Original Article

Effect of left gastric artery embolization on obesity and ghrelin/leptin levels in pigs

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Abstract: Objective: To investigate the influence of left gastric artery embolization (LGAE) on obesity and Ghrelin/Leptin levels in pigs. Methods: Healthy female Wuzhishan piglets aged 3-4 months were allocated into obesity + LGAE group, obesity group, control group (fed with a normal diet; n=8 for each group). Body weight, serum Ghrelin, Leptin, glucose and insulin levels were compared before surgery, 1 month and 3 months after surgery. Results: Piglets in obesity group and obesity + LGAE group were heavier than those in control group before and 1 month after surgery (P<0.001), and in obesity group were heavier than those in obesity + LGAE group 1 month after surgery (P<0.05). A significant decrease in body weight was observed in obesity + LGAE group 3 months after surgery (P<0.05), and the obesity group showed heavier weight than the other two groups (P<0.001). Ghrelin levels in obesity group and obesity + LGAE group were higher than those in control group before and 1 month after surgery (P<0.001), and in obesity group were higher than those in obesity + LGAE group 1 month after surgery (P<0.05). They showed an evident decrease in obesity + LGAE group 3 months after surgery (P<0.05), and in obesity group were higher than that in the other two groups (P<0.01). Leptin levels in obesity group and obesity + LGAE group were higher than those in control group before and 1 month after surgery (P<0.001), and in obesity group were higher than those in obesity + LGAE group 1 month after surgery (P<0.05). They decreased significantly in obesity + LGAE group 3 months after surgery (P<0.01), and in obesity group were higher than those in the other two groups (P<0.01). Conclusion: LGAE greatly contributes to weight loss possibly by reducing the secretion of Ghrelin and Leptin.

Keywords: Left gastric artery embolization, wuzhishan piglet, intervention, obesity, Ghrelin/Leptin

Introduction

The improvement of living standards has led to an upward trend in annual incidence of obesity throughout the world, and the number of obese patients in China has been increasing. The global incidence of obesity among female adolescents has increased from 0.7% in 1976 to 5.6% in 2016; therefore, considerable attention has been devoted to the treatment of obesity [1]. There is evidence that the high prevalence of obesity has also resulted in an increase in the incidence of type 2 diabetes, hypertension, hyperlipidemia and nephropathy in obese people [2-4]. For those patients, diet control and reasonable exercise are priorities because a healthy lifestyle is the prerequisite of weight loss [5, 6]. If the effect of diet control is not as expected, drug interventions such as targeting the central nerve, fat and digestive tract are needed [7-9]. Diet and exercise are able to reduce body weight by 5-10% in the short term, but adiposity rebound is likely to occur in the later period [10]. Moreover, drug-induced side effects may lead to unsatisfactory weight loss [11]. Thus, surgical treatment is adopted clinically to achieve weight loss. Owing to the advance of technology, effective bariatric surgeries such as gastric bypass bariatric surgery, gastric banding, and sleeve gastrectomy have emerged, especially for obese patients with diabetes, which can also reduce the risk of cardiovascular events [12]. Surgical treatment is traumatic after all, and there is a risk of complications [13]. It is reported that the principle of bariatric surgery is to accelerate energy consumption and metabolism that are closely associated with hormone secretion. Ghrelin, Leptin and glucagon-like peptide-1 are metabolism-related hormones, of which Ghrelin is the
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most relevant [14]. Ghrelin is primarily produced in the gastric fundus (four times as much as in other parts) to promote appetite, hunger and gastrointestinal motility [15]. The left gastric artery (LGA) is the main blood supply artery in the gastric fundus, and its embolization (LGAE) induces mucosal ischemia and reduces Ghrelin levels, thereby achieving the purpose of weight loss [16]. The production of Leptin, another hormone closely related to weight regulation, by peripheral mature adipocytes is able to inhibit hunger signals [17]. In addition, a study proposes that bariatric surgery may play a role by reducing Leptin levels [18].

Wuzhishan piglets are easy to breed and raise, and have high disease resistance and environmental adaptability; in addition, their gene sequences are more than 90% similar to those of humans. Therefore, highly homozygous Wuzhishan piglets are an option for artery modeling of a variety of human diseases [19]. Beyond that, Ghrelin levels secreted from the gastric fundus, as well as origin and branching pattern of gastric artery of those piglets are also highly similar to those of humans [20]. Therefore, Wuzhishan piglet models of obesity were induced to estimate the effect of LGAE on obesity and Ghrelin/Leptin levels.

