Effect of piperine in the regulation of obesity-induced dyslipidemia in high-fat diet rats

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Received: 30-06-2010
Revised: 26-10-2010
Accepted: 23-02-2011

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Introduction

Greater consumption of energy leads to an increase in the fat mass (adiposity) and fat-cell enlargement (hypertrophy), producing the characteristic pathology of obesity.[1] The rising tide of obesity is one of the most pressing health issues of our time. Increase in fat mass increases the associated risk conditions such as dyslipidemia, type 2 diabetes mellitus, and coronary heart disease, termed as excessive fat-related metabolic disorders (EFRMD).[2] The brain controls fat storage (i.e. energy homeostasis) by regulating food intake and energy expenditure. Sensory input is received from the body in the form of circulating hormones (leptin, ghrelin, etc.), fuels (glucose, fatty acids, etc.), and vagal efferents from the gut.[3] This information is integrated with clues from the outside world as well as the emotional state of the organism. The brain then initiates the appropriate alterations in food intake and energy expenditure with the ultimate goal of maintaining energy balance. Obesity develops when this system malfunctions.[4]

One of the most important of such centers is the hypothalamus, especially the arcuate nucleus.[5] Among the hypothalamic neuropeptide systems regulating feeding, melanocortins play a prominent role.[6]

Melanocortins (MC) cleaved from pro-opiomelanocortin (POMC), exert their effects by binding to the members of the melanocortin receptor family, in the brain.[7] Increase in the MC-4 receptor activity leads to a decrease in appetite, increased energy expenditure, and increased insulin sensitivity. Thus, an increase in MC-4 activity helps in reducing adiposity (obesity) and its related metabolic syndromes like dyslipidemia.

Increase in MC-4 activity can be achieved by increasing CNS leptin and / or insulin activity, which is dependent upon the peripheral leptin / insulin production, transport across the blood-brain barrier, and effect upon the CNS target...
receptors. Melanocortin activity may also be increased by an endogenous inhibition of inverse agonists (agonist-related peptide) of melanocortin receptors. Alternatively it can also be achieved through selective melanocortin receptor agonists such as piperazine, piperidine, pyridazinone, tetrahydropyran, thiazazole, and diazole derivatives. The diminished activity of MC-4 receptors not only increases the adiposity, but also increases the risk of its associated metabolic syndromes.[8] Therefore piperine, a piperidine derivative can be used as a melanocortin agonist.

Piper nigrum commonly known as black pepper and Piper longum commonly known as long pepper are highly reputed plants in the ayurvedic system of medicine. A phytochemical review reveals the presence of piperine (1-piperoyl piperidine), the major constituent in these plants, which is isolated from its fruits. This constituent of the Piper species has been found to possess a number of therapeutic properties, mainly indicating its use as a bioavailability enhancer. Other indications are in bronchitis, chronic cold, cough, congestion, hemorrhoids, hepatitis, arthritis, chronic dyspepsia, anorexia, chronic asthma, burning heart, colic, rheumatoid / osteoarthritis, juvenile asthma, and so on.[9] Piperine, which is 1-piperoyl piperidine, can be proposed to be used as an a melanocortin-4 agonist. In the light of above mentioned reports, the present investigation was undertaken to study the potential use of piperine in improving the lipid profile in obese animals without suppressing the appetite.

Materials and Methods

Materials

Piperine was purchased from Sigma Aldrich Co., St Louis, USA, and Sibutramine was a generous gift from Intas Pharmaceuticals Ltd, Ahmedabad. All other chemicals used were of analytical grade.

Animals

Male Sprague-Dawley rats weighing 400 – 450 g were used for the present study. They were housed in clean polypropylene cages (three rats / cage) and maintained under controlled room temperature (22 ± 2°C) and humidity (55 ± 5%), with a 12 : 12 hour light and dark cycle. All the rats were fed normal pellet diet (NPD) (commercial rat pellets) and were given water ad libitum before the dietary manipulation. The guidelines of the committee for the purpose of control and supervision of experiments on animals (GPCSEA), Government of India, were followed, and prior permission was sought from the Institutional Animal Ethics Committee for conducting the study.

Experimental Protocol

Male Sprague-Dawley rats were used for the present investigation. The rats were divided into four groups of six animals each.

Group I — Control group
Group II — High-fat diet (HFD) — control group
Group III — HFD + Piperine (suspended in 0.5% carboxy methylcellulose (CMC), p.o), for the last three weeks.
Group IV — HFD + Sibutramine (solution in deionized water, p.o.), for the last three weeks.

