Original Article

Relation of plasma somatostatin levels with malondialdehyde in hyperlipidemic patients

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Somatostatin (SST) may protect organism from overnutrition-induced insulin resistance and oxidative stress by inhibiting pancreatic endocrine and exocrine secretion, gastrointestinal digestion and absorption. Many studies clearly show its release becomes perturbed in diabetes and obesity. Therefore, in the present study we first aimed to investigate whether or not plasma somatostatin level was different in patients with hyperlipidemia and normolipidemic controls. We also assessed the relationship between plasma somatostatin levels with atherosclerotic index (AI) and malondialdehyde (MDA) in non-diabetic dyslipidemic patients. Subjects with hyperlipidemia have insulin resistance and high levels of oxidative stress. Median somatostatin (57.2±19.2 *vs* 68.0±21.9 pg/mL; $p<0.05$) levels were lower in hyperlipidemic than in normolipidemic subjects. Significant inverse relationships between SST level and AI ($r=-0.21$, $p< 0.05$), or MDA ($r=-0.31$, $p<0.01$) were observed. These results suggest a possible protective role of endogenous SST, at least on hyperlipidemia and atherosclerosis that are attributed to excess energy intake and physical inactivity. Of course these preliminary results should be supported by prospective studies.

Key Words: somatostatin, insulin resistance, oxidative stress, malondialdehyde, hyperlipidemia

INTRODUCTION

The prevalence of dyslipidemia resulting from excess energy intake and physical inactivity is increasing not only in Western countries,[1] but also in Asian societies.[2] A fat enriched diet is regarded as an important factor in the development of cardiac diseases since it leads to the development of abnormal lipid metabolism, hyperlipidemia and atherosclerosis. Higher rates of macronutrients (refined sugar, saturated fat) oxidation caused by an intake that considerably exceeded the actual needs is related to an increased electron flow along the mitochondrial respiratory chain, resulting in increase of free radicals and further inducing oxidative stress.[3] Clearly, glucose, fat and macronutrient intake induces oxidative stress.[4] It has been well documented that reactive oxygen species (ROS) play an important role in the pathogenesis of high energy intake-induced obesity, insulin resistance, and cardiovascular disease.[5,6]

Somatostatin (SST) is a natural and ubiquitous neurohormone produced in the central nervous system and in most major peripheral organs including the salivary glands, stomach, pancreas, and intestine.[7] It has been recognized as a peptide that exerts a negative action on a variety of physiologic functions. Somatostatin inhibits gastric emptying and the release of gastric acid as well as pancreatic exocrine and endocrine secretions.[8] Similarly, intestinal absorption of key nutrients (glucose, fat, and amino acids) is inhibited by SST.[9,10] SST might be a physiological regulator in the homeostasis of ingested nutrients by modulating intestinal absorption rate. Wenger FA *et al*. demonstrated that octreotide inhibit lipid peroxidation and increased the activity of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) of Syrian hamsters fed a high-fat diet (HFD).[11] We also found that simultaneous administration of SST analog octreotide to HFD-fed mice significantly reduced ROS production of the digestive system and resulted in the improvement of HFD-induced metabolic syndrome.[12] More recently, SST has been used to treat hyperinsulinemia associated with obesity and also primary insulin hypersecretion.[13,14]

Given the accumulating evidence that insulin resistance may play an important role in dyslipidemia, and that SST is important for regulating insulin secretion and maintenance of the homeostasis of ingested nutrients, the purpose of this study was to: 1) determine if the plasma SST levels were lower in hyperlipidemic subjects compared to controls, and 2) explore the association of plasma SST level with atherosclerotic index (AI) and malondialdehyde (MDA) in this population.

