated by insulin [13] and TNF-α [14]. So far, the regulation of APJ expression in skeletal muscle in relation to muscle exercise has not yet been fully investigated. Moreover, the impact of muscle exercise on the expression of APJ expression in AT and skeletal muscle remains to be addressed.

Therefore the aim of the present work is to study:

1- The effect of muscle exercise on APJ receptor gene expression in both AT and skeletal muscle in control and obese rats.

2- Tissue specificity of Apelin/APJ expression.

3- The impact of muscle exercise on proinflammatory markers IL-6 and TNF-α in control and obese rats.

4- Possible interaction between Apelin/APJ expression and inflammatory markers, TNF and IL-6.

Material and Methods

Experimental animals and diet:

A total of 40 male albino rats were used in this study weighing 160-180 grams. Rats were treated in accordance with the guidelines approved by the Animal Use Committee of Cairo University. The animals were housed in Animal House of Faculty of Medicine, Cairo University during 2014, in wire mesh cages at room temperature, with 12 hour light dark cycle. They had free access to water and were either maintained on a normal chow diet (control group) or fed a High Fat Diet (HFD). The HFD are consisted of 45% (kcal%) fat, 20% protein and 35% carbohydrate [15].

Rats were divided into the following groups:

Group 1: Control group (fed normal chew diet).

Group 2: Obese group (fed HFD).

At the end of 12 weeks, each group was subdivided to the following subgroups:

Subgroup A: Non exercising group.

Subgroup B: Exercising group.

Experimental protocol:

Exercise protocol:

Swimming exercise training was done for a period of four weeks which was initially carried out once per day for a period of one week. Training was then increased to twice daily separated by one hour rest period in the second week. Exercise was increased to three bouts per day in the third week with one hour intervening between each bout and the successive one. In the fourth week, training was increased to four bouts per day with one hour separating each bout from the next. The training regimen was designed similar to those established in other studies [16-18].

Body weight gain and biochemical analysis:

Body weight for all rats in every group was recorded before study initiation (Day 0) and then weekly till the end of experimental period.

Blood samples were taken after a 12 hour overnight fast. Blood samples were withdrawn through retro-orbital route using heparinized capillary tubes in 1 ml eppendorf tubes, then centrifuged (6000xg for 3min) to separate serum from which biochemical parameters were measured within 24 hours.

- Fasting serum Triglycerides (TG), High Density Lipoproteins (HDL), and Total Cholesterol levels (TC): TC was assayed as described by Siedel et al., [19], while the protocols of T. Gordon and M. Gordon [20] and Jacobs and Van Denmark [21] were adopted for the determination of HDL and TG.

- Fasting serum glucose was measured using oxidase-peroxidase method [22].

- Fasting serum insulin levels were analyzed using enzyme-linked immunosorbent assay ELISA (Dako, Carpinteria, CA) according to the manufacturer’s instructions [23].

- Serum IL-6 and TNF-α were measured by using ELISA (quantikine R & D system USA) according to the manufacturer’s instructions.

Sacrification and tissue extraction:

After blood collection, animals were sacrificed by cervical dislocation and then gastrocnemius muscle and VAT (peri renal) were dissected then washed with phosphate buffer saline, parts of the muscle and adipose tissue were kept frozen at −80°C till analyzed for determination of gene expression of Apelin/APJ receptor.
Homeostasis model assessment of insulin resistance (HOMA-IR):

Insulin resistance was assessed and calculated using HOMA-IR index \[24\], as the product of fasting insulin (in µU) and fasting glucose (in mmol/l) divided by 22.5 (I0 X G0/22.5). A lower index indicates greater insulin sensitivity.

Real-time quantitative analyses for apelin gene expression:

The relative abundance of mRNA species was assessed using the SYBR Green method using an ABI prism 7500 sequence detector system (Applied Biosystems, Foster City, CA). PCR primers were designed with Gene Runner Software (Hasting Software, Inc., Hasting, NY) from RNA sequences from GenBank (Table 1). All primer sets had a calculated annealing temperature of 60º. Quantitative RT-PCR was performed in duplicate in a 50-µl reaction volume consisting of 2X SYBR Green PCR Master Mix (Applied Biosystems), 2µl of each primer and 0.5µl of cDNA. Amplification conditions were 2min at 50º, 10min at 95º and 40 cycles of denaturation for 15s and annealing/extension at 60º for 10min. The real time-PCR result was analyzed with the step one applied biosystem software. Relative expression of target gene mRNA was calculated using the applied biosystem software R \[25\].