Group I was fed NPD, while Groups II, III, and IV were fed HFD for eleven weeks, that is, throughout the study. At the end of the eighth week, groups III and IV were treated with piperine (40 mg / kg)[9] and sibutramine (5 mg / kg), respectively, for three weeks. The composition of HFD[11] is given in Table 1. The following parameters were measured: physical parameters like body weight and food intake[12] and biochemical parameters.[12] At the end of the study, four rats from each group were sacrificed and the fat mass was collected and immediately weighed.[12]

Collection of Blood Samples

At the end of the fourth, eighth, and eleventh weeks, blood was collected under inhalation anesthesia by retro-orbital puncture from overnight fasted animals. Blood was allowed to clot for 30 minutes at room temperature. Serum was separated by centrifugation at 4,000 – 5,000 rpm for 15 minutes and analyzed for serum cholesterol (CHOD-PAP), HDL (PEG-CHOD-PAP), and triglyceride (GPO-PAP) levels using the commercially available diagnostic kits (Span Diagnostics Ltd., Surat, India).

Fat-pad Analysis

At the end of the eleventh week, animals were decapitated between 09:00 and 12:00 hours. They were free to access food and water. After sacrificing by decapitation, the epididymal white adipose tissue and interscapular brown adipose tissue (BAT) were dissected out. The collected fat was weighed immediately and compared with the other groups.

Statistical Analysis

All the values were expressed as mean ± SEM, n = 6 in each group. The statistical analysis for determining the significant difference was performed using the Student’s paired t-test and the Tucky (one way ANOVA test) test. Value of P less than 5% (P < 0.05) was considered statistically significant.

Results

Effect of Piperine on the Physical Parameters

a. Effect of piperine on body weight

Body weight was measured every week till eleven weeks. The body weight of all the HFD groups (groups II, III, IV) was significantly increased compared to the control group (group I) for first eight weeks. Piperine-treated group showed significant reduction in body weight, by 12 – 15% as compared to the HFD-control group (P < 0.05), while the sibutramine-treated group (group IV) exhibited 35 – 40% weight reduction [Figure 1].

b. Effect of piperine on food intake

Supplementing piperine for three weeks with the HFD, exhibited no significant alteration in the food intake as compared to the HFD-control and control group (p < 0.05).

Table 1:

| Ingredients          | (g / kg) |
|----------------------|----------|
| Powdered NPD         | 300      |
| Lard                 | 275      |
| Casein               | 200      |
| Cholesterol          | 10       |
| Vitamin and mineral mix | 60     |
| dl-methionine        | 03       |
| Sodium chloride      | 02       |
| Sucrose              | 150      |

For example: 60 mg / kg

Sucrose 150

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Indian Journal of Pharmacology | June 2011 | Vol 43 | Issue 3 297
Sibutramine treated group exhibited a significant reduction in food intake as compared to the HFD-control group. This indicated a protective effect of piperine in reducing body weight without any alteration in food intake [Figure 2].