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MATERIALS AND METHODS

Subjects

Recruitment of subjects for this study was carried out at the Jiangnan University hospital. Eighty nine subjects with hyperlipidemia (57 males and 32 females) and 45 control subjects (27 males and 18 females) (Table 1) participated in the study. Lifestyle information, such as the past history of exercise, current exercise, history of dieting, smoking status, and alcohol intake was obtained from a self-reported questionnaire. All subjects have almost similar economic status, food habits, and physical activity. Lactating mother, cigarette smokers and alcoholics were excluded from the study. Hyperlipidemia with other complications like diabetes mellitus, hypertension and pregnant women were also excluded from the study. None of the subjects were taking antioxidant vitamin supplements, or lipid-lowering drugs. Written consent was obtained from all individuals after the purpose and nature of the study had been explained. Institutional ethics committee approved the study protocol was obtained. Body mass index (BMI) was calculated as weight (kilograms)/height (meters^2).

Sample collection

For all patients and healthy subjects, venous blood was collected in the outpatient department from 0830–0930 h after at least 10 hours of overnight fasting. Plasma was obtained from blood samples after centrifugation (1000 *g* for 15 min at 4°C) and divided into aliquots and stored at −70 °C until assayed.

Laboratory procedures

For determination of plasma total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C) and TG concentrations, the corresponding diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, PR China) were used according to the instructions of the manufacturer. The lipoproteins LDL-C and HDL-C were fractionated by a dual precipitation technique.[15] After fractional precipitation, lipoprotein cholesterol was estimated. The AI was calculated as (TC-HDL-C)/HDL-C. Plasma thiobarbituric acid reactive substances (TBARS),[16] plasma superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were also assayed.[17,18] Glucose values were measured by the glucose oxidase method, and plasma insulin was measured by RIA (Beijing Huaying Bioengineering Institute, Beijing, PR China). Insulin resistance was evaluated by the homeostasis model assessment.[19] The HOMA index was calculated as the product of the fasting plasma insulin level (μIU/mL) and the fasting plasma glucose level (mmol/L), divided by 22.5. Circulating plasma levels of SST were assessed by RIA method (Beijing Huaying Bioengineering Institute, Beijing, PR China). Measurements were made in a blinded manner. All samples were assayed in duplicate, and those showing values above the standard curve were retested with appropriate dilutions. Intra- and inter-assay coefficients of variation for both insulin and SST immunoassays were less than 5% and 10%, respectively.

Table 1. Clinical characteristics of controls and hyperlipidemic patients

	normolipidemic subjects (n = 45)	hyperlipidemic subjects (n = 89)
Age (years)	41.8±7.6	45.4±7.9
BMI (kg/m^2)	22.6±2.7*	23.1±2.6*
TC (mmol/L)	4.75±0.79*	5.50±0.88**
TG (mmol/L)	1.27±0.28*	2.78±0.79**
HDL-C (mmol/L)	1.29±0.26*	1.09±0.22**
LDL-C (mmol/L)	3.05±0.63*	3.50±0.80**
Fasting glucose (mmol/L)	5.10±0.34*	5.98±1.32**
Fasting insulin (uIU/mL)	15.3±4.52*	19.0±5.56**
HOMA index	3.48±1.11*	5.14±2.19**
AI	2.76±0.66*	4.14±0.92**
SOD (U/mL)	105±13.4*	91.0±11.7**
GSH-PX (U/mL)	344±28.4*	254±29.5**
CAT (U/mL)	7.01±2.63*	6.74±2.40*
MDA (nmol/mL)	5.73±1.25*	8.77±1.88**

Values are expressed as mean ± SD. Values in the same row with different superscripts are significantly different at $p<0.05$ level.

Statistical analysis

All results are expressed as mean ± standard deviation. Comparisons across groups were performed by one-way ANOVA. Pearson's correlation coefficient (r) was used to determine the relation of AI and MDA to plasma SST level. A $p<0.05$ was considered statistically significant. Analysis was done with SPSS 11.5 (SPSS, Inc, Chicago, IL, USA).

