ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Impact of Obesity on Malondialdehyde and Certain Antioxidants in North Indian Obese Punjabi Population.

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ABSTRACT

Obesity is one of the most common health problems in developed and developing nations like India. In addition, obesity is considered as a principal risk factor in the initiation of various non-communicable chronic diseases such as dyslipidemia, atherosclerosis, cardiovascular, non-insulin dependent diabetes mellitus etc. The pathogenesis of these diseases is associated with oxygen-derived free radicals. The aim of the study was to analyze oxidative status in obese subjects with respect to normal healthy subjects. The present study was performed in 50 obese men (BMI >46kg/m^2) and equal number of normal healthy men (BMI < 27kg/m^2) and the levels of malondialdehyde, antioxidant enzymes/molecules like superoxide dismutase, catalase reduced glutathione, glutathione reductase, glutathione peroxidase, antioxidant vitamin-E & vitamin-C along with total antioxidant activity were evaluated. A significant increase ($p < 0.01$) in the concentration of malondialdehyde by 61.07% was observed in obese men subjects in comparison to normal healthy men subjects while a significant fall was recorded in the levels of vitamin-E (19.54%, $p < 0.05$), vitamin-C (18.07%, $p < 0.05$), superoxide dismutase (19.51%, $p < 0.05$), catalase (20.28%, $p < 0.05$), GSH (36.56%, $p < 0.05$), GR (33.28%, $p < 0.01$),GPx (19.14%, $p < 0.05$) and a significant decrease by 33.33% ($p < 0.01$) was observed in the total antioxidant activity in obese subjects as compared to normal healthy control subjects. All these observations suggested that oxidative stress induced in the obese subjects (BMI > 46kg/m^2) of North Indian Punjabi population even in the absence of non-communicable chronic diseases and hence should be treated with same attention as the obesity with complications such as diabetes mellitus, hypertension, hyperlipidaemia, renal or liver disease, smoking, alcohol.

Keywords: Obesity, Oxidative stress, Vitamin-E, Vitamin-C, Lipoproteins, Lipid peroxidation, antioxidants and cardiovascular disease (CVD).

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ISSN: 0975-8585

INTRODUCTION

Obesity is a growing epidemic in developed and developing nations like India, with the estimated prevalence ranging from 10 to 50 percent or more in the adult population [1]. Many studies had confirmed the role of obesity in diabetes mellitus, hyperlipidemia, colon cancer, sudden death and other cardiovascular diseases has been confirmed [2-4]. It is also well reported in the literature that oxidative stress is responsible for the pathophysiology of all these disease [3, 5-7]. Many cellular processes such as control of membrane permeability, secretion of extra cellular matrix proteins, cell proliferation, cell migration and cell deaths are found to be promoted by oxygen free radicals that have contributed to the development of diabetes and cancer [5]. Free radicals may produce their deleterious effects by; a) Deoxidizing cell membrane lipids thus altering the structural integrity of the membrane; b) Peroxidizing the lipids in maintaining the micro environment of membrane proteins or reacting both with proteins and with membrane lipids separately or concurrently.

In addition, oxygen radicals and products of the reaction may alter the structure of DNA and RNA. It is apparent therefore that an excessive stress imposed upon the cell could lead to the cellular modifications and thereby, essential chemical components of the cells leading to alter function. The cell and plasma contain a variety of antioxidants and free radical scavenging molecules and enzymes like superoxide dismutase (SOD), catalase (CAT), xanthine oxidase (XOD), glutathione (GSH), glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-S-Transferase (GST) etc., which under normal circumstances help the cell to maintain a reducing environment by preventing the potentially deleterious effects of free radical on cell membrane and organelles [8]. Any changes in the activity of these enzymes and antioxidants increase oxidative stress and could lead to the development of various diseases like cardiovascular diseases, arthritis, diabetes, cancer etc.