**Effect of Piperine on Serum Lipid Profile**

Serum triglyceride, cholesterol, LDL, and VLDL levels

### Table 2:

| Parameters          | Duration | Control Group-I | HFD-control Group-II | Piperine Group-III | Sibutramine Group-IV |
|---------------------|----------|-----------------|----------------------|--------------------|----------------------|
| Serum Cholesterol   | Fourth week | 73.5 ± 6.8 | 145.8 ± 11.5* | 150.4 ± 14.2* | 156.2 ± 11.3* |
|                     | Eighth week | 88.5 ± 8.6  | 162.9 ± 14.2* | 173.5 ± 14.9* | 160.9 ± 7.1* |
|                     | Eleventh week | 98.9 ± 5.4 | 193.9 ± 16.2* | 131.9 ± 11.7** | 96.8 ± 5.8** |
| Serum HDL-Cholesterol | Fourth week | 46.9 ± 5.3 | 31.9 ± 2.9* | 33.5 ± 3.5* | 30.5 ± 4.5* |
|                     | Eighth week | 55.3 ± 3.7  | 25.1 ± 1.8* | 25.6 ± 1.9* | 23.9 ± 1.9* |
|                     | Eleventh week | 66.3 ± 3.8 | 23.2 ± 1.3* | 34 ± 3** | 46.1 ± 3.7** |
| Serum LDL-Cholesterol | Fourth week | 13.7 ± 4.5 | 92.3 ± 13.8* | 94.2 ± 15.6* | 105.2 ± 15.8* |
|                     | Eighth week | 17.6 ± 5.2  | 102.2 ± 14.4* | 108.7 ± 15.7* | 99.6 ± 6.9* |
|                     | Eleventh week | 14.4 ± 3.8 | 119.9 ± 29.4* | 32.3 ± 2.6** | 28.9 ± 12.9** |
| Serum VLDL-Cholesterol | Fourth week | 12.9 ± 0.8 | 21.8 ± 1.5* | 22.8 ± 1.8* | 20.5 ± 2.9* |
|                     | Eighth week | 15.7 ± 5.2  | 35.7 ± 1.3* | 39.3 ± 0.7* | 37.5 ± 1.3* |
|                     | Eleventh week | 12.1 ± 11.8 | 46.9 ± 11.8* | 22.03 ± 1.5** | 10.8 ± 8.4** |
| Serum Triglyceride  | Fourth week | 64.7 ± 4.2 | 108.6 ± 7.6* | 113.8 ± 9* | 102.4 ± 14.6* |
|                     | Eighth week | 78.4 ± 9.4  | 178.4 ± 6.7* | 196.3 ± 3.1* | 187.1 ± 6.6* |
|                     | Eleventh week | 91.4 ± 3 | 252.9 ± 15.2* | 146 ± 5.2** | 107.2 ± 4.7** |

All values are expressed as mean ± SEM, n = 6; *P < 0.05 compared to Group-I (control); **P < 0.05 compared to Group-II (HFD-control)

**Figure 3:** Effect of piperine on epididymal fat mass in high-fat diet animals

**Figure 4:** Effect of piperine on interscapular fat mass in high-fat diet animals
were significantly increased, while the serum HDL level was significantly decreased in all the HFD groups for the first eight weeks compared to the control group. On treatment with piperine, serum triglyceride, cholesterol, LDL, and VLDL levels were significantly reduced, while the HDL level was significantly increased compared to the HFD control group ($P < 0.05$) [Table 2]. Very similar results were observed with the sibutramine treated group. Thus treatment with piperine showed a significant reduction in the lipid profile related to obesity.

**Fat Pad Analysis**

As the animals were kept on HFD for 11 weeks, there was an accumulation of visceral, subcutaneous, and interscapular fat. There was a significant reduction in the epididymal (visceral VAT) and interscapular (BAT) fat mass in the piperine-treated group, compared to the HFD-control group [Figures 3 and 4]. This showed the protective effect of piperine in the increased fat mass condition.

**Discussion**

The present study was undertaken with a therapeutic approach to develop strategies to reduce the worldwide obesity epidemic and a research goal to develop safe and effective drugs, which will not only reduce excessive fat mass, but also its related metabolic syndromes. High-fat diet is one of the main causes leading to excessive fat mass accumulation — obesity — which in turn leads to other metabolic syndromes like dyslipidemia. Thus, a high-fat diet (HFD) model was used to produce dyslipidemia similar to humans.

Increase in body weight and fat deposition are the chief indicators for the gradual progress of obesity. As the animals were fed with HFD, there was an increase in the adiposity, which in turn increased the fat cell mass. Thus, there was an overall increase in body weight. The increased body weight found in HFD rats might be due to the consumption of a diet rich in energy, in the form of saturated fats (lard) and its deposition in various body fat pads, and decreased energy expenditure as compared to NPD-fed animals. However, on treatment with piperine there was a significant decrease in body weight and fat mass, which proved its antiobese action.

Dyslipidemia is the most important relationship of obesity to coronary artery disease. The most common characteristics of dyslipidemia related to obesity are characterized by (i) increased triglycerides, (ii) decreased HDL levels, and (iii) increased dyslipidemia related to obesity are characterized by (i) increased small intestine following the intake of HFD, which proved its antiobese action.

Thus the above results suggests that piperine significantly possesses a lipid lowering effect and anti-obesity activity without any change in appetite. The possible hypothesis, seeing to the structural similarity, is that, piperine being a piperidine derivative, works as an MC-4 receptor agonist. The other mechanism piperine possesses is the thyrogenic activity, thus modulating apolipoprotein levels and insulin resistance in HFD-fed rats, and opening a new window in the management of dyslipidemia by dietary supplementation with nutrients. Moreover piperine also inhibits lipid and lipoprotein accumulation by significantly modulating the enzymes of the lipid metabolism, like Lecithin–cholesterol acyltransferase (LCAT) and Lipoproteins lipase (LPL).

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