Table (1): Primer sequences used for RT-PCR.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apelin</td>
<td>5’- CAT CAT GAG GAG ACG GGG-3’</td>
</tr>
<tr>
<td>Apelin</td>
<td>5’- TCC AAG TGG ACA A GT AAG CC-3’</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Forward: 5’- CTCCCCATCTCTCCACCTTGTG-3’</td>
</tr>
<tr>
<td>GAPDH</td>
<td>reverse: 5’-CTTGCTTCAGTATCCTTTCG-3’</td>
</tr>
</tbody>
</table>

Statistical analysis:

Data were coded and entered using the statistical package SPSS version 21. Data was summarized using mean ± standard deviation. Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test when comparing more than 2 groups and unpaired t-test when comparing 2 groups. \(p\)-values less than 0.05 were considered as statistically significant.

Results

Comparison between body weight, lipid profile (TG, Cholesterol and HDL) and glycemic profile (glucose, insulin, HOMA-IR) in all studied groups:

Table (2) and Figs. (1-3) showed that at the end of experimental protocol, body weight, serum TG, cholesterol, glucose, insulin, and HOMA-IR were significantly elevated \((p<0.05)\) while HDL was significantly decreased \((p<0.05)\) in obese non exercising group (Group IIA) compared to control non exercising group (Group IA).

Moreover, (Table 2) and Figs. (1-3) showed that exercise had no effect on body weight, serum TG, cholesterol, HDL, glucose, insulin, and HOMA-IR when comparing control exercising group (Group IB) to control non exercising group (Group IA), as they showed no statistical significant difference between both groups \((p<0.05)\).

On the other hand, exercise resulted in statistical significant reduction of serum TG, cholesterol, glucose, insulin, and HOMA-IR and statistical significant elevation of serum HDL when comparing obese exercising group (Group IIB) to obese non exercising group (Group IIA) \((p<0.05)\).

Table (2): Comparison between mean ± SD of body weight, lipid profile (TG, Cholesterol & HDL) and glycemic profile (glucose, insulin, HOMA/IR) in all studied groups.

<table>
<thead>
<tr>
<th></th>
<th>Control Group IA</th>
<th>Control+exercise Group IB</th>
<th>% Change</th>
<th>Obese Group IIA</th>
<th>Obese+exercise Group IIB</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (gm)</td>
<td>247.00±29.08</td>
<td>196.00±5.16</td>
<td>-20.65</td>
<td>306.00±11.74</td>
<td>254.00±20.11*</td>
<td>-20.47</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>60.75±10.06</td>
<td>57.15±5.69</td>
<td>-5.93</td>
<td>106.57±8.08*</td>
<td>77.85±5.36*</td>
<td>-36.89</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>128.95±4.72</td>
<td>129.50±6.77</td>
<td>0.43</td>
<td>214.15±17.03*</td>
<td>161.60±14.06*</td>
<td>-32.52</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>56.07±4.43</td>
<td>61.05±3.16</td>
<td>8.88</td>
<td>28.95±2.63*</td>
<td>43.88±3.35*</td>
<td>34.02</td>
</tr>
<tr>
<td>Glucose (mmol/dl)</td>
<td>5.30±0.63</td>
<td>5.04±0.44</td>
<td>-4.91</td>
<td>13.4±2.13*</td>
<td>7.8±0.65*</td>
<td>-71.68</td>
</tr>
<tr>
<td>Insulin (uIU/ml)</td>
<td>9.12±1.07</td>
<td>8.87±1.35</td>
<td>-2.74</td>
<td>16.20±3.11*</td>
<td>12.33±1.46$</td>
<td>-31.39</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.13±0.24</td>
<td>1.99±0.39</td>
<td>-6.57</td>
<td>9.59±1.82*</td>
<td>4.30±0.66$</td>
<td>-123.02</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD. *: Statistically significant compared to corresponding value in control group \((p<0.05)\).
$: Statistically significant compared to corresponding value in Obese group \((p<0.05)\).
**Impact of Exercise on Apelin/APJ Expression in Skeletal Muscle & AT in Normal**

Weight of rats at 4 th week

![Graph showing weight of rats at 4th week for different groups.](image)

Fig. (1): Comparison between mean ± SD of body weight in all studied groups. Values are represented as mean ± SD.