Materials and methods

Establishment of piglet models of obesity

All operations in this experiment were approved by Animal Ethics Committee of our hospital. Healthy female Wuzhishan piglets aged 3-4 months were allocated into obesity + LGAE group, obesity group, control group (fed with a normal diet; n=8 for each group). Piglets in the first two groups were fed with a high-fat diet for 2 months. Normal diet: rice, bran, semolina, soybeans, fish meal, rape seed cake dregs, yeast, calcium and other trace elements. High-fat diet: meat, eggs, milk. Feed composition: crude protein, 27%; crude fat, 8%; crude fiber, 2.7%; calcium, 1.2%; phosphorus, 0.9%. High-fat feed formula was provided by Department of Animal Husbandry & Veterinary Medicine, Hainan Academy of Agricultural Sciences. The food was prepared by a specialized breeder and the piglets were fed twice a day (11:00 am and 18:00 pm), 500 g/time. They were allowed to drink water freely. Patients with a weight ≥30% of the standard weight were considered obese [19].

Surgery procedures

Femoral artery catheterization: The piglets were deprived of feed and water for 12 hours before surgery. Atropine was injected intramuscularly (0.03 mg/kg, CR Double-crane Pharma, China). Ten minutes later, Sumianxin (0.05 mL/kg, Department of Animal Husbandry & Veterinary Medicine of Hainan Academy of Agricultural Sciences, China) was injected intramuscularly, followed by intravenous injection of ketamine (7 mg/kg, Fujian Gutian Pharma, China) + diazepam (0.3 mg/kg, Harbin Pharmaceutical Group Sanjing Pharmaceutical Co., Ltd., China). After anesthesia, the piglets were fixed on the operating table in a supine position. The area from the right groin to the knee joint was shaved and locally disinfected prior to draping. A 2 cm transverse incision was made 4-5 cm above the knee joint. The fascia and muscle covering branches of the femoral artery were bluntly dissected, and the superficial branches were dissociated. Afterwards, the distal end of the isolated femoral artery was ligated. Next, the artery was cut and a 4F catheter sheath was inserted along the introduced guide wire. At last, the proximal end was ligated and the sheath was fixed.

Super-selective LGAE: First, the 4F catheter was inserted into abdominal artery for angiography, then a 3F microcatheter was inserted into the LGA for complete angiography. Then polyvinyl alcohol (PVA) granules of 300-500 µm (Hangzhou Alicon Pharma, China) were used for LGAE.

Criteria for LGAE: Complete occlusion of the LGA: See Figure 1.

Postoperative extubation: After the surgery, catheters were removed, then the proximal end of the artery was ligated and the incision was sutured layer by layer. Metoclopramide (Jiangsu Pengyao Pharma, China) and amoxicillin (Shengda Pharma, China) were taken orally once a day for 10 days. The stitches were removed 10 days after surgery.

Outcome measures

Main outcome measures: Body weights of fasted piglets were measured and venous blood (2 mL) was sampled from the forelimb superficial vein at 10:00 am before surgery, 1 month and
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3 months after surgery. And serum Ghrelin and Leptin were quantified by enzyme-linked immunosorbent assay (ELISA).

Secondary outcome measures: Venous blood (2 mL) was sampled from the forelimb superficial vein at 10:00 am before surgery, 1 month and 3 months after surgery. Glucose oxidase method and ELISA were used to measure glucose and insulin, respectively.

Statistical indicators

SPSS 17.0 was employed for statistical analysis. The continuous variables were expressed by mean ± standard deviation (\( \bar{x} \pm sd \)). Inter-group comparisons were analysed by independent samples t test, intra-group comparisons were conducted by paired samples t test. One-way ANOVA test was used for multi-group comparison, and Tukey’s HSD for pairwise comparison. The categorical variables were expressed in cases/percentage (n/%), and analysed with Pearson Chi-square test and Fisher exact probability test (denoted by Chi-square). A value of \( P<0.05 \) was considered statistically significant.

Results

Comparison of body weight

Piglets in obesity group and obesity + LGAE group were heavier than those in control group before and 1 month after surgery (\( P<0.001 \)), and in obesity group were heavier than those in obesity + LGAE group 1 month after surgery (\( P<0.05 \)). Three months after surgery, a significant decrease in body weight was observed in obesity + LGAE group (\( P<0.05 \)), and the obesity group showed heavier weight than the other groups (\( P<0.001 \)), whereas there was no significant difference between obesity + LGAE group and control group (\( P>0.05 \); Table 1 and Figure 2).