RESULTS

Clinical characteristics of the study participants

Anthropometric and clinical characteristics of the subjects are shown in Table 1. The hyperlipidemic patients showed high levels of TG, TC and LDL-C, and low level of HDL-C as compared to controls. A higher AI was found in hyperlipidemic patients than in normolipidemic subjects. Fasting plasma insulin and glucose levels were higher in the hyperlipidemic subjects compared to controls. Values of HOMA index was almost 148% greater in hyperlipidemic subjects (5.14±2.19) than in controls (3.48±1.11). MDA levels increased in hyperlipidemic subjects (8.77± 1.88 nmol/mL) compared with that in controls (5.73±1.25 nmol/mL, $p<0.05$). Activities of SOD and GSH-Px significantly decreased in hyperlipidemic patients compared with that in the normolipidemic subjects.

Somatostatin in subgroups

As detailed in Figure 1, median somatostatin (57.2± 19.2 *vs* 68.0±21.9 pg/mL; $p<0.05$) levels were lower in hyperlipidemic than in normolipidemic subjects.

Correlation of SST level with AI and MDA

The correlation of plasma SST level with AI and MDA is shown in Figure 2. A significant negative correlation was found between SST and AI (r=–0.21, $p<0.05$). There was a negative correlation between SST and MDA (r=–0.31, $p<0.01$). These data suggested that plasma SST level was directly related to oxidative stress and SST secretion level has a role in atherosclerosis.

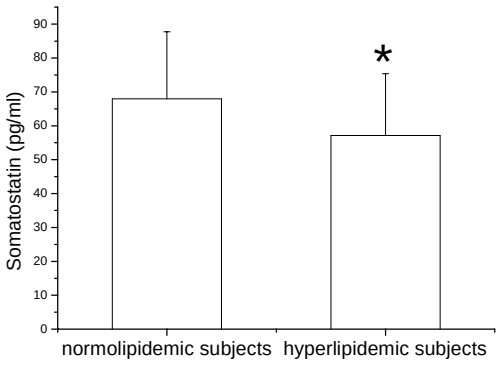

Figure 1. Plasma somatostatin levels in hyperlipidemic and normolipidemic subjects.

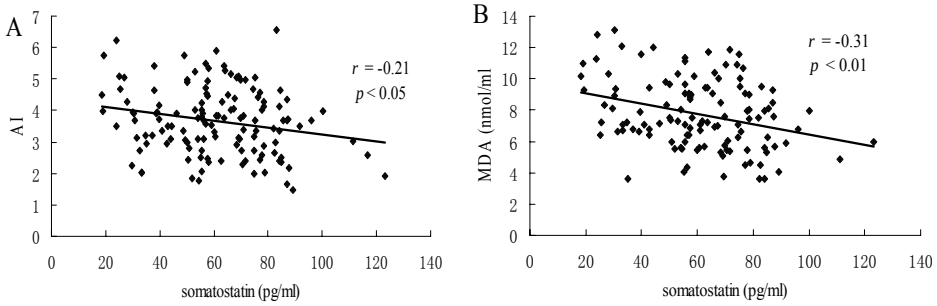

Figure 2. The correlation of plasma SST level with AI (A) and MDA (B).

DISCUSSION

We hypothesized that SST could be implicated in the insulin resistance process and oxidative stress described in hyperlipidemic patients, possibly contributing to the well-known association between insulin resistance and cardiovascular disease risk. Therefore, in the present study we have measured circulating plasma SST levels in a sample of non-diabetic dyslipidemic patients and then evaluated its relationship with AI and MDA. Our results demonstrate that a decreased SST levels accompanied by insulin resistance and oxidative stress are found in hyperlipidemic subjects. Suppressed SST levels may contribute to the metabolic derangements associated with insulin resistance and oxidative stress in dyslipidemia.

