Correlated Increase of Omentin-1 and Adiponectin by Exenatide, Avandamet and Dietary Change in Diet-Induced Obese Rats

(exenatide / avandamet / dietary change / obesity / adipokines)

W.-H. FENG1, X.-W. YUAN2, G.-Y. TONG1, W.-M. WANG1, Y. HU1, S.-M. SHEN1, P. LI1, Y. BI1, J. HU1, L.-L. SHAO3, Y.-Y. DAI4, Y.-Q. LIU4, S.-K. XIANG5, D.-H. YANG1, D.-L. ZHU1

1Department of Endocrinology, Drum Tower Hospital Affiliated to Nanjing Medical University, Nanjing, China
2Department of Endocrinology, Nanjing Children’s Hospital Affiliated to Nanjing Medical University, Nanjing, China
3Nanjing University of Chinese Medicine, Nanjing, China
4Medical School of Southeast University, Nanjing, China
5Department of Endocrinology, Changzhou First People’s Hospital, Nanjing, China

Abstract. Adipokines omentin-1 and adiponectin have been reported to improve insulin resistance. It is known that insulin sensitizers exenatide, avandamet, or diet change from high-fat to normal chow ameliorate metabolic disorders. However, whether these treatments increase omentin-1 levels in high fat-diet animals and the relationship between omentin-1 and adiponectin remain largely unknown. We investigated the effect of insulin sensitizers exenatide and avandamet, and of dietary change on these adipokine levels, body weight, and insulin sensitivity in diet-induced obese rats. Obesity was induced in rats by high-fat diet feeding for 8 weeks, and then the rats were given exenatide, avandamet and diet change to normal chow, respectively, for additional 8 weeks. Compared to the high-fat control group, exenatide and avandamet treatment significantly induced adipose gene expression and elevated the circulation levels of omentin-1 and adiponectin, whereas they decreased the leptin gene expression and circulation level, which is associated with improvement of systemic insulin sensitivity and the glucose and lipid profile. Notably, there was a significant positive correlation between omentin-1 and adiponectin in the above regimens, suggesting that omentin-1 and adiponectin may contribute to the insulin-sensitizing effect of exenatide and avandamet.

Introduction

Adipose tissue produces a number of adipokines, which play an important role in modulating energy homeostasis and insulin sensitivity. Adipokines are known to be dysregulated in and contribute to the development of obesity and its related diseases (Weisberg et al., 2003; Kadowaki et al., 2006; Yang et al., 2006; Xu et al., 2011). Omentin-1, a recently identified adipocytokine, is expressed in stromal vascular cells of visceral adipose tissue, small intestine, and endothelial cells and has insulin-sensitizing effects (Yang et al., 2006). Plasma omentin-1 is down-regulated in the conditions of obesity, type 2 diabetes mellitus (T2DM), polycystic ovary syndrome (PCOS), dyslipidaemia and hypertension (de Souza Batista et al., 2007; Tan et al., 2008; Yan et al., 2011a; Shibata et al., 2012) and increased after weight loss in obese people or aerobic training in overweight and obese men (Moreno-Navarrete et al., 2010; Saremi et al., 2010).

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Adiponectin is a known insulin-sensitizing adipokine with anti-inflammatory and antiatherogenic properties (Kadowaki et al., 2006). Low adiponectin levels contribute to insulin resistance in obesity, T2DM and PCOS (Kadowaki et al., 2006; Li et al., 2008; Singh et al., 2012), while high adiponectin levels can increase insulin sensitivity (Li et al., 2008; Singh et al., 2012). Positive correlation of omentin-1 with adiponectin levels has been reported in patients with obesity and T2DM (de Souza Batista et al., 2007; Yan et al., 2011a). On the other hand, high leptin and leptin resistance promote the development and progression of insulin resistance and related diseases (Xu et al., 2011).