*: Statistically significant compared to corresponding value in control group ($p<0.05$).

$: Statistically significant compared to corresponding value in Obese group ($p<0.05$).

HOMA IR

![Graph showing HOMA-IR values for different groups.](image)

Fig. (3): Comparison between mean ± SD of HOMA-IR in all studied groups.

*: Statistically significant compared to corresponding value in control group ($p<0.05$).

$: Statistically significant compared to corresponding value in Obese group ($p<0.05$).

**Comparison between the inflammatory markers (IL-6 and TNF-α) in all studied groups:**

Table (3) and Fig. (4) showed that both IL-6 and TNF-α were significantly elevated ($p<0.05$) in obese non exercising group (Group IIA) compared to control non exercising group (Group IA).

Table (3) and Fig. (4) showed that exercise causes statistical significant elevation of IL-6 when comparing control exercising group (Group IB) to control non exercising group (Group IA) ($p<0.05$). However, exercise had no effect on TNF-α as it showed no statistical significant difference between both groups ($p<0.05$).

On the other hand, exercise resulted also in statistical significant elevation of IL-6 but statistical significant reduction of TNF-α when comparing obese exercising group (Group IIB) to obese non exercising group (Group IIA) ($p<0.05$).

<table>
<thead>
<tr>
<th></th>
<th>Control Group IA</th>
<th>Control+exercise Group IB</th>
<th>% Change</th>
<th>Obese Group IIA</th>
<th>Obese+exercise Group IIB</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 (pg/ml)</td>
<td>31.98±4.88</td>
<td>129.05±2.69 *</td>
<td>303.53</td>
<td>117.45±7.93*</td>
<td>931.32±105.69* $</td>
<td>873.9</td>
</tr>
<tr>
<td>TNF-α (ng/ml)</td>
<td>33.03±4.66</td>
<td>32.42±3.48</td>
<td>−1.85</td>
<td>120.23±9.43*</td>
<td>67.87±9.57* $</td>
<td>−77.15</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD.

*: Statistically significant compared to corresponding value in control group ($p<0.05$).

$: Statistically significant compared to corresponding value in Obese group ($p<0.05$).

![Graph showing lipid profile for different groups.](image)

Fig. (2): Comparison between mean ± SD of lipid profile (TG, Cholesterol & HDL) in all studied groups. Values are represented as mean ± SD.

*: Statistically significant compared to corresponding value in control group ($p<0.05$).

$: Statistically significant compared to corresponding value in Obese group ($p<0.05$).
Comparison between Apelin/APJ expression in Adipose tissue and skeletal muscles in all studied groups:

Table (4) and Fig. (5) showed that Apelin/APJ expression in Adipose tissue were significantly elevated ($p<0.05$) in obese non exercising group (Group IIA) compared to control non exercising group (Group IA). However, Apelin/APJ expression in skeletal muscles showed no statistical significant difference between both groups.

Table (4) and Fig. (5) showed that exercise causes statistical significant elevation of Apelin/APJ expression in skeletal muscles when comparing control exercising group (Group IB) to control non exercising group (Group IA) ($p<0.05$). However, exercise had no effect on Apelin/APJ expression in adipose tissue as it showed no statistical significant difference between both groups ($p<0.05$).

On the other hand, exercise resulted also in statistical significant elevation of Apelin/APJ expression in skeletal muscles but statistical significant reduction of Apelin/APJ expression in adipose tissue when comparing obese exercising group (Group IIB) to obese non exercising group (Group IIA) ($p<0.05$).

Table (4): Comparison between mean ± SD of Apelin/APJ in Adipose tissue and skeletal muscles in all studied groups.

