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Original Research

High Fat Content in Diets and Oxidative Stress in Livers of Non-Diabetic and Diabetic Rats

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Abstract

Environmental factors such as high fat content in a diet affect pro- and antioxidative balances in tissues. Our study was designed to determine whether a four-week diet enriched to a total of 15% fat content with either a polyunsaturated (linoleic) or saturated (palmitic) fatty acid predisposes or protects the liver tissue against oxidative stress in both non-diabetic and diabetic rats. In the rat liver the activity of catalase, superoxide dismutase, glutathione peroxidase, and the level of thiobarbituric acid reactive substances were determined. Our study suggests that both diets induce oxidative stress in livers of non-diabetic rats. However, in diabetic rats a diet enriched in linoleic acid appears to attenuate oxidative stress.

Keywords: oxidative stress, liver, fat diet, diabetes

Introduction

Environmental factors, including nutrition, affect the risk, progression and mortality in type 1 and 2 diabetes mellitus [1-4]. Dietary fat content and composition have been considered the most important factors [1, 3, 4]. Detrimental effects of excessive fat intake were initially attributed solely to the excess of saturated fat and cholesterol in diets. However, in the last two decades of the 20th century oxidative stress mechanisms, including lipid peroxidation, began to be recognized. Since then the initial views on the beneficial effects of dietary unsaturated fatty acids and disadvantageous effects of saturated fatty acids continue to be verified.

The effect of dietary fat content and composition on tissue oxidative status is undoubted [5-9], as is the fact that diabetes is a factor that induces oxidative stress in human and animal tissues [10, 11]. However, available data concerning these issues are contradictory. It remains unclear whether a diet rich in either saturated or polyunsaturated fatty acid protects or rather predisposes the tissues to the oxidative stress and which diet should be considered as a potentially harmful factor in healthy and diabetic subjects. Diets rich in saturated fatty acids have been reported to create oxidative stress-resistant states in the liver and protect it against ethanol-induced oxidative stress [5, 6]. In other reports, long-term feeding of a high-saturated fat diet acts as an inducer of oxidative stress, since it significantly attenuates the hepatic enzyme antioxidant system [7], and increases the levels of lipid peroxidation products in the liver [7] and plasma [8]. A diet rich in linoleic acid has been found to increase oxidative stress in the liver [9]. However, in diabetic subjects, some polyunsaturated fatty acids appear to attenuate rather than promote diabetes-induced oxidative stress [12].

Because of these limited and contradictory data, the present study was designed to determine the effect of a four-week diet enriched in the individual and nutritionally significant fatty acid, either a polyunsaturated fatty acid – linoleic acid (LA – C18:2 ω-6) or saturated fatty acid – palmitic acid (PA – C16:0) on the activity of antioxidant enzymes: catalase (CAT), superoxide dismutase (Cu, Zn-SOD), glutathione peroxidase (GSH-Px), and the level of thiobarbituric acid reactive substance (TBARS) in the rat liver of both non-diabetic subjects and individuals with streptozotocin-induced diabetes.

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Experimental Procedures

Healthy, adult female Wistar rats weighing 230 ± 20 g were used for the study. The animals were placed in a temperature- and light-controlled room with free access to food and water. The experiments were always performed at the same daily periods. Our study was approved by the local ethics committee.

The rats were divided into three groups of 16 animals each. The first group was fed a standard laboratory diet (Labofeed B, 3.5% fat content). The second group was fed a diet composed of the standard diet enriched to a total of 15% fat content with linoleic acid (C18:2 ω-6, Sigma) - the LA diet. The third group was fed a standard diet enriched to a total of 15% fat content with palmitic acid (C16:0, Sigma) – the PA diet. After two weeks of feeding, diabetes was induced in half of the animals in each group with a single dose of streptozotocin (Sigma) at 65 mg/kg body weight administered intraperitoneally. Streptozotocin (STZ) was dissolved in 1.0 ml of 0.1 mol/l citrate buffer pH 4.5. Diabetes was verified by the measurement of plasma insulin level using a radioimmunoassay (Linco Research kit, St. Charles, MO, USA) and plasma glucose level (Cormay kit). The remaining rats, which served as non-diabetic groups of reference received 1.0 ml of 0.1 mol/l citrate buffer pH 4.5, intraperitoneally. Both diabetic and non-diabetic rats were fed their respective diets for the next two weeks. They were named as follow:

Non-diabetic rats:

Group I (control) - rats fed the standard laboratory diet,

Group II (LA diet) - rats fed the LA diet,

Group III (PA diet) - rats fed the PA diet,

Diabetic rats:

Group IV (diabetic control) - rats fed the standard laboratory diet with STZ-induced diabetes,

Group V (LA diet + STZ) - rats fed the LA diet with STZ-induced diabetes,

Group VI (PA diet + STZ) - rats fed the PA diet with STZ-induced diabetes.