For treatment of obesity and associated insulin resistance, glucagon-like peptide 1 (GLP-1) analogue, metformin, thiazolidinediones and high-fat to low-fat dietary change are commonly employed in clinic. The effect of these regimens on selective adipokines has been studied separately. For example, exenatide (synthetic exendin-4) and liraglutide, analogues of GLP-1, can increase adiponectin and omentin-1 levels and decrease the leptin level in T2DM patients, which is associated with improved insulin resistance (Li et al., 2008; Verzegnassi and Chinello, 2010; Sathyanarayana et al., 2011; Yan et al., 2011b). Rosiglitazone, a thiazolidinedione acting on the nuclear transcription factor peroxisome proliferator-activated receptor γ (PPAR-γ), and metformin, which are known to relieve insulin resistance in T2DM patients, can increase adiponectin and decrease leptin levels (Gupta et al., 2008; Kadoglou et al., 2011; Cicero et al., 2012). Recently, metformin has been reported to raise omentin-1 levels in PCOS women (Tan et al., 2010). Changing from high-fat diet to normal diet has also been found to increase insulin sensitivity (Silva et al., 2011). Apparently, the above-mentioned drug treatment and dietary change can increase insulin sensitivity and, at the same time, increase adiponectin levels, suggesting that adiponectin may be a mediator of the insulin sensitivity. However, our knowledge on whether and how omentin-1, another insulin-sensitizing adipokine, is regulated under these insulin-sensitizing regimens is relatively poor, which impairs our understanding of its pathophysiological role in obesity and diabetes.

This study mainly aims to investigate the effect of exenatide, avandamet (a mixture compound of rosiglitazone and metformin) and high-fat to low-fat dietary change (referred to as dietary change hereafter) on omentin-1 and its relationship with adiponectin in diet-induced obese rats.

Material and Methods

Animal studies

Male Sprague-Dawley (SD) rats, aged 4–5 weeks, were purchased from Shanghai SLAC Laboratory Animal Centre (Shanghai, China). All experimental animals were maintained in a 12:12 h light:dark cycle with the light on at 06:00 h and off at 18:00 h, with temperature control at 25 °C and allowed ad libitum access to chow and water. All animal care and use procedures were in accordance with the guidelines of the Institutional Animal Care and Use Committee at Drum Tower Clinical Medical College of Nanjing University.

The animals were acclimatized for one week and then randomly divided into two groups: the regular chow control group and the high-fat diet (HFD) group. Rats in the control group were maintained on a standard chow diet (calorie-based components: 65 % carbohydrates, 22 % proteins, and 13 % lipids) for 16 weeks. Obesity was induced by feeding rats with a high-fat diet (HFD, 33 % carbohydrates, 12 % proteins, and 55 % lipids) for eight weeks and then the HFD animals were randomly divided into the exenatide group, where the animals were given both HFD and exenatide (10 μg/kg twice a day via subcutaneous injection), the avandamet group, where both HFD and avandamet (metformin 500 mg/kg and rosiglitazone 2 mg/kg once a day via gavage) were given, the dietary change group (change of diet from high-fat to low-fat), and the HFD group, where only HFD was given for additional eight weeks. Animal body weight was measured weekly.

Biochemical analyses

At the end of a 16-week study, oral glucose tolerance test (OGTT) was conducted by administering an oral dose of glucose (2 g/kg body weight) and tail vein glucose levels were measured. Overnight fasting blood samples were obtained for serum chemistry measurements of glucose, total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by using a semi-automatic biochemical analyser (Olympus Corporation, Tokyo, Japan) at the Central Laboratory of Tower Drum Hospital. Serum-free fatty acid (FFA) concentrations, fasting insulin (FINS), adiponectin and leptin levels were measured by using respective ELISA kits (Sirocoo Biotechnology, Shanghai, China) and serum omentin-1 was measured by ELISA kit (Beijing Adiboshengwu Inc, China). Homeostasis model assessment (HOMA) was calculated as FBG (fasting blood glucose) × FINS / 22.5 (Bonaora et al., 2000).