Although the exact biochemical mechanisms responsible for the association between obesity and the development of various non-communicable diseases like atherosclerosis, arthritis, diabetes, cancer etc. have not been completely elucidated, and data on the change in antioxidant molecules/enzymes such as SOD, CAT, GR, GPx, GSH is scant in obese North punjabi population of Indian. So, the present work was designed, to study the change in oxidative stress markers by analyzing MDA, SOD, CAT, GSH GR, GPx along with change in antioxidant vitamins such as vitamin-E & vitamin-C levels in obese North Punjabi population of India.

MATERIAL AND METHODS

The present study was carried out in the Department of Biochemistry, Govt. Medical College Amritsar on 50 male obese individuals in age group ranging from 20 to 35 years with BMI > 46kg/m^2. The obese subjects, who were suffered with complication such as diabetes mellitus, hypertension, hypothyroidism, renal failure, hepatic diseases, acute illnesses, recurrent myocardial infarction, unstable angina and those not on any weight lose treatment & inherited obese individuals were not taken for the study and equal number of normal healthy male subjects in same age group ranging from 20 to 35 years with BMI < 27 kg/m^2 were taken as control group.

All subjects recruited for the study were vegetarian, non-smoker and non-alcoholic, and there was no positive family history of diabetes, cardiovascular diseases (CVD) in these subjects. These subjects were interviewed and by giving a questionnaire regarding the detailed information on their lifestyle, medical history, diet etc. and after obtaining the written consent these were considered for the study.

Study design

This study was a case control cross-sectional prospective study. This study protocol was approved by the Ethical Committee of the Institution. A detailed history, physical, and systemic examination, including measurement of height, weight, heart rate, blood pressure, and body mass index (BMI), complete lipid profile, fasting and post-pranendial glucose levels was carried out in every subject who entered the study as per a pre-designed proforma for assessing the signs of chronic heart failure, diabetes and also the presence of any exclusion criteria.

Data Collection

Measurements of Anthropometric Parameters

The examination of body weight was done by taking weight in kilogram (kg) and height was measured in centimeters. The BMI was calculated from the formula: BMI = weight in kg/(height in meters)^2.

Blood sampling

A volume of 5 ml of peripheral venous blood was collected by vein puncture using a dry, disposable syringe between 8 and 9 AM after an overnight fast from both groups (control and Obese patients) in heparin containing vial. Blood samples were immediately centrifuged at 4000 rpm at 4 ◦C and plasma was stored at -20 ◦C until analysis. The 100µl of RBC were taken and lysed with 2.0ml ice cold water and the clear lysate obtained after spinning down the cell debris at 8500 g for 10 min at 4 ◦C was used for various biochemical assays.

Biochemical Assays

Estimation of Glucose levels: Fasting and post pranendial glucose levels were estimated spectrophotometrically using an enzymatic test kit based on GOD-POD method supplied by Transasia Biomedical Private Limited, Mumbai (India).

Estimation of Lipoprotein levels: The total cholesterol, triglyceride, high density lipoprotein cholesterol, were analyzed by using commercial available kits procured from Transasia Biomedical Private Limited, Mumbai (India). Low density lipoprotein-cholesterol levels were estimated by the primary measurements by using the empirical equation of Bates and Warren, 1989 [9]. Serum LDL-cholesterol= Total cholesterol - [HDL + Triglyceride/5] and very low density lipoprotein cholesterol was calculated by dividing triglyceride concentration with 5.

Estimation of hemoglobin (%): Hemoglobin (Hb) from whole blood was estimated spectrophotometrically by using a kit supplied by Transasia Biomedical Private Limited, Mumbai (India).

Estimation of malondialdehyde (MDA) levels: MDA level, an end product of lipid peroxidation of erythrocytes was assayed using a diagnostic kit supplied by Transasia Biomedical Private Limited, Mumbai (India).

Estimation of Superoxide dismutase (EC 1.15.1.1): SOD levels were measured in erythrocyte by using Jaiswal et al[10] method in RBC hemolysate.

Estimation of Catalase (EC 1.11.1.6): CAT activity was measured spectrophotometrically according to the method of Al-Essa et al[11] in erythrocyte by following the decomposition rate of H_2O_2 at 240 nm.

Estimation of Reduced Glutathione (GSH): The levels of GSH from whole blood was estimated by applying the method of Giustarini et al[12].