Comparison of serum ghrelin levels

Ghrelin levels in obesity group and obesity + LGAE group were higher than those in control group before and 1 month after surgery (\( P<0.001 \)), and in obesity group were higher than those in obesity + LGAE group 3 months after surgery (\( P<0.05 \)), and in obesity group were higher than those in obesity + LGAE group and control group (\( P<0.01 \)), but no differences between this two groups were found (\( P>0.05 \); Table 2).

Comparison of serum leptin levels

Leptin levels in obesity group and obesity + LGAE group were higher than those in control group before surgery (\( P<0.01 \)) and 1 month after surgery (\( P<0.05 \)). They decreased significantly in obesity + LGAE group 3 months after surgery (\( P<0.01 \)), and in obesity group were higher than those in obesity + LGAE group and control group (\( P<0.01 \)), but no differences between this two groups were found (\( P>0.05 \); Table 3).

Comparison of insulin levels

There was no difference in insulin levels among obesity group, obesity + LGAE group and control group before surgery, 1 month and 3 months after surgery (all \( P>0.05 \); Table 4).

Comparison of glucose levels

There was no difference in glucose levels among the three groups before surgery, 1 month and 3 months after surgery (all \( P>0.05 \); Table 5).

Discussion

The incidence of obesity is on the rise worldwide. Obesity usually leads to intestinal dysbacteriosis, inflammation, impairment of glucose...
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Table 1. Comparison of weight changes in three groups

<table>
<thead>
<tr>
<th>Body weight</th>
<th>Obesity group (n=8)</th>
<th>Obesity + LGAE group (n=8)</th>
<th>Control group (n=8)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before operation (kg)</td>
<td>9.79±0.63***</td>
<td>9.84±0.67***</td>
<td>7.53±0.41</td>
<td>41.212</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>1 month after operation (kg)</td>
<td>9.95±0.79***,#</td>
<td>9.05±0.62***</td>
<td>7.71±0.49</td>
<td>24.421</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3 months after operation (kg)</td>
<td>10.31±0.88***###</td>
<td>8.73±0.59@</td>
<td>8.12±0.57</td>
<td>21.181</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: ***P<0.001 vs. control group in the same period; #P<0.05, ###P<0.001 vs. obesity + LGAE group in the same period; @P<0.05 vs. same group before surgery.

Figure 2. Comparison of weight changes in three groups.

and lipid metabolism and other internal changes, thereby resulting in arteriosclerosis, abnormal blood glucose, and renal hemodynamic alterations [21-23].

Although effective in the treatment of obesity, dietary and drug interventions still have low efficacy in some patients. Surgical intervention has become a new means to facilitate weight loss, and LGAE is a minimally invasive one with less trauma [16]. As the therapeutic effect of LGAE has not been assessed, it has not been widely used in the clinic. In the present study, the body weight of piglets decreased significantly after LGAE, as well as Ghrelin and Leptin levels. A previous study indicated that there was no significant difference in serum Leptin levels between pigs treated by LGAE with sodium morrhuate (study group) and with normal saline (control group) before surgery (1006.3 pg/dL vs. 1078.0 pg/dL). After complete embolization of the LGA, the levels in the study group were lower than those in the control group (684.3 pg/dL vs. 1014.0 pg/dL), and greater weight loss was achieved 4 weeks after surgery (15.0% vs. 7.8%) [24]. Recanalization may occur after embolization with sodium morrhuate, so the effects of different embolic agents on body weight, Ghrelin and fat area after LGAE are still under investigation. In another study, ghrelin decreased by 15.8% and 30.2% after embolization with PVA and bleomycin-lipiodol emulsion (BLE), lower than that in the control group. In addition, the reduction of body weight and fat area was lower than that in control the group, and both PVA and BLE showed a high degree of safety [25]. LGAE helps control and stop bleeding under microscope in patients with gastric bleeding. It is reported that the body weight of patients treated with LGAE decreased by 7.2%, higher than those treated with abdominal artery embolization (2.0%) [26]. Moreover, after 6 months of follow-up of 5 obese patients undergoing LGAE, a foreign study found that the weight of patients decreased by 8.5%, Leptin decreased by 24.1%, however, Ghrelin increased by 5.3%, and the increase was considered to associate with the activation of negative feedback mechanism [27]. Limitations and prospects: The sample size of this study is small, so the sample will be expand to explore the therapeutic effect of LGAE in obesity. In addition, in the absence of clinical trials, the effectiveness and safety of LGAE for obese patients remain to be investigated.