<table>
<thead>
<tr>
<th></th>
<th>Control IA</th>
<th>Control+exercise IB</th>
<th>% Change</th>
<th>Obese IIA</th>
<th>Obese+exercise IIB</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apelin/APJ (Adipose tissue)</td>
<td>1.06±.16</td>
<td>1.59±.46</td>
<td>50</td>
<td>10.73±1.94</td>
<td>5.48±2.73 *$</td>
<td>−95.8</td>
</tr>
<tr>
<td>Apelin/APJ (Skeletal muscle)</td>
<td>.14±.04</td>
<td>.34±.09 *</td>
<td>142.86</td>
<td>.16±.07</td>
<td>.68±.14 *$</td>
<td>76.47</td>
</tr>
</tbody>
</table>

Values are represented as mean ± SD.

*: Statistically significant compared to corresponding value in control group ($p<0.05$).

$: Statistically significant compared to corresponding value in Obese group ($p<0.05$).
Correlation between TNF-α, Apelin/APJ expression in Adipose tissue and Apelin/APJ expression in skeletal muscles with all studied parameters (Table 5):

When Pearson’s correlation was performed in studied groups, the level of Apelin/APJ expression in adipose tissue showed significant positive correlation with body weight ($r=0.776^{**}$), TG ($r=0.929^{**}$), Cholesterol ($r=0.871^{**}$), HOMA-IR ($r=0.884^{**}$), and TNF-α, ($r=0.936^{**}$), and significant negative correlation with HDL ($r=0.880^{**}$). On the other hand, Apelin/APJ expression in skeletal muscle showed only a significant positive correlation with IL-6 ($r=0.886^{**}$).

Moreover, the level of serum TNF-α showed significant positive correlation with body weight ($r=0.840^{**}$), TG ($r=0.930^{**}$), Cholesterol ($r=0.933^{**}$), HOMA-IR ($r=0.939^{**}$), and significant negative correlation with HDL ($r=0.944^{**}$).

<table>
<thead>
<tr>
<th>Table (5): Correlation between TNF-α, Apelin/APJ in Adipose tissue and skeletal muscles and all studied parameters.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TNF-α:</strong> Body weight</td>
</tr>
<tr>
<td>r</td>
</tr>
<tr>
<td>p-value</td>
</tr>
<tr>
<td>N</td>
</tr>
<tr>
<td><strong>Apelin/APJ (Adipose tissue):</strong></td>
</tr>
<tr>
<td>r</td>
</tr>
<tr>
<td>p-value</td>
</tr>
<tr>
<td>N</td>
</tr>
<tr>
<td><strong>Apelin/APJ (Skeletal muscle):</strong></td>
</tr>
<tr>
<td>r</td>
</tr>
<tr>
<td>p-value</td>
</tr>
<tr>
<td>N</td>
</tr>
</tbody>
</table>

****: Correlation is significant at the 0.001 level (2-tailed).

**Discussion**

Many epidemiological studies have shown the preventive effects of regular exercise training on obesity and related diseases [26,27]; however, there is a lot of controversy about the underlying mechanisms. There has been great interest in the role of proinflammatory adipokines in the pathophysiological changes of obesity and on the impact of exercise on them. In our study, we investigated the adipokine response to regular swimming exercise in obese and non-obese rats.

As expected, induction of obesity in our sedentary experimental rats by HFD for 12 weeks was accompanied by impairment of lipid profile as reflected by the significant rise in TG, cholesterol and decrease in HDL ($p<0.05$). In addition, there was a significant increase in of glucose and insulin serum levels with rise in insulin resistance ($p<0.5$), which indicates the induction of insulin resistance. This whole picture of "diseasome of obesity and physical inactivity" was significantly reversed when the obese rats were subjected to regular swimming exercise for 4 weeks.

Our results also showed a significant rise in circulating levels of inflammatory markers, TNF α and IL-6 ($p<0.5$) in obese non exercising rats.

In accordance with our results, Ouchi N. et al., [28] demonstrated that obese AT mainly releases proinflammatory cytokines among which are TNF-α, IL-6, leptin, visfatin, resistin, angiotensin II, and plasminogen activator inhibitor 1.