Studies were performed after a total of four weeks of the experiment on non-diabetic and diabetic rats. The rats were anaesthetized with ketamine and xylazyne (90 mg/kg body weight and 10 mg/kg body weight, respectively, intraperitoneally) and blood samples were taken from the right ventricle. The animals were killed by cardiac excision and their livers were taken for analysis.

To obtain the enzyme assays the liver samples were homogenized in 10 vol. of 50 mM TRIS – 0.1 mM EDTA (pH 7.6) at 4ºC for 30 s with a Teflon homogenizer [13]. To prepare the postmitochondrial fractions, the homogenates were centrifuged for 20 minutes at 12,000 x g (+ 4ºC) in a Janetzki K-24 centrifuge [14]. The supernatant (postmitochondrial fractions) was used to measure CAT activity by the Beers and Sizer method [15]. The activity of CAT was expressed as Bergmayer units per gram of protein (1.0 Unit of Bergmayer is the amount of enzyme that catalyzes the breakdown of 1000 mg of hydrogen peroxide per minute at + 25ºC, pH 7.0). The cytosolic fractions were prepared by supernatant centrifuging for 20 minutes at 105,000 x g (+ 4ºC) in a Janetzki VAC 602 ultracentrifuge. In the supernatant, the activity of Cu, Zn-SOD and GSH-Px was measured [14]. Cu, Zn-SOD activity was determined in terms of its ability to inhibit the autoxidation of epinephrine to adrenochrome in alkaline solution [16]. It was expressed as units of Cu, Zn-SOD per milligram protein (1.0 U is the amount of enzyme that causes a 50% inhibition of the reaction at a rate of the absorbency increase of 0.025 unit per minute). The activity of GSH-Px was measured by the Little and O'Brien method [17], with cumene hydroperoxide as a substrate. It was expressed in units per gram protein (1.0 Unit is the amount of enzyme that decreases GSH content by 10% of the initial value in 1 minute at pH 7.0).

Protein was measured by the Lowry et al. technique [18].

The concentration of thiobarbituric acid reactive substance (TBARS) was measured by the Okhawa et al. method [19], based on the reaction of thiobarbituric acid (TBA) with malondialdehyde (a product of lipid, protein, carbohydrate, and DNA peroxidation) and other TBA-reactive compounds.

The values are expressed as means ±SD. Statistical analysis was performed with two-tailed Student's t-test for unpaired variables at a significance level of $p < 0.05$, n=8.

Table 1. The level of plasma glucose and insulin in the rats fed the standard, LA or PA diet with and without STZ-induced diabetes.

	n	Glucose mM/l x ± SD	Insulin μM/ml x ± SD
Non-diabetic rat			
Group I (control)	8	7.93 ± 0.31	51.44 ± 1.80
Group II (LA diet)	8	8.42 ± 0.57	53.11 ± 2.05
Group III (PA diet)	8	8.29 ± 0.79	55.89* ± 2.32
Diabetic rats (*STZ-induced diabetes*)			
Group IV (diabetic control)	8	21.97* ± 1.39	2.96* ± 0.51
Group V (LA diet + STZ)	8	22.37 ± 1.17	3.89 ± 1.52
Group VI (PA diet + STZ)	8	22.56 ± 0.98	4.11† ± 1.32

STZ - streptozotocin
values are expressed as means ± SD;
n – the number of rats in the group
* $P < 0.05$ vs. non-diabetic control
† $P < 0.05$ vs. diabetic control

Results

STZ administration to the control group significantly increased plasma glucose concentration by 177.1% (from 7.93 ± 0.31 to 21.97 ± 1.39, $P < 0.05$) and decreased plasma insulin level by 94.3% (from 51.44 ± 1.80 to 2.96 ± 0.51, $P < 0.05$) compared to the control values (Table 1).

