Laminaria japonica Polysaccharide Reduces Lipids and Leptin Levels in Hyperlipidemic Mice

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The aim of this study is to examine the effects of Laminaria japonica polysaccharide (LJPS) on lipids and leptin metabolism in hyperlipidemic mice. The hyperlipemia models were established by feeding fat-rich forage to Kunming mice for four weeks and administered with different doses of LJPS (75mg/d, 150mg/d, 300mg/d). Triglyceride (TG) and total cholesterol (TC) in serum were determined with enzymic method and cholesterol oxidation method. The free fatty acid (FFA) and leptin in serum were determined by enzyme linked immunosorbent assay (ELISA). The results showed that the serum levels of TG, TC, FFA and leptin in the hyperlipidemic group were higher than those in control group (\(P < 0.01\)). The serum levels of TG, TC and leptin in treated groups were lower than those in the hyperlipidemic group (\(P < 0.05\)), and the serum level of FFA in middle and high dose groups was lower than those in the hyperlipidemic group (\(P < 0.01\)). The serum levels of TG, TC and FFA in middle and high dose groups were lower than those in low dose group (\(P < 0.05\)), while the serum level of leptin in high dose group was lower than low dose group (\(P < 0.05\)). The results suggested that LJPS could reduce blood lipid level in hyperlipidemic mice and improve the leptin resistance with dose-dependent relationship.

Keywords: Laminaria japonica polysaccharide; hyperlipemia; leptin; mice

INTRODUCTION

Hyperlipidemia and atherosclerosis are the common causes of cardio-cerebrovascular diseases (Arenillas et al., 2007), and related to the risk factors of blood glucose, low density lipoprotein, apolipoprotein A, non-high density lipoprotein, homocysteine, 6-keto-prostaglandin F1 alpha, high-sensitivity C-reactive protein (Zhou et al., 2010; Wang, 2012). As a polypeptide hormone secreted by white adipose tissue, leptin not only regulates appetite, affects adipose metabolism and consumption of energy (Caro et al., 1996; Kita et al., 2003), but also has some biological effects like affecting thyroid hormone (Zimmermann-Belsing et al., 2004), insulin (Benoit et al., 2004) and sympathetic nervous system (Correia et al., 2004). So leptin is closely related to cardio-cerebrovascular diseases (Mundy et al., 2007), but whether it could be an independent risk factor of hyperlipidemia and atherosclerosis is not clear (Ku et al., 2011). Previous studies indicate that many obesity patients had their serum leptin level increased significantly, which means leptin resistance occurred in individual patient (Qiu et al., 2001). Leptin resistance mainly takes place in the following three component elements: transport dysfunction into brain through blood-brain barrier (Pan et al, 2008); central resistance occurred by mutation of leptin receptors (most of them are hypotype B) (Lahlou et al., 2002); abnormality of signal transduction behind the receptor (Munzberg et al., 2004). Absence or abnormality in each component elements may result in hyperlipidemia and atherosclerosis. Free fatty acid (FFA) is a nonesterified fatty acid and a hydrolysate of triglyceride, which may accommodate the transport of glucose and the metabolism of lipid. At
physiological status, FFA is released into blood circulation after release from adipose tissue (Li et al., 2011), while in obesity patient, especially patient with hyperlipidemia, high FFA syndrome occurs because of adipose metabolic disorder (Chen et al., 2011; Franssen et al., 2011).

*Laminaria japonica* polysaccharide (LJPS), which is composed of Alginate and Fucoidan (Yang et al., 2007), is a polysaccharide purified from Laminaria japonica and has some biological activities such as improving individual's immunity, anti-aging, anti-oxidative stress and antitumor (Zhou et al., 2009). However, few reports are on LJPS regulation of lipid and anti-atherosclerosis (Wang et al., 2007). In the present study, we examined the effects of LJPS on serum lipid and leptin in hyperlipidemic mice.