Estimation of Glutathione peroxidase (EC 1.11.1.9): The activity of GPx in erythrocytes was estimated spectrophotometrically by applying the method of Paglia et al [13].

Estimation of Glutathione reductase (1.6.4.2): Erythrocyte GR activity was determined by using the method described by Worthington et al[14].

Estimation of Vitamin C: Plasma vitamin C was estimated by Natelson, 1971 [15] dinitrophenyl hydrazine method. Vitamin C is oxidized to diketogluconic acid which reacts with 2, 4-dinitrophenyl hydrazine to form diphenylhydrazone. The hydrazone dissolves in strong acid solution to form orange-red colored complex at 520 nm. Vitamin C was expressed in mg/dl.

Estimation of Vitamin E: Plasma vitamin E was estimated by the method of Baker and Frank, 1968 [16]. Vitamin E reduces ferric ion to ferrous ion, which forms a red colored complex with α, α'-bipyridyl. Vitamin E was expressed in mg/dl.

Estimation of Total Antioxidant Activity (TAA): Plasma total antioxidant capacity was estimated in plasma by the FRAP (Ferric Reducing Ability of Plasma) assay by applying the method of Benzie & Strain, 1996 [17].

Statistical Analysis

The data was expressed as Mean ± SD and analyzed with the SPSS 16.0.7 statistical software package. Differences between the obese and control subjects were evaluated using the Student's independent samples t test. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Anthropometric Parameters

The Anthropometric measurements of both obese and normal healthy control subjects are summarized in the Table-1. The body weight, height, BMI, BP systolic and BP-diasystolic was 137±25Kg , 236.6 ± 0.11cm, 46.33±0.40Kg/m^2, 127.45 ± 12.53 mmHg, 85.29 ± 8.74 mmHg respectively in obese subjects in comparison to 62±14Kg, 249.12± 0.16cm, 26.80±0.48Kg/m^2, 128.15 ± 10.53 mmHg and 85.01 ± 7.32 mmHg respectively of healthy control subjects.

Glucose, Hemoglobin and Lipid levels

The levels of fasting and post pranendial glucose levels, hemoglobin and lipoprotein fractions are summarized in Table-1. The fasting and post pranendial plasma glucose levels was 89.65 ± 11.21mg/dl &124 ± 8.29mg/dl respectively in obese patients with respect to 84.56 ± 9.23mg/dl & 121.16 ± 9.11mg/dl fasting and post pranendial glucose levels in healthy control subjects. A hemoglobin level 13.89 ± 2.91g/dl in obese subjects and 14.81 ± 1.16 g/dl in normal healthy subjects was observed. The lipoprotein fractions such as total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol and VLDL-cholesterol levels was 178.70±34.57mg/dl, 156.96±24.62mg/dl, 109.5±20.6mg/dl, 38±18.70mg/dl, 31.8±14.65mg/dl in obese patients in comparison to 140.88±28.92mg/dl, 104.45±18.65mg/dl, 94.85±15.40mg/dl, 45.8±14.60mg/dl & 21.1±11.41mg/dl total cholesterol, triglycerides, LDL-cholesterol, HDL- cholesterol and VLDL- cholesterol respectively in normal healthy control subjects. There were statistical significant difference in the glucose, hemoglobin and various lipid levels in obese and normal healthy subjects and these biochemical parameters were remained within the normal limit.

Lipid Peroxidation

A significant increase was recorded in the MDA levels (from 132.93±11.23 to 214.12±12.27 nmol MDA/gHb) in obese subjects by 61.07% ($p < 0.001$) with respect to normal healthy subjects (Figure-1)