Quantitative PCR analysis (qPCR)

Total RNAs from liver samples and different adipose depots (small intestine linked to stomach, inguinal subcutis, and paraepididymis) were extracted using TRizol (Invitrogen, San Diego, CA) and reverse transcription (RT) was performed using the SYBR® PrimeScript® RT-PCR Kit (Takara, Dalian, China) according to the manufacturer’s protocol. Quantitative real-time PCR reaction was conducted in an ABI 7500 real-time PCR system. The primers used are shown in Table 1. Relative expression to β-actin was calculated by the comparative CT method. The experiments were repeated three different times.
Statistical analysis

All statistical analyses were performed using SPSS software (version 16.0) and data were expressed as the mean ± SE. One-way ANOVA with the least significant difference was used to compare the group means. A P value of less than 0.05 was considered statistically significant. The relationship between different serum adiponectin and the same adiponectin between different depots of adipose tissues was evaluated by linear regression and Pearson’s correlation analyses.

Results

Effect of exenatide, avandamet and diet change from high-fat to normal chow on diet-induced obesity and blood biochemical abnormalities

As expected, rats fed with HFD gained more body weight than those fed with regular chow. At the end of eight weeks of feeding, the body weight of the HFD group was 376.1 ± 34.3 g (mean ± SD), more than that of 346.6 ± 34.2 g of the regular chow group (P < 0.05). The HFD group animals were then randomly divided into the exenatide, avandamet, diet change from high-fat to normal chow, and HFD group from the ninth week thereafter. As shown in Fig. 1, the exenatide group stopped gaining weight. In contrast, all other groups continued to gain weight. At the end of eight weeks of exenatide administration, the body weight of the exenatide group was 367.9 ± 41.0 g, compared to 454.0 ± 36.9 g of HFD, 419.9 ± 41.4 g of avandamet treatment, 453.0 ± 32.6 g of dietary change, and 403.2 ± 23.0 g of the regular diet controls, respectively (P < 0.05 for exenatide vs. other groups).

Blood biochemistry analyses showed that the serum TG and FAA levels were significantly elevated in HDF rats (0.71 ± 0.08 mmol/l, 71.91 ± 29.80 ng/ml, respectively), compared to the control group (0.18 ± 0.05 mmol/l, 22.45 ± 2.15 ng/ml) (P < 0.01 for TG and FAA), exenatide and avandamet treatment lowered these levels (0.45 ± 0.22 mmol/l and 23.79 ± 8.20 ng/ml) respectively (P < 0.01 for all comparisons of exenatide or avandamet vs. HDF, Table 2). In addition, the diet change from high-fat to normal chow also resulted in lower TG levels (0.51 ± 0.14 mmol/l), compared to the HFD group (0.71 ± 0.08 mmol/l, P < 0.05). FAA levels treated by exenatide and avandamet, and TG levels treated by avandamet were significantly lower than those of the diet change group. Interestingly, exenatide or avandamet treatment reduced total cholesterol by 22.16 % (P < 0.01) or 20.45% (P < 0.05), compared to the HFD group (Table 2).

Table 1. List of primer sequences for real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward/Primer (5’ to 3’)</th>
<th>Reverse</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Actin</td>
<td>F: 5’-CACCCGCGAGTACAACTTC-3’</td>
<td>R: 5’-CCCATCCACCACCATCAC-3’</td>
</tr>
<tr>
<td>Omentin-1</td>
<td>F: TTGTGTGCTGGCATGAAGGTC</td>
<td>R: GTGAGTTCGATCGTCCAAATC</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>F: CCCCTCCACCCAAGGAACTT</td>
<td>R: GGTATCCCATTGACCCAGGA</td>
</tr>
<tr>
<td>Leptin</td>
<td>F: ATGACACAAACCCTCATCAAG</td>
<td>R: TGAAGTCCAAACCGTACC</td>
</tr>
</tbody>
</table>