Over the last decade, evidence accumulated that, obesity has been closely associated with low-grade chronic inflammatory response, termed "metabolic inflammation" [29,30]. Indeed, an increased number of infiltrating macrophages and dysregulated expression of some inflammation-related adipokines, such as TNF-α and IL-6, have been observed in the VAT of obese mice [31,32].

The proinflammatory adipokines modulate insulin resistance either directly by affecting the insulin signaling pathway or indirectly via stimulation of inflammatory pathways. Indeed, serine phosphorylation of Insulin Receptor Substrate (IRS) by various adipokines directly or via inflammatory pathways including the c-Jun N-terminal Kinase (JNK) pathway and I-kappa B kinase β (IKKβ)/NFκB pathway disrupts the insulin signaling pathways, possibly giving rise to insulin resistance [33]. Indeed, our results showed a positive correlation between TNF-α and insulin resistance in the obese non-exercising and exercising group of rats.
Regular exercise appears to suppress systemic low-grade inflammation [28]. However, the mediators of this effect are unresolved. A number of mechanisms have been proposed to explain the anti-inflammatory effects of exercise. Exercise increases the release of epinephrine, cortisol, growth hormone and other factors that have immunomodulatory effects [34] or decreases the expression of inflammation-related adipokines by reducing oxidative stress in VAT [35].

Indeed, swimming exercise in our study significantly improved lipid and glycemic profile and insulin sensitivity represented by HOMA-IR. Concurrently, there was a significant drop in circulating levels of TNF-α, thus confirming the anti-inflammatory effect of exercise mentioned earlier.

On the other hand, our results showed a considerably exercise-induced upregulation of plasma levels of IL-6 by 303.53% in non-obese and 873.9% in obese rats.

In agreement with our results, Bonyadi et al., (2009) [36] found that regular swimming for 8 weeks increased plasma levels of IL-6 by 9-times in healthy rats and 23-times in diabetic ones. Pedersen et al., (2001) [37] found that the IL-6 level is increased 100 fold in marathon athletes, and is related to exercise duration. The latter observation may be related to differences in type, intensity, or duration of exercise.

Identification of IL-6 production by skeletal muscle during physical activity generated renewed interest in the metabolic role of IL-6 because it created a paradox. On one hand, IL-6 is markedly produced and released in the post exercise period when insulin action is enhanced but, on the other hand, IL-6 has been associated with obesity and reduced insulin action [38].

It is well accepted that contracting skeletal muscle secretes enhanced levels of myokines which have a beneficial endocrine effect on other organs, presenting novel targets for the treatment of metabolic diseases and type 2 diabetes [39]. Interestingly, many of the contraction-regulated myokines are additionally known to be secreted by adipocytes and thus termed adipomyokines [35]. Within our work, we tried to elaborate on the question why pro-inflammatory adipokines on the one hand are upregulated in the obese state, and have beneficial effects after exercise on the other hand.

Recent work has shown that both upstream and downstream signalling pathways for IL-6 differ markedly between myocytes and macrophages. It appears that unlike IL-6 signalling in macrophages, which is dependent upon activation of the NFκB signalling pathway, intramuscular IL-6 expression is regulated by a network of signalling cascades, including the Ca2+/NFAT and glycogen/p38 MAPK pathways. Thus, when IL-6 is signalling in monocytes or macrophages, it creates a pro-inflammatory response, whereas IL-6 activation and signalling in muscle creates an anti-inflammatory response that is totally independent of a preceding TNF-response or NFκB activation [40].

Just recently, Apelin emerged as a novel adipomyokine being secreted from AT and muscle cells and has the potential to act on both tissues [36,37]. However, there are controversies in the literature regarding the co-expression of apelin in different tissues with altered glucose metabolism. Moreover, the regulation of APJ receptor gene expression in skeletal muscle has not yet been studied.

As shown in our study, apelin/APJ receptor expression profile was different in VAT and skeletal muscle after the onset of obesity and insulin resistance in rats.

Indeed, data from our study showed that induction of obesity by HFD resulted in upregulation of Apelin/APJ expression in VAT (912%) but not in muscle tissue compared to chow-fed control rats. More importantly, apelin receptor expression in adipose tissue positively correlated with body weight, TG and HOMA-IR.