**Experimental design**

**Mouse models**

Total of 64 healthy male Kunming mice (SPF grade and weighting 22g to 27g) were supplied by the Experiment Animal Center of Qingdao Drug Inspection Institute (SCXK (LU) 20110010). The experiment was approved by the Ethics Committee of Qingdao University Medical College (No. QUMC 2011-09). All the mice were acclimatized for 3 days with general forage which was composed of corn flour (32%), bean flour (20%), wheat flour (29%), wheat bran (7%), fish flour (7%), yeast flour (3%) and bone meal (2%) before experiment. After 3 days, 10 mice were randomly sacrificed and sampled to determine the normal serum lipid level. Then 10 mice were taken as control group which were fed with general forage, and other 44 were fed for 4 weeks with fat-rich forage which was composed of general forage (59%), sucrose (20%), pig fat oil (10%), egg yolk powder (10%) and cholic acid sodium (1%) to establish hyperlipemia model (Zhang et al., 2007). At the end of 4th week, 8 fat-rich forage given mice were randomly sacrificed and sampled to determine the serum lipid level. Then 10 mice were taken as control group which were fed with general forage, and other 44 were fed for 4 weeks with fat-rich forage which was composed of general forage (59%), sucrose (20%), pig fat oil (10%), egg yolk powder (10%) and cholic acid sodium (1%) to establish hyperlipemia model (Zhang et al., 2007). At the end of 4th week, 8 fat-rich forage given mice were randomly sacrificed and sampled to determine the serum lipid level. The hyperlipemia models (TG=4.49±0.82mmol/L, TC=6.48±0.25mmol/L) were considered successful when TG and TC level differed by more than two standard deviations from the control group (TG=1.31±0.73mmol/L, TC=3.23±0.52mmol/L). The 36 successful hyperlipidemic mice were divided into hyperlipidemic control group (n=9), low dose LJPS treated group (n=9), middle dose LJPS treated group (n=9) and high dose LJPS treated group (n=9).

**Treatment method**

From the 5th week, control group mice were fed with general forage for 2 weeks; hyperlipemia model group mice were fed with fat-rich forage continually; low, middle and high dose LJPS treated group mice were fed with different dose of LJPS (each mouse of these three groups was fed with 75mg/d, 150mg/d and 300mg/d) for 2 weeks. The LJPS powders originated from “Zhongke NO.1” Laminaria japonica in the Rongcheng sea area of Shandong Province. The Laminaria japonica was pulverised and extracted after ultrasonic concussion, filtration and concentration. Protein was removed by Sevage method and extraction was washed repeatedly with ethanol and acetone, frozen and dried to obtain the delicate LJPS which accounted for 17.9% weight of Laminaria japonica. All of the LJPS powders are provided by Institute of Oceanology, Chinese Academy of Science.

**Specimen collection**

At the end of this experiment, all the mice that were given absolute diet for 8 hours before specimen collection, and 1 ml blood samples from orbital plexuses were collected for each mouse, centrifuged for 10 minutes at 4000 r/min (Eppendorf 5801, Genman) to separate 0.5ml serum and stored at -20℃.

**Determination of Serum TG and TC**

The triglyceride (TG) and total cholesterol (TC) levels in serum were determined by enzymic method and cholesterol oxidation method with the unit of mmol/L (Zeng, 2012).

**Determination of Serum FFA and Leptin**

The free fatty acid (FFA) and leptin levels in serum were determined according to the description of FFA and leptin enzyme linked immunosorbent assay (ELISA) kit (T-30315, T-32203, Andy Sci. and Tech. Co. Ltd., USA) with the unit of μmol/L and μg/L (Jin et al., 2006).
Table 1. Comparison between serum TG and TC level (mmol/L, \(\bar{x} \pm s\))

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>TG</th>
<th>TC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>9</td>
<td>1.14±0.10</td>
<td>3.20±0.26</td>
</tr>
<tr>
<td>Model group</td>
<td>9</td>
<td>4.33±0.20 (^a)</td>
<td>6.58±0.36 (^a)</td>
</tr>
<tr>
<td>Low dose group</td>
<td>9</td>
<td>3.49±0.32 (^b)</td>
<td>5.22±0.37 (^b)</td>
</tr>
<tr>
<td>Middle dose group</td>
<td>9</td>
<td>1.45±0.17 (^b\ \ ^c)</td>
<td>3.07±0.20 (^b\ \ ^c)</td>
</tr>
<tr>
<td>High dose group</td>
<td>9</td>
<td>1.32±0.14 (^b\ \ ^c)</td>
<td>3.17±0.15 (^b\ \ ^c)</td>
</tr>
</tbody>
</table>