Table 2. The activity of antioxidant enzymes and the level of MDA in the liver of rats fed the standard, LA diet, or PA diet with and without STZ-induced diabetes.

	n	CAT	GSH-Px	Cu, Zn-SOD	TBARS
		Bergmayer U/ g protein	U/g protein	U/mg protein	nmol/g tissue
		x ± SD	x ± SD	x ± SD	x ± SD
Non-diabetic rats					
Group I (control)	8	21.47 ± 0.78	79.65 ± 9.99	41.01 ± 2.19	17.0 ± 1.2
Group II (LA diet)	8	37.53* ± 2.88	238.96* ± 9.71	62.13* ± 5.25	23.6* ± 1.9
Group III (PA diet)	8	26.03* ± 2.52	86.82 ± 8.12	17.01* ± 3.25	19.7* ± 1.1
Diabetic rats (STZ-induced diabetes)					
Group IV (diabetic control)	8	6.57* ± 1.76	140.09* ± 8.80	27.55* ± 2.96	30.5* ± 1.8
Group V (LA diet + STZ)	8	12.42†a ± 1.57	183.41†a ± 9.52	20.34†a ± 2.50	26.3†a ±1.3
Group VI (PA diet + STZ)	8	9.75†b± 1.35	147.32b ± 7.60	22.19†b ± 3.99	29.1b ±1.2

STZ - streptozotocin
values are expressed as means ± SD;
n – the number of rats in the group
* $P < 0.05$ vs. non-diabetic control
† $P < 0.05$ vs. diabetic control
a $P < 0.05$ vs. group II (non-diabetic rats - LA diet)
b $P < 0.05$ vs. group III (non-diabetic rats - PA diet)

Feeding the rats the LA diet did not significantly change glucose and insulin levels in the plasma of both the non-diabetic and diabetic animals, while the PA diet resulted in increased plasma insulin level in both the non-diabetic and diabetic animals by 8.7% (from 51.44 ± 1.80 to 55.89 ± 2.32, $P < 0.05$) and 38.9% (from 2.96 ± 0.51 to 4.11 ± 1.32, $P < 0.05$), respectively, in comparison to the respective control groups (Table 1).

In the non-diabetic rats, a diet enriched in LA caused a significant increase in the activity of all determined antioxidant enzymes: CAT activity by 74.8% (from 21.47 ± 0.78 to 37.53 ± 2.88, $P < 0.05$), GSH-Px activity by 200.0% (from 79.65 ± 9.99 to 238.96 ± 9.71, $P < 0.05$), and Cu, Zn-SOD activity by 51.5% (from 41.01 ± 2.19 to 62.13 ± 5.25, $P < 0.05$) that was associated with a rise of TBARS level by 38.8% (from 17.0 ± 1.2 to 23.6 ± 1.9, $P < 0.05$) compared to the non-diabetic values (Table 2). Feeding the control rats the PA diet increased the activity of CAT by 21.2% (from 21.47 ± 0.78 to 26.03 ± 2.52, $P < 0.05$) and decreased that of Cu, Zn-SOD by 58.5% (from 41.01 ± 2.19 to 17.01 ± 3.25, $P < 0.05$) with concomitant increase in the level of TBARS by 15.9% (from 17.0 ± 1.2 to 19.7 ± 1.1, $P < 0.05$) in comparison to the non-diabetic control (Table 2).

The administration of STZ to the rats fed the standard diet significantly lowered the activity of CAT by 69.4% (from 21.47 ± 0.78 to 6.57 ± 1.76, $P < 0.05$) and Cu, Zn-SOD by 32.8% (from 41.01 ± 2.19 to 27.55 ± 2.96, $P < 0.05$), and increased the activity of GSH-Px by 75.9% (from 79.65 ± 9.99 to 140.09 ± 8.80, $P < 0.05$) and the level of TBARS by 79.4% (from 17.0 ± 1.2 to 30.5 ± 1.8, $P < 0.05$) compared to the control values (Table 2).

The LA diet partially neutralized diabetes-induced decline in hepatic CAT activity, producing an increase in CAT activity by 89.0% (from 6.57 ± 1.76 to 12.42 ± 1.57, $P < 0.05$) compared to the diabetic control, but enhanced STZ-induced changes in Cu, Zn-SOD and GSH-Px activity (Table 2). In comparison to diabetic control, the activity of Cu, Zn-SOD was reduced by 26.2% (from 27.55 ± 2.96 to 20.34 ± 2.50, $P < 0.05$), while that of GSH-Px was increased by 30.9% (from 140.09 ± 8.80 to 183.41 ± 9.52, $P < 0.05$) in the LA group with STZ-induced diabetes (Table 2). TBARS level was decreased by 13.8% (from 30.5 ± 1.8 to 26.3 ± 1.3, $P < 0.05$) compared to the diabetic control (Table 2).

In diabetic animals fed the PA diet, CAT activity was increased by 48.4% (from 6.57 ± 1.76 to 9.75 ± 1.35, $P < 0.05$), while the activity of Cu and Zn-SOD was decreased by 19.5% (from 27.55 ± 2.96 to 22.19 ± 3.99, $P < 0.05$) compared to the diabetic control (Table 2). PA diet did not modify diabetes-induced changes in the activity of GSH-Px and the level of TBARS (Table 2).