Fig. 1. Exenatide suppresses diet-induced obesity. Mean body weight of rats fed with HFD (○; N = 31) or normal chow (△; N = 8) for 8 weeks. The HFD group was then divided into the HFD group (○; N = 7), exenatide group (□; N = 8), avandamet group (◇; N = 8) and diet change group (▽; N = 8) (* P < 0.05 vs. control group; # P < 0.05, ## P < 0.01 vs. HFD group; ♦ P < 0.05, ♦♦ P < 0.01 vs. exenatide group).
Improvement of insulin sensitivity by exenatide, avandamet and dietary change

In HFD rats, the insulin resistance index HOMA-IR was more than one-fold higher than that in control rats; exenatide and avandamet treatment decreased the index by 38 % (P < 0.05) and 34 %, respectively, and the increased HOMA-IR was also somewhat decreased by the diet change from high-fat to normal chow, but still significantly higher compared to the control group (P < 0.05) (Table 2). Furthermore, OGTT showed that the blood glucose levels were lowered by exenatide or avandamet treatment when compared with the high-fat and control groups, but no hypoglycaemia occurred, and the diet change also improved blood glucose levels compared to the high-fat group (Fig 2).

Correlated increase of circulating omentin-1 and adiponectin by exenatide, avandamet and diet change

Adipokines omentin-1, adiponectin and leptin are known to regulate insulin sensitivity. We next examined the serum levels of adiponectin, omentin-1 and leptin by exenatide, avandamet or diet change from high-fat to normal chow. In circulation, omentin-1 and adiponectin levels were reduced, whereas leptin was elevated in HFD rats (Fig. 3a) (P < 0.01–0.05 vs. control). Remarkably, exenatide and avandamet treatment increased the serum omentin-1 levels by 159 % and 187 %, respectively (P < 0.01 vs. HFD), and adiponectin levels by 80 % (P < 0.01 vs. HFD) and 67 % (P < 0.05 vs. HFD), respectively, to near-normal levels (Fig. 3a).

Table 2. Effects of exenatide, avandamet and diet change from high-fat to normal chow on blood biochemistry

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group (N = 8)</th>
<th>HFD group (N = 7)</th>
<th>Exenatide group (N = 8)</th>
<th>Avandamet group (N = 8)</th>
<th>Diet change group (N = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFA (ng/ml)</td>
<td>22.45 ± 2.15</td>
<td>71.91 ± 29.80**</td>
<td>23.79 ± 2.80***</td>
<td>21.81 ± 8.12****</td>
<td>61.63 ± 23.13**</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>0.18 ± 0.05</td>
<td>0.71 ± 0.08**</td>
<td>0.45 ± 0.22**</td>
<td>0.32 ± 0.15***</td>
<td>0.51 ± 0.14**</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>1.54 ± 0.16</td>
<td>1.76 ± 0.37</td>
<td>1.37 ± 0.16**</td>
<td>1.40 ± 0.40</td>
<td>1.59 ± 0.16</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.16 ± 0.11</td>
<td>1.03 ± 0.31</td>
<td>1.19 ± 0.35</td>
<td>1.15 ± 0.26</td>
<td>1.20 ± 0.17</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>0.20 ± 0.03</td>
<td>0.24 ± 0.04</td>
<td>0.16 ± 0.08*</td>
<td>0.18 ± 0.07</td>
<td>0.17 ± 0.06</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>12.20 ± 1.56</td>
<td>26.48 ± 8.64***</td>
<td>16.85 ± 7.68*</td>
<td>18.28 ± 10.64</td>
<td>22.21 ± 8.41</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.85 ± 0.77</td>
<td>6.55 ± 2.49**</td>
<td>4.06 ± 2.16*</td>
<td>4.32 ± 2.80</td>
<td>5.44 ± 2.17*</td>
</tr>
</tbody>
</table>

Control group: the regular chow control group; HFD group: the high-fat diet group; Exenatide group: rats were given both HFD and exenatide; Avandamet group: rats were given both HFD and avandamet; Diet change group: rats returned to regular chow after HFD.