Hyperlipidemic subjects had higher fasting insulin levels. HOMA index in hyperlipidemic subjects is significantly higher than normolipidemic subjects. HOMA assessment of insulin sensitivity takes into account the effect of variation in fasting blood glucose levels on the insulin levels, which has been shown to be a reliable estimate of insulin resistance. The insulin resistance observed in hyperlipidemic subjects is in agreement with those reported in other studies.[20,21] Insulin resistance is associated with an atherogenic lipid profile, such that resistance to the action of insulin leads to enhanced very-low-density lipoprotein (VLDL) synthesis, resulting in hypertriglyceridemia, small, dense LDL and reduced HDL levels.[22-24] The development of insulin resistance is linked to both genetic and environmental factors. A key environmental element is the diet. Excessive fat intake induces hypersecretion of insulin, which increases nutrient uptake and triglyceride accumulation in the adipocytes. The hyperinsulinemia that develops secondary to the insulin resistance should further increase nutrient uptake, completing a vicious cycle in the development of the metabolic syndrome.[25] We found that this vicious cycle may correlate with reduced SST secretion. SST is known to be a potent inhibitor of insulin secretion from pancreatic ß-cells and pancreatic exocrine secretion. Decreased SST secretion may elevate blood insulin concentrations. Several studies have found that compounds that reduce insulin release (the SST analog octreotide) have been successfully used to treat obesity in a group of patients that exhibited insulin hypersecretion.[26] These data are consistent with our suggestions that decreased SST secretion may participate in insulin resistance in hyperlipidemic subjects.

In particular, we found that SST was negatively correlated with MDA. Although correlation does not prove causation, this observation suggests that decreased SST may increase oxidative stress in hyperlipidemic subjects. It was recently described that somatostatin and related analogs (octreotide) inhibit lipid peroxidation. Arias-Diaz *et al*. demonstrated the decrease of typical markers of lipid peroxidation in diaphragms of septic rats treated with somatostatin.[27] Several studies have shown that SST might be a physiological regulator in the homeostasis of ingested nutrients by modulating the intestinal absorption rate. The mitochondria are one of macronutrient catabolic sites, and its redox chain is a physiologic source of free oxygen radicals. Free radical generated during mitochondrial oxygen reduction may damage tissues and organs if the antioxidant potential is insufficient to quench the extra free radical production. This could be the result that overnutrition increased generation of free radicals and further led to oxidative stress. More recently, Aljada A *et al.* demonstrated that a moderately sized high fat high carbohydrate meal (900 kcal) also induced oxidative stress and inflammation in normal subjects.[28] Clearly, macronutrient intake is a key factor in generating oxidative stress. Since

SST is known to be a potent inhibitor of intestinal absorption of nutrients (glucose, fat, and amino acids), it is relevant that a decrease in SST release is causative for overnutrition and oxidative stress. Thus, it is not surprising that a challenge with excess energy intake resulted in a further increase in oxidative stress, which was significantly greater in the hyperlipidemic subjects with lower plasma SST level than that observed in normal subjects. There is evidence that oxidative stress is involved in the pathogenesis of hyperlipidemia and atherosclerosis. It is likely that such a practice on a regular basis further enhances the cumulative atherogenic risk.

In conclusion, the present study has provided evidence that plasma SST level decreased in Chinese dyslipidemic subjects, and showed a negative correlation with AI and MDA. It suggested that SST may play a role in the pathogenesis of hyperlipidemia and atherosclerosis that are attributed to excess energy intake and physical inactivity. This proposal is under current study in our laboratory.

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AUTHOR DISCLOSURES

None of the authors has conflict of interest.

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Original Article

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高脂血症患者血浆生长抑素水平与氧化应激的关系

生长抑素具有抑制胰腺内外分泌和胃肠消化吸收的功能，可能对机体高能摄入导致的胰岛素阻抗和氧化应激具有保护作用。许多研究也表明糖尿病和肥胖个体的生长抑素分泌紊乱。本研究目的是调查高脂血症患者的血浆生长抑素水平是否低于正常个体，并进而分析血浆生长抑素水平与动脉粥样硬化指数与 MDA 的关系。结果表明：高脂血症患者存在明显的氧化应激和胰岛素阻抗现象。高脂血症患者血浆生长抑素水平显著低于正常对照组，而且生长抑素水平与动脉粥样硬化指数和 MDA 水平呈显著负相关。這些數據说明，生长抑素分泌水平降低，可能与长期高能量摄入和体能活动不足攸关的高脂血症和动脉粥样硬化的发生密切相关。

关键词：生长抑素，胰岛素阻抗，氧化应激，MDA，高脂血症