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Diet high in lipid hydroperoxide by vitamin E deficiency induces insulin resistance and impaired insulin secretion in normal rats

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Abstract

To clarify the effect of dietary lipid hydroperoxide (LPO) on development of glucose intolerance, we fed Sprague–Dawley rats on a diet containing elevated LPO level for 10 weeks and measured both insulin sensitivity and insulin secretion. The contents of LPO in both plasma and skeletal muscle in the LPO-fed rats were significantly higher than those in the controls. Both insulin resistance evaluated by steady-state blood glucose (SSBG) methods and impaired insulin secretion evaluated by oral glucose tolerance test (OGTT) were found in the LPO-fed rats as compared with control rats. Furthermore, the levels of insulin receptor substrate (IRS)-1 protein in the skeletal muscle were significantly lower in the LPO-fed rats. Those impairments were not reversed in LPO-fed rats with supernormal levels of plasma vitamin E following vitamin E supplementation for 5 weeks. Moreover, the immunohistochemical study revealed that NF-κB-p50 protein was found in the nucleus of pancreatic β-cells of the LPO-fed rats, whereas it was not observed in the nucleus of the islets in the control rats. These findings indicate that NF-κB is activated in response to oxidative stress in pancreatic islet cells in LPO-fed rats. In conclusion, our studies reveal that diet high in LPO by vitamin E deficiency accelerates glucose intolerance through impairments of both sensitivity and secretion of insulin.

Keywords: Lipid peroxide; Insulin resistance; Insulin secretion; IRS-1; NF-κB

1. Introduction

It is well known that diabetic condition is associated with increased content of lipid hydroperoxide (LPO) [1,2] and that hyperglycemia is one of the major causes of enhanced free radical accumulation [3,4]. Moreover, our group reported that intestinal absorption of glucose as well as lipid is increased in diabetic patients and rats [5,6]. Recently, we have also shown

Abbreviations: FFA, free fatty acids; IRI, immunoreactive insulin; IRS, insulin receptor substrate; LPO, lipid hydroperoxide; OGTT, oral glucose tolerance test; SSBG, steady-state blood glucose; SSPI, steady-state plasma insulin; TBARS, thiobarbituric acid reactive substances; PI3-kinase, phosphatidylinositol 3-kinase

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that insulin resistance produces oxidative stress in vascular cells [7,8]. Thus, oxidative stress is one of the typical characteristics of type 2 diabetes mellitus. However, it is still unclear whether the elevated level of LPO plays any role in steps leading to diabetes, such as impairment of either insulin secretion or action. In spite of a number of studies of the relation between insulin resistance and circulating LPO content [9,10], we do not know whether dietary LPO accelerates insulin resistance and impaired insulin secretion from pancreatic β cells. Ihara demonstrated that the pancreatic β cells of GK rats were oxidatively stressed, and that chronic hyperglycemia might be responsible for the oxidative stress observed in the pancreatic β cells [11]. This study provides us the evidence that LPO from diet origin may contribute to the loss of insulin secretory activity in pancreatic β cells.

Vitamin E is one of the typical lipid-soluble antioxidant vitamins and is efficient in protection against cell membrane damage by LPO [12]. Supplementation with α-tocopherol, one of the main components of vitamin E, ameliorates the impairment of insulin action in Type 2 diabetic patients [13]. These effects of vitamin E supplementation thus suggest the involvement of free radical attack in the development of insulin resistance [11]. Staprans et al. [14] also demonstrated that dietary oxidized lipids made a major contribution to the levels of oxidized lipids in circulating lipoproteins and indicated that increased absorption of oxidized lipids in diabetic animals might play a role in the elevation of oxidized lipoproteins. However, it is unknown whether dietary lipid hydroperoxide caused by the depletion of vitamin E may be absorbed and contribute to impaired glucose metabolism in normal animals. To clarify whether prolonged, low-grade oxidative stress from the diet accelerated insulin resistance and impairment of insulin secretion, we fed rats on a diet containing high LPO content, which was made by removing α-tocopherol from the laboratory chow, for 10 weeks. We then assessed insulin sensitivity using steady-state blood glucose (SSBG) method and insulin secretion by the oral glucose tolerance test (OGTT). Furthermore, to investigate the causes of insulin resistance, we measured the levels of insulin receptor substrate (IRS)-1 in skeletal muscle and analyzed pancreatic β cells by immunohistochemical approach.

2. Materials and methods

2.1. Animals and the experimental diet

Five-week-old male Sprague–Dawley rats weighing 110–130 g were divided into two groups. Control rats were fed on a chow for 10 weeks. High LPO laboratory chow was made by removing vitamin E and subjecting air-dried for 7 days to increase LPO content in the chow. The LPO containing diet was fed for 10 weeks in the experimental rats (LPO-fed rats). Standard chow contained 69% carbohydrates (sucrose and corn starch), 21% protein (mainly casein), 10% fat (mainly safflower oil), and a vitamin and mineral mixture (Clea, Japan) as described elsewhere [15]. The α-tocopherol-free laboratory chow contained the same components as the standard chow except vitamin E. The LPO levels in LPO-containing chow was significantly higher than that of the control chow (5.9 ± 1.8 nmol/g, 0.5 ± 0.1 nmol/g, respectively; $P < 0.01$). To study the effect of vitamin E supplementation on LPO contents and biological effects, 30 mg of vitamin E was added to 100 g of LPO-containing chow, which was three times higher than that in standard chow. Moreover, to clarify the time-dependent and dose-dependent effects of vitamin E-deficient diet on LPO production, three different amounts of LPO containing chow (50, 70 and 100% amount of LPO chow) were arranged by mixing the control and vitamin E-deficient chow. Then rats were fed on these chows for 10 days to measure plasma LPO and vitamin E levels.

2.2. Measurement of lipid peroxide content

The LPO content of the laboratory chow, plasma, and soleus muscle were assessed by measuring the reaction products of malondialdehyde, one of the thiobarbituric acid-reactive substances (TBARS) [16]. Plasma LPO concentrations were adjusted for the plasma levels of total cholesterol and triglyceride. To prevent artificial auto-oxidation under the assay condition, *t*-butyl-4-hydroxyanisole, at a final concentration of 10 μmol/l, was added to the specimens. The LPO content in the specimens was measured within 3 weeks. Vitamin E concentrations were measured by HPLC (high performance of liquid chromatography).