Antioxidants vitamin and Enzymes

A significant decrease ($p<0.05$) was seen in plasma vitamin-E (From 0.87 ± 0.16 to 0.70± 0.10mg/dl) & vitamin- C (From 0.83 ± 0.06 to 0.68 ± 0.06 mg/dl) by 19.54% and 18.07% respectively in obese with respect to normal healthy subjects (Figure-2). A significant decrease in the levels of SOD (from 239.52 ± 8.37 to 192.78 ± 6.72U/g Hb), CAT (from 81.4 ± 2.71 to 69.89 ± 4.19 mg/g Hb), GSH (71.55 ± 1.92 to 35.39 ± 2.14 mg/g Hb), GR (from 45.4 ± 3.16 to 30.29 ± 3.62) and GPx (from 39.49 ± 2.61 to 32.93 ± 3.92mg/gHb) by19.51% ($p < 0.05$), 20.28% ($p < 0.05$), 36.56% ($p < 0.001$), 33.28% ($p < 0.001$), 19.14% ($p < 0.05$) respectively was observed in erythrocyte of obese patients in comparison to normal healthy control subjects (Figure-1).

Total Antioxidant Activity

The levels of Total Antioxidant Activity was found 799.26 ± 19.69 µ mol /L in plasma of obese individuals and 613.23± 11.07 µ mol /L in normal healthy control individuals. This fall in plasma total

ISSN: 0975-8585

antioxidant activity by 23.30% was found to be statistically significant ($p < 0.01$) in obese subjects with respect to control healthy subjects (Figure-3).

Table 1: General characteristics of normal healthy control and obese population

Anthropometric and Biochemical Assays	Normal Healthy Control Subjects	Obese Subjects
Subject Number	50	50
Sex	Male	Male
Height (cm)	158.12± 0.16	161.6 ± 0.11
Weight (kg)	67.00±14	121.00±25
Age (years)	31.17±5.45	33.34±6.51
Body mass index (Kg/m^2)	26.80±0.48	46.33±0.40
Blood pressure systolic (mmHg)	128.15 ± 10.53	127.45 ± 12.53
Blood pressure diasystolic (mmHg)	85.01 ± 7.32	85.29 ± 8.74
Hemoglobin (g/dl)	14.81 ± 1.16	13.89 ± 2.91
Fasting Glucose (mg/dl)	84.56 ± 9.23	89.65 ± 11.21
Postprandial Glucose(mg/dl)	121.16 ± 9.11	124.00 ± 8.29
Total Cholesterol (mg/dl)	140.88±28.92	158.70±34.57
Triglycerides (mg/dl)	80.01±18.65	86.06±24.62
LDL- Cholesterol (mg/dl)	94.85±15.40	96.09±16.05
HDL- Cholesterol (mg/dl)	45.80±14.60	48.09±18.70
VLDL- Cholesterol (mg/dl)	16.00±11.41	17.01±14.65

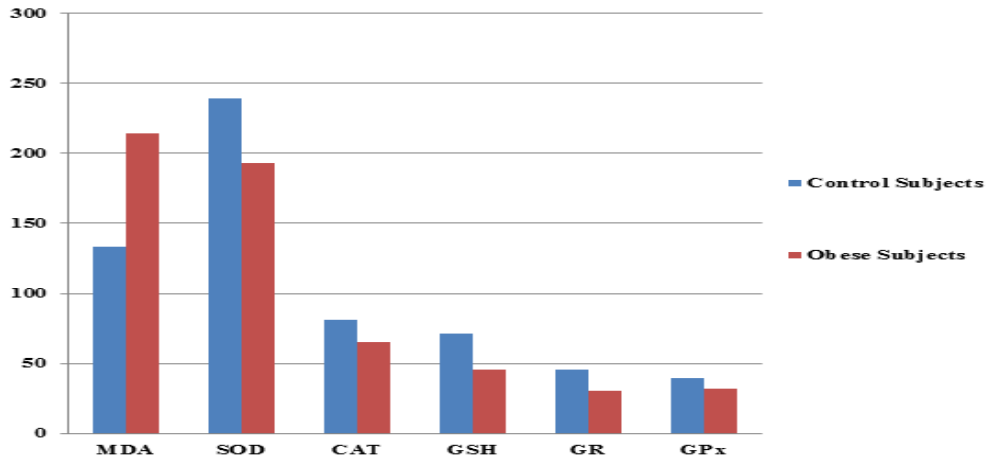

Figure 1: Changes in MDA, SOD, CAT, GSH, GR and GPx in normal healthy controls subjects (*n* = 50) and obese patients (*n* = 50). Values in figure are expressed as mean ± standard deviation.
The levels of MDA are expressed in nmol/g Hb, SOD levels are expressed in U/g Hb, and levels of CAT, GSH, GR & GPx are expressed in U/g Hb, mg/g Hb, mU/g Hb, and mU/g Hb respectively.