* P < 0.05, ** P < 0.01 vs. control group; * P < 0.05, ** P < 0.01 vs. HFD group. ● P < 0.05, ●● P < 0.01 vs. diet change group.

Fig. 2. OGTT shows that exenatide, avandamet or diet change from high-fat to normal chow improved glucose disposal in different degrees. * P < 0.05 and * * P < 0.01 vs. control group; # P < 0.05 and # # P < 0.05 vs. HFD group; ● P < 0.05 and ●● P < 0.01 vs. exenatide group.
change also slightly elevated serum omentin-1 and adiponectin levels, but serum omentin-1 levels increased by the diet change were still lower than those elevated by the avandamet treatment and serum adiponectin levels were lower than those increased by exenatide treatment (Fig. 3a). Circulating leptin levels were decreased by 32% and 48% in exenatide- and avandamet-treated rats, respectively, vs. the HFD group, but remained higher in the diet change group (Fig. 3a). Intriguingly, serum omentin-1 was positively associated with the serum adiponectin correlation index, $R = 0.45$, $P = 0.004$ (Fig. 3b). Both serum omentin-1 and adiponectin levels appeared negatively associated with serum leptin, but without statistical significance (Fig. 3c-d).

**Induction of omentin-1 and adiponectin gene expression by exenatide, avandamet, or dietary change**

We next examined the effect of exenatide, avandamet or the diet change on the gene expression of these adipokines. HFD feeding decreased omentin-1 and adiponectin gene expression in mesenteric and visceral fat and adiponectin gene expression in subcutaneous fat (P < 0.01–0.05 vs. control) (Fig. 4a-b), but increased the leptin gene expression in mesenteric and subcutaneous fat tissues (P < 0.01 vs. control) (Fig. 4c). Remarkably, exenatide treatment increased omentin-1 and adiponectin gene expression and decreased the leptin gene expression in all fat depots (P < 0.01–0.05 vs. the HFD group) (Fig. 4a-c). There was no elevated omentin-1 tissue gene expression in the avandamet or diet change-treated group (Fig. 4a). Induced gene expression of adiponectin and decreased gene expression of leptin were found in mesenteric and subcutaneous fat in avandamet-treated rats (P < 0.01 vs. the HFD group) (Fig. 4b-c). The diet change from high-fat to normal chow led to an increase in gene expression of adiponectin in visceral and subcutaneous fat depot, but a decrease of leptin gene expression in mesenteric and subcutaneous fat de- pots (P < 0.01-0.05 vs. the HFD group) (Fig. 4b-c). Significantly, in all the five groups of rats, gene expression of omentin-1 (R = 0.42, P < 0.01) and adiponectin (R = 0.39, P < 0.05) in mesenteric fat was positively associated with that in visceral or subcutaneous fat (Fig. 5a-b). There was also significant positive association between mesenteric and subcutaneous fat leptin gene expression (R = 0.62, P < 0.01) (Fig. 5c).

**Discussion**

In this study, we demonstrated that exenatide and avandamet increased serum omentin-1 and adiponectin levels and decreased the leptin levels in diet-induced obese rats, which is associated with an improvement of biochemical abnormalities and insulin sensitivity. We also found that circulating omentin-1 and adiponectin were positively associated.
**Fig. 4.** Effect of exenatide, avandamet or diet change from high-fat to normal chow on gene expression of omentin-1 and adiponectin. Quantitative real-time PCR analysis of gene expression of omentin-1 (a), adiponectin (b) and leptin (c) in mesenteric, visceral and subcutaneous adipose tissue in relation to the expression of β-actin in control, high-fat diet (HFD), exenatide-treated HFD (exenatide), avandamet-treated HFD (avandamet) and diet change to regular chow (diet change) rats. Data are expressed as mean ± SE. * P < 0.05 and ** P < 0.01 vs. control group; # P < 0.05 and ## P < 0.01 vs. HFD group; ♦ P < 0.05 and ♦♦ P < 0.05 vs. exenatide group.