Discussion

Our study suggests that high consumption of lipids promotes an oxidative stress in the liver of non-diabetic rats. Oxidative stress–inducing effect appears to be evoked by both the linoleic and palmitic acid-enriched diets. However, in diabetic subjects, the LA diet appears to attenuate STZ-induced oxidative stress, while the PA diet does not seem to modify it effectively.

Feeding the non-diabetic rats both the LA and the PA diets caused a significant increase in the activity of CAT; if compared to the diabetic control, higher activity of hepatic catalase was also observed in the diabetic rats fed both diets (Table 2). High fat diets increase oxidation of fatty acids through the peroxisomal oxidation pathway [20] that is associated with increased generation of hydrogen peroxide (H_2O_2). Catalase, an enzyme localized in peroxisomes, catalyses dismutation of H_2O_2 [21]. Therefore, the increased activity of CAT would suggest a compensatory response of hepatic defence system under condition of enhanced H_2O_2 generation. In vitro studies have shown that H_2O_2 increases CAT mRNA levels [22, 23], but inhibits the action of superoxide dismutase [24].

Both in the non–diabetic and diabetic rats, the PA diet resulted in reduced activity of hepatic Cu, Zn-SOD (Table 2). Since insulin may induce the synthesis of SOD and activate its proenzyme [25], either insulin resistance present in the rats fed a saturated fat diet [26] or hypoinsulinemia found in diabetic animals (Table 1) may contribute to the decrease in Cu, Zn-SOD activity observed under such conditions. Especially that consuming a saturated fat diet enhances a diabetes-induced drop of Cu, Zn-SOD activity (Table 2).

It has been reported recently that insulin increases, while conditions associated with insulin resistance decrease, fatty aldehyde dehydrogenase (FALDH) gene expression in rat liver [27]. FALDH appears to detoxify the oxidized lipid species, thus acting against oxidative stress associated with lipid peroxidation [27]. Therefore, under conditions of hypoinsulinemia and/or insulin resistance, deregulation of FALDH may be considered one of the factors involved in the oxidative stress mechanisms and increased TBARS production. Both in the control and diabetic rats the PA diet did not modify the activity of GSH-Px (Table 2) that catalyzes the reduction of hydrogen and organic peroxides, including lipid peroxides [21]. Unchanged activity GSH-Px observed under conditions of increased generation of reactive oxygen species [20] and reduced Cu, Zn-SOD activity may be partly responsible for increased levels of TBARS observed in control rats fed the PA diet. However, in diabetic rats, the PA diet did not affect an STZ-induced increase in the level of hepatic TBARS.

Feeding the non-diabetic rats a diet rich in LA resulted in increased activity of all determined antioxidant enzymes in the liver (Table 2). In spite of this, the hepatic TBARS level increased (Table 2). Our data confirm that in healthy subjects, high dietary content of linoleic acid acts itself as an inducer of oxidative stress [5]. However, in diabetic subjects the LA diet slightly, but significantly, reduces the diabetes-induced increase in the level of TBARS (Table 2). This effect, at least in part, may be attributed to diet-induced changes in the activity of antioxidant enzymes, especially of GSH-Px, that controls the rate of lipid peroxidation [21]. STZ-induced increase in GSH-Px activity, unaffected by the PA diet, was enhanced by feeding the rats the LA diet (Table 2). Besides, the LA diet partially prevented STZ-induced decrease in CAT activity and this effect was stronger than that observed in diabetic rats fed the PA diet (Table 2). In the diabetic rats fed the LA diet, the activity of CAT and GSH-Px finally appears to compensate for a marked decrease in the third enzymatic component of the antioxidative barrier – the activity of Cu, Zn-SOD (Table 2).

Conclusions

Our study suggests that a four-week diet rich in either palmitic acid or linoleic acid induces an oxidative stress in the rat liver of non-diabetic rats, as shown by increased levels of hepatic TBARS and changes in the activity of antioxidant enzymes. Therefore, in healthy subjects high content in a diet of both saturated (palmitic) and polyunsaturated (linoleic) fatty acids should be considered a potentially harmful factor with respect to pro- and antioxidative balance in the liver. However, in diabetic rats a diet rich in linoleic acid partially prevents STZ-induced generation of peroxidation products, thus it appears to attenuate the oxidative stress in the liver. A diet rich in palmitic acid does not seem to modify diabetes-induced oxidative stress effectively, since it does not change the level of TBARS, despite significant diet-induced changes in antioxidant enzyme activity.

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