\(^a\) P<0.01 vs control group, \(^b\) P<0.01 vs model group, \(^c\) P<0.01 vs low dose group

Table 2. Comparison between serum FFA and leptin level (\(\bar{x} \pm s\))

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>FFA ((\mu)mol/L)</th>
<th>Leptin (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>6</td>
<td>47.83±1.53</td>
<td>0.50±0.05</td>
</tr>
<tr>
<td>Model group</td>
<td>6</td>
<td>77.44±2.49 (^a)</td>
<td>0.91±0.45</td>
</tr>
<tr>
<td>Low dose group</td>
<td>5</td>
<td>63.88±2.58</td>
<td>0.77±0.23</td>
</tr>
<tr>
<td>Middle dose group</td>
<td>6</td>
<td>52.96±4.62 (^b\ \ ^c)</td>
<td>0.70±0.20 (^b)</td>
</tr>
<tr>
<td>High dose group</td>
<td>7</td>
<td>54.71±2.59 (^b\ \ ^c)</td>
<td>0.65±0.31 (^b\ \ ^c)</td>
</tr>
</tbody>
</table>

\(^a\) P<0.01 vs control group; \(^b\) P<0.05 vs model group; \(^c\) P<0.05 vs low dose group

Statistical Analysis

SPSS17.0 software was used for statistical analysis and data were expressed as \(\bar{x} \pm S\). Multi-group comparison was via analysis of variance (ANOVA) and Student's test and two-group comparison was by LSD-t test. Values were considered to be significant when \(P<0.05\).

RESULTS

Serum TG and TC Level

At the end of experiment, the serum TG and TC levels of each group had significant differences \((F_{TG}=54.40, F_{TC}=32.19, P<0.01)\). The serum TG and TC level in hyperlipidemic group were significantly higher than in control group \((t_{TG}=11.38, t_{TC}=8.51, P<0.01)\); LJPS treated groups were significantly lower than those of hyperlipidemic group \((t_{TG}=3.00-10.72, t_{TC}=3.18-8.80, P<0.01)\); middle and high dose groups were significantly lower than those in low dose group \((t_{TG}=7.28-7.72, t_{TC}=5.37-5.62, P<0.01)\); but there were no significant differences between middle and high dose groups \((t_{TG}=0.44, t_{TC}=0.26, P>0.05)\). (See Table 1).

Serum FFA Level

At the end of the 6th week, the serum FFA levels of each group had significant differences \((F=10.10, P<0.01)\). The serum FFA level in hyperlipidemic model group was significantly higher than those in control group \((t=5.62, P<0.05)\); middle and high dose LJPS treated groups were significantly lower than those in model group \((t=4.13-4.40, P<0.05)\); there were no significant differences between low dose group and model groups \((t=1.80, P>0.05)\); middle and high dose groups were significantly lower than those in low dose group \((t=2.26-2.60, P<0.05)\); but there were no significant differences between middle and high dose groups \((t=0.43, P>0.05)\). (See table 2).

Serum Leptin Level

At the end of the 6th week, the serum leptin levels of each group had significant differences \((F=17.24, P<0.01)\). The serum leptin level in hyperlipidemic group was significantly higher than that in control group \((t=7.97, P<0.05)\); the values of LJPS treated groups were significantly lower than those in model group \((t=2.73-5.16, P<0.01)\). Among the three LJPS treated groups, the values of high dose group were significantly lower than those of low dose group \((t=2.34, P<0.05)\); but there were no significant differences between the two other LJPS treated groups \((t=1.05-1.24, P>0.1)\). (See Table 2).