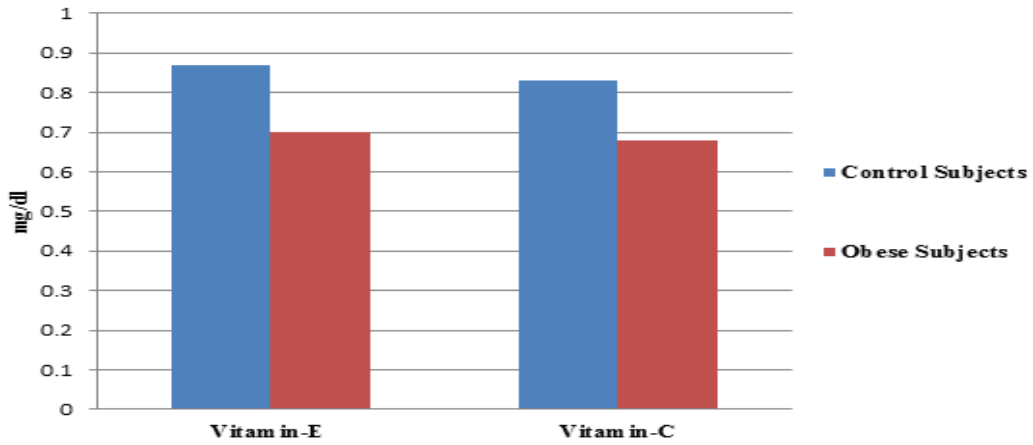

Figure 2: Alterations in vitamin –E and Vitamin-C levels in normal healthy controls subjects (*n* = 50) and obese patients (*n* = 50). Values in figure are expressed as mean ± standard deviation.
The levels of vitamin –E and Vitamin-C are expressed in mg/dl.

ISSN: 0975-8585

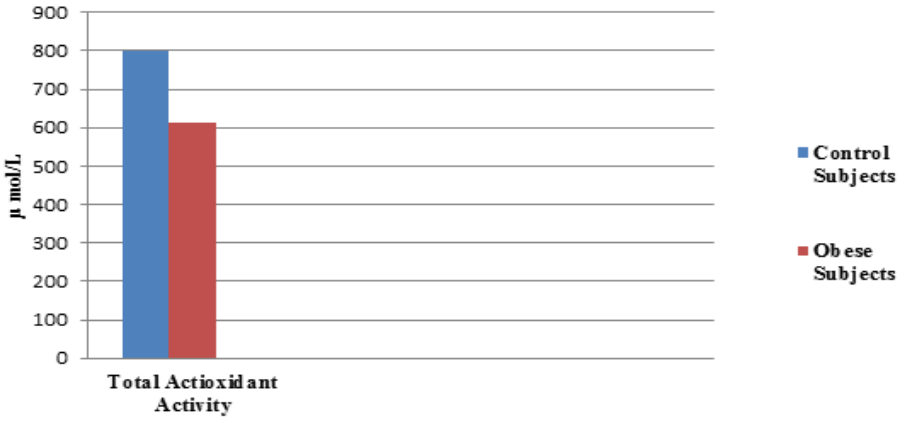

Figure 3: Changes in total antioxidant activity in normal healthy controls subjects (*n* = 50) and obese patients (*n* = 50). Values in figure are expressed as mean ± standard deviation. The levels of total antioxidant activity are expressed in µ mol/L.