Thus, our study supports previous findings that higher apelin serum concentrations in humans are associated with higher BMI as well as traits of the metabolic syndrome including high serum glucose, TG, and insulin resistance [41,42].

In agreement to our results, Dray et al., (2010) observed in obese and insulin-resistant mice, an increase in apelin and APJ expression in AT but not in skeletal muscles [43]. This suggests that apelin and APJ expression are differently regulated according to the tissue and the severity of insulin resistance. This means that, in a less deleterious state of insulin resistance (observed mainly in HF-fed mice), apelin expression can be upregulated specifically in AT. These data suggest that insulin sensitivity of AT is preserved or higher when compared with skeletal muscle in the model of HF-induced insulin resistance. This is supported by a recent study showing that, after 14wk of HFD in mice, AT was more sensitive in response to insulin regarding protein kinase B phosphorylation compared with liver or muscle [44].
Our results disagree with the widely accepted paradigm that “unbeneficial” adipokines are upregulated, whereas “beneficial” ones are downregulated in obese persons. The present work focuses on the recently described adipokine, apelin, which is unique in that its production is upregulated in some models of obesity and it exerts predominantly “beneficial” effects on insulin sensitivity and cardiovascular physiology.

Accumulating evidence have shown that Apelin production in AT is strongly upregulated by insulin, and that plasma concentrations are increased in obese and hyperinsulinemic mice and humans [48]. Most of the studies have linked apelin to insulin resistance and considered this adipokine as an important factor for the maintenance of insulin sensitivity and the stimulation of glucose utilization by the peripheral tissues [46-48]. The decrease in apelin levels after weight loss [49] supports the hypothesis of the interaction between apelin and insulin resistance. The increased levels of apelin observed in obesity could be a mechanism to counteract insulin resistance. Another study in mice revealed the existence of the APJ receptor in the pancreatic islets and concluded that apelin inhibits the glucose-stimulated insulin secretion [50]. Thus, one could speculate that “hyper-apelinemia” is a sort of negative feedback mechanism which combats hyperinsulinemia in obesity. It remains to be established if obesity is associated with apelin resistance analogous to insulin resistance and leptin resistance.

A potential link between VAT inflammation and elevated apelin secretion is further supported by the finding that TNF-α correlates with circulating apelin [51].

Indeed, our current study showed a positive correlaton between VAT apelin/APJ expression and TNF-α plasma level in HFD-induced obesity and insulin resistance.

It is tempting to speculate that TNF-α mediates the increase in apelin expression in AT during the development of obesity and insulin resistance. As a means with which to signal skeletal muscle to increase oxidative capacity [52].

It is well accepted that physical activity exerts multiple beneficial effects on the prevention of chronic diseases, both due to an improved energy balance and due to effects independent of obesity. It is assumed that contraction-regulated adipomyokines play a pivotal role in the communication between muscle and AT [38,39,53].

We propose a different influence of apelin depending on the target tissue. In energy-supplying tissues like the AT, the insulin signal is attenuated, whereas in energy-utilizing tissues like the skeletal muscle, insulin action is improved.

Within this context, the purpose of the present study was to investigate the impact of muscle exercise on apelin/APJ expression both in AT and in skeletal muscle in both control and obese rats.

Our results showed that swimming exercise resulted in upregulation of apelin/APJ expression in skeletal muscle both in non-obese and obese rats and the effect was more pronounced in the obese group (+142% vs +325%). On the contrary, swimming exercise resulted in down regulation of Apelin/APJ expression in VAT (−95%).

These results thus provide evidence that apelin/APJ mRNA expression exhibit tissue-specific regulation and are differentially regulated between exercising and non-exercising obese and non-obese rats.

In accordance with our results, Besse Patin showed a twofold increase in apelin mRNA level was found in muscle but not in AT in obese subjects. Interestingly, apelin was significantly expressed and secreted in primary human myotubes. Apelin gene expression was upregulated by cyclic AMP and calcium, unlike the other myokines investigated. Importantly, changes in muscle apelin mRNA levels were positively related to whole-body insulin sensitivity improvement [54].