DISCUSSION

Dysfunction of lipid metabolism plays an important role in the development of cardio-cerebrovascular diseases and atherosclerosis (Ding, 2011). Several clinical reports confirmed that the elevation of serum TG, TC, LDL-C, ApoB100, LP(a) and ApoB100/ApoA1 and the reduction of serum HDL-C and ApoA1 could promote the incidence
of atherosclerosis (Tan et al., 2007), which it is now considered as the imbalance of oxidation and antioxidation, oxygen radical increasing to destroy endothelial cell structure and enhance the lipid peroxidation and atherosclerotic plaques formation (Xu et al, 2010). In the present study, the serum levels of TG and TC in hyperlipidemic model group increased significantly when compared with control group, while decreased significantly in the treated groups after two weeks of LJPS intervention, which was consistent with Huang’s (Huang et al., 2010) finding. Moreover, the effects in middle and high dose groups were obviously superior to low dose group. After giving middle or high dose of LJPS, the serum lipid levels in hyperlipemia mice came close to the level in the control group, which indicated that LJPS could reduce the serum TG and TC effectively and dependence on the LJPS dose. Under the principle of minimizing dose, the middle dose (3 g/kg) may provide an ideal therapeutic effect.

At general condition of high blood FFA patient, the rising FFA enter into skeletal muscles and then elevate the TG level in muscle cells to activate protein kinase C, reduce tyrosine phosphorylation of insulin receptor and transportation of glucose to result in insulin resistance (Chen et al., 2011), meanwhile, insulin resistance may further aggravate the glucolipid metabolism disorders to form leptin resistance. FFA also plays an important role in the process of atherosclerosis. The elevation of serum FFA may cause mitochondrial disorder and oxidative stress, increase active oxygen and damage antioxidation ability, both of them combined to induce pathological changes (Villareal et al., 2006). In this experiment, the mice in hyperlipemia model group have abnormally high serum levels of lipid with high FFA levels at the same time, which indicated that the adipose tissue of hyperlipemia mice decompose and produce large amount of FFA to form a high blood FFA condition, and the high serum lipid and high blood FFA may promote each other.

In LJPS treated groups, the serum FFA levels decreased significantly which suggested that LJPS could reduce the serum FFA level efficiently, alleviate peroxidation condition, improve lipid metabolism disorders and reduce the risk factors and incidence of atherosclerosis, which has been demonstrated by other hyperlipidemic animals (Li et al, 2005). Though no significant statistical differences were found between low dose group and hyperlipidemic group, the trend of reducing FFA could be seen obviously in this experiment, which further explained the dose-effect relationship of the drug LJPS.

It is revealed that leptin restrained the formation of TG, promoted the oxidation of fatty acid and the aggregation of platelets with concentration-dependence (Park et al., 2001). Leptin could also increase vascular endothelial growth factor (VEGF) and matrix metalloproteinase in atheromatous plaque, which resulted in blood vessel remodeling and influenced the formation and progress of hyperlipemia directly or indirectly (Saginova et al., 2011). Leptin could influence obesity receptor B (Ob-Rb) in hypothalamus directly, influence hypothalamus to secrete and release neuropeptide Y (NPY) (Ahima RS et al., 1996), and reduce appetite, reduce ingestion, and increase the consumption of energy. At the same time, leptin could also take part in lipid metabolism via peripheral tissue pathway such as liver and adipose (Rhee et al., 2008). In the present experiment, the serum leptin level elevated significantly in hyperlipidemic group mice, which suggested that hyperlipemia itself was not sensitive to leptin and physiological effect of leptin was reduced to form leptin resistance. After intervention with LJPS, the leptin level of hyperlipemia mice reduced to some extent and then improves the leptin resistance. Compared with each treated group, the effect of reduced leptin was different in high dose group in comparison to low dose group; both of high and low dose groups had change in tendency but no differences. All of these observations are consistent with the “gradual principle” of using drug. Therefore, the serum leptin level could reflect serum lipid metabolic condition and the extent of hyperlipemia in a certain angle.

CONCLUSIONS

This study showed that LJPS could reduce blood lipid level in hyperlipemia mice and decrease the leptin resistance with dose dependent relationship.

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