**Fig. 5.** Correlations of gene expression of mesenteric and visceral fat omentin-1 (a), mesenteric and subcutaneous fat adiponectin (b), mesenteric and subcutaneous fat leptin (c).
Omentin-1, mainly synthesized by visceral adipose tissue, promotes insulin signal transduction by activating Akt signalling and stimulating insulin-stimulated glucose transport in isolated human adipocytes (Yang et al., 2006). Like adiponectin, lower omentin-1 levels were associated with insulin resistance-related diseases such as obesity, T2DM and PCOS, and omentin-1, therefore, is considered as an insulin sensitizer and as acting protectively against cardiovascular diseases (de Souza Batista et al., 2007; Tan et al., 2008; Yan et al., 2011a; Shibata et al., 2012).

Exenatide, as a GLP-1 analogue for T2DM treatment, has been reported to improve insulin resistance, inhibit food intake and reduce body weight (Li et al., 2008; Verzegnassi and Chinello, 2010; Sathyanarayana et al., 2011). Boc5, a substituted cyclobutane, as a non-peptide GLP-1 receptor agonist or other GLP-1 analogues increase adiponectin and decrease leptin levels in high-fat-fed animals and T2DM patients, and adiponectin secretion from 3T3-L1 adipocytes is enhanced by GLP-1-stimulated macrophages (Li et al., 2008; Cummings et al., 2010; He et al., 2010; Verzegnassi and Chinello, 2010; Sathyanarayana et al., 2011; Shiraishi et al., 2012). Circulating omentin-1 levels were decreased in T2DM patients, but were increased significantly by treatment with another GLP-1 analogue liraglutide (Yan et al., 2011b). Here, we have shown that exenatide significantly suppressed weight gain as well as food intake (not quantified) in HFD rats, and importantly, exenatide elevated adiponectin and omentin-1 adipose expression and circulating levels. Thus, the increased omentin-1 levels by exenatide in the present study may contribute to the exenatide’s insulin-sensitizing effect.

Avandamet consists of a combination of metformin belonging to biguanides and rosiglitazone belonging to thiazolidinediones, two commonly used glucose-lowering agents, and also exerts a number of pleiotropic effects in T2DM, such as reducing insulin resistance, displaying anti-inflammation and anti-atherogenesis effects (Gupta et al., 2008; Barnett, 2009; Tan et al., 2010; Kadoglu et al., 2011; Cicero et al., 2012). Metformin has been found to elevate omentin-1 levels in PCOS women (Tan et al., 2010), but there are no data about rosiglitazone effects on omentin-1. In the present study, we found that avandamet improved impaired glucose tolerance without effect on body weight. Significantly, lowered circulating omentin-1 and adiponectin concentrations were largely restored by avandamet, along with improvement of HOMA-IR compared with HFD rats.

Lowering fat intake will help weight loss and ameliorate metabolic disorders (Nordmann et al., 2006). We did not observe any significant weight loss after the diet change from high-fat to normal chow, and further, omentin-1 and adiponectin levels remained lower and the leptin level remained higher, suggesting that the diet change only is not sufficient to correct the adverse metabolic profiles and that weight loss by strict diet control with exercise or pharmaceutical intervention might be required to inverse the metabolic disorders (Moreno-Navarrete et al., 2010; Saremi et al., 2010).

A significant and novel finding of the study is the correlated increase between omentin-1 and adiponectin in the circulation by exenatide, avandamet or diet change from high-fat to normal chow. Subsequent gene expression analyses indicated that adipose expression of both omentin-1 and adiponectin were induced by those treatments, suggesting a possible co-regulation at the gene expression level.

In summary, we have found that exenatide and avandamet increased adiponectin and omentin-1 and improved insulin sensitivity in HFD-fed rats, suggesting that these adipokines may partially mediate the drugs’ insulin-sensitizing action. The mechanism for the concerted induction of omentin-1 and adiponectin deserves further investigation.

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