These results were also supported by our results which showed a positive correlation between skeletal muscle apelin/APJ expression and improvement of insulin resistance.

Similarly, a positive correlation was observed in our study between skeletal muscle Apelin/APJ expression and serum levels of IL-6.

Accumulating evidence suggest that apelin is involved in the regulation of skeletal muscle metabolism. For instance, acute apelin treatment has been shown to increase rates of skeletal muscle glucose disposal [47], while long-term treatment has been reported to lead to reductions in weight gain and fat pad mass while increasing the expression of uncoupling protein 3 (UCP3) in murine skeletal muscle [55]. Moreover, apelin has been shown to increase the activity of 5′-AMP-activated protein kinase (AMPK) [56], a reputed mediator of mitochondrial biogenesis [55,56]. Collectively these results suggest that apelin may be involved in the regulation of skeletal muscle mitochondrial content.
In conclusion:

It is worth noting that the high apelin expression in skeletal muscle in exercising obese, insulin-resistant rats, is associated with increases in skeletal muscle mitochondrial content and oxidative capacity [55,56] which could help mitigate the degree of obesity-induced lipotoxicity [60]. This supports the hypothesis that an exercise-induced increase in skeletal muscle apelin/APJ expression might contribute to the beneficial effects of exercise training on obesity and related risk factors.

In conclusion:

The upregulation of Apelin/APJ expression in VAT in obesity and in muscle tissue with muscle exercise, support the hypothesis that apelin may act as a feedback control to limit obesity-associated disorders and may also contribute to beneficial effects of exercise.

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الملخص العربي

المخلاصة: إن الآلية هو أديبوكي فيكون حديثاً وجد أن له علاقة بالإعراض المصاحبة للبدانة وخلفيات النسب للجلوكوز بالبدن، وحتى الان استجابة لتمارين الرياضة غير معرفة. يهدف هذا العمل إلى دراسة تأثير ممارسة التمارين الرياضية بانتظام مثل السباحة على التعبير الجيني لل أليلين/أبيج في الأنسجة الشحميةحوشية والعضلات في الفئران المصابة بالبدانة، عن طريق نظام غذائي يحتوي على دهون عالية الكثافة.

المؤلفون: تم استخدام 60 ذكر الفئران البيضاء (160-180 جم) وتم تقسيمهم إلى المجموعات التالية تحتوي كل منها على 10 الفئران.


وتم تقسيم ووزن الجسم، أسباب التحكم بالسكين، ونسب الدهون، نسبة IL-6 وTGF-α في الدم وتم قياسهم في بداية العمل وبدأت العملية بعد 4 أسابيع

النتائج: أظهرت الفئران السنية بسبب نظام غذائي يحتوي على دهون عالية الكثافة خلق في مستوى الدهون في الدم مع ارتفاع في مقاومة الأنسولين، مع ارتفاع ملحوظ في مؤشرات الالتهاب، IL-6 و TGF-α. أما بعد ممارسة الفئران الرياضية فقد لوحظ تحسن صورة الدهون، زيادة الحساسية للأنسولين وانخفاض وزن الجسم.

أما بالنسبة للتغيير الجيني في مستقبلات أليلين/أبيج في فئران التحكم والفئران البدينة فقد أظهر زيادة التغيير الجيني في الأنسجة الشحمية الحيوية ولكن ليس في العضلات في الفئران البدينة، ولكن الفئة النتائج بعد نهاية تمارين السباحة فقد زاد التغيير الجيني لل أليلين/أبيج في العضلات وليس في الأنسجة الشحمية الحيوية في فئران التحكم، بالإضافة إلى ذلك تمارين السباحة بانتظام قد قامت بتفتيض التغيير الجيني لمستقبلات أليلين/أبيج في الأنسجة الشحمية الحيوية وزيادتها في العضلات.

والخلاصة: نتائجنا تشير إلى أن ارتفاع التغيير الجيني لمستقبلات أليلين/أبيج في أنسجة العضلات وتفتيضها في الأنسجة الشحمية الحيوية قد تكون واحدة من الآليات المفصلة لدراسة رياضة السباحة للكفاءة البدنية والإمكانيات ذات الصلة بها.