To sum up, LGAE greatly contributes to weight loss in pigs, which may be related to the reduction of Ghrelin and Leptin secretion.

Acknowledgements

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Table 2. Comparison of serum Ghrelin levels in three groups

<table>
<thead>
<tr>
<th></th>
<th>Obesity group (n=8)</th>
<th>Obesity + LGAE group (n=8)</th>
<th>Control group (n=8)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ghrelin (pg/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before operation</td>
<td>6.23±2.89***</td>
<td>6.27±2.94***</td>
<td>2.43±1.36</td>
<td>6.195</td>
<td>0.008</td>
</tr>
<tr>
<td>1 month after operation</td>
<td>6.25±2.92***</td>
<td>4.03±1.69*</td>
<td>2.39±1.32</td>
<td>6.863</td>
<td>0.005</td>
</tr>
<tr>
<td>3 months after operation</td>
<td>6.35±2.78***,*#</td>
<td>2.57±1.42**##</td>
<td>2.42±1.33</td>
<td>10.340</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Note: **P<0.01 and ***P<0.001 vs. control group in the same period; *P<0.05, **P<0.01 vs. obesity + LGAE group in the same period; *P<0.05 vs. same group before surgery.

Table 3. Comparison of serum Leptin levels in three groups

<table>
<thead>
<tr>
<th></th>
<th>Obesity group (n=8)</th>
<th>Obesity + LGAE group (n=8)</th>
<th>Control group (n=8)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ug/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before operation</td>
<td>87.23±7.82**</td>
<td>88.11±6.74</td>
<td>80.22±7.11</td>
<td>2.695</td>
<td>0.095</td>
</tr>
<tr>
<td>1 month after operation</td>
<td>87.82±7.72**</td>
<td>84.34±6.92</td>
<td>79.63±7.24</td>
<td>2.487</td>
<td>0.107</td>
</tr>
<tr>
<td>3 months after operation</td>
<td>87.34±7.77**##</td>
<td>80.36±6.22</td>
<td>80.31±7.22</td>
<td>2.697</td>
<td>0.098</td>
</tr>
</tbody>
</table>

Note: **P<0.01 and ***P<0.001 vs. control group in the same period; **P<0.01 vs. obesity + LGAE group in the same period; ##P<0.01 vs. same group before surgery.

Table 4. Comparison of insulin levels in the three groups

<table>
<thead>
<tr>
<th></th>
<th>Obesity group (n=8)</th>
<th>Obesity + LGAE group (n=8)</th>
<th>Control group (n=8)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin (pmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before operation</td>
<td>87.23±7.82**</td>
<td>88.11±6.74</td>
<td>80.22±7.11</td>
<td>2.695</td>
<td>0.095</td>
</tr>
<tr>
<td>1 month after operation</td>
<td>87.82±7.72**</td>
<td>84.34±6.92</td>
<td>79.63±7.24</td>
<td>2.487</td>
<td>0.107</td>
</tr>
<tr>
<td>3 months after operation</td>
<td>87.34±7.77**##</td>
<td>80.36±6.22</td>
<td>80.31±7.22</td>
<td>2.697</td>
<td>0.098</td>
</tr>
</tbody>
</table>

Table 5. Comparison of the glucose level of the three groups of experimental pigs

<table>
<thead>
<tr>
<th></th>
<th>Obesity group (n=8)</th>
<th>Obesity + LGAE group (n=8)</th>
<th>Control group (n=8)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (pmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before operation</td>
<td>5.72±0.63</td>
<td>5.74±0.66</td>
<td>5.18±0.56</td>
<td>2.238</td>
<td>0.132</td>
</tr>
<tr>
<td>1 month after operation</td>
<td>5.71±0.65</td>
<td>5.41±0.59</td>
<td>5.21±0.54</td>
<td>1.431</td>
<td>0.261</td>
</tr>
<tr>
<td>3 months after operation</td>
<td>5.74±0.66</td>
<td>5.26±0.58</td>
<td>5.20±0.52</td>
<td>2.017</td>
<td>0.161</td>
</tr>
</tbody>
</table>

Disclosure of conflict of interest

None.

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