DISCUSSION

In the present case control study, we recorded a significant increase ($p< 0.001$) by 61.07%) in MDA (representing the lipid peroxidation) levels in obese individuals even in the absence of any chronic diseases such as diabetes, hypertension, hyperlipidemia etc in comparison to normal healthy individuals (Figure-1). Lipid peroxidation causes polymerization of membrane components and induce changes in the membrane permeability resulting in hemolysis, would relate to the degree of intravascular red blood cell (RBC) destruction. Extravascular mechanisms of RBC destruction may involve changes in cell deformability and antigenicity. Our observation of increased malondialdehyde levels is agreement with the literature reports and to decrease the membrane fluidity, deformability, visco-elasticity & life span of erythrocytes in the obese subjects[18-21], which might be responsible for the initiation of complications such as diabetes, hypertension, hyperlipidemia etc. in obese subjects later on in the north western Indian population.

The biological effects of free radicals are normally controlled in vivo by a wide range of antioxidants such as vitamin E, the main liposoluble chain breaking antioxidant in human beings, scavenges peroxyl radicals produced during lipid peroxidation. Reduced glutathione and vitamin C regenerate vitamin E. A significant decreased in vitamin-E and vitamin-C, have potential to protect both cytosolic and membrane components of cells from oxidant damage [21] levels were falls significantly in the plasma of obese subjects with respect to normal healthy subjects (Figure-2) indicated the accumulation of free radicals.

SOD, a superoxide radical scavenging enzyme is considered the first line of defense against the deleterious effect of oxygen radicals in the cells and it scavenges reactive oxygen radical species by catalyzing the dismutation of O_2- radical to H_2O_2 and O_2 [5-8] A significant inhibition by 25.63% in SOD activity in diabetics (Figure-1) may results in an increased flux of $O_2^{\cdot}$- radical and hence reflects the tissue damage/injury. The activity of CAT, another potent antioxidant enzyme, especially against the $O_2^{\cdot-}$ radicals and singlet oxygen, was also found to significantly ($p< 0.01$) decreased by 20.28% in erythrocyte of obese subjects by with respect to control subjects (Figure-1) CAT, protects cells from the accumulation of H_2O_2 by dismutating it to form H_2O and O_2 or by using it as an oxidant, in which it works as a peroxidase[6, 22]. Therefore, the decrease in the activity of CAT suggested the accumulation of H_2O_2 in the obese subjects.

Glutathione (GSH), a tripeptide is maintained in reduced state by an efficient glutathione peroxidase/glutathione reductase system. Glutathione is a potent endogenous antioxidant that helps to protect cells from a number of noxious stimuli including oxygen derived free radicals [7, 23, 24]. In the present work, the level of glutathione significantly decreased by 36.56% ($p<0.01$) in obese subjects with respect to normal healthy control subjects (Figure -1). A significant fall in GSH levels, confirm an increased susceptibility to oxidative damage. The activity of GPx, a selenium-containing enzyme was found to be decreased by 19.14% ($p<0.05$) in obese individuals in comparison to control healthy individuals (Figure-1). GPx catalyses the reduction of variety of hydrogen peroxide (ROOH and H_2O_2) using glutathione as a substrate, thereby protecting mammalian cells against oxidative stress [25]. The significant inhibition ($p<0.001$, 44.53%) in the activity of GR in diabetics (Figure-1) attributed to increased oxidation or decreased synthesis of GSH. The less

availability of NADPH may also cause a decrease in GR activity [26]. Our observations of significant reduction in GSH levels are is an agreement with the literature reports that inverse relationship exists between LPO and GSH status, can impair the cell defense against the toxic action of xenobiotic and may lead to cell injury/death [24].A significant fall in GPx activity may render the tissue more susceptible to LPO damage. Accordingly, in the present work, we observed a significant decrease in GPx activity upon increase in LPO level. This observation is in accordance with the hypothesis that LPO and GPx might play a role in tissue damage [27-29] that oxidative stress is induced in the obese subjects. Our observations of decreased total antioxidant activity suggesting that oxidative stress induced in the obese subjects (Figure-3).

CONCLUSION

All these observations suggested that oxidative is induced in the obese subjects of North Indian Punjabi population even in the absence of chronic disease such as diabetes mellitus, hypertension, hyperlipidaemia, renal or liver disease by altering the levels of MDA, vit.-E, vit.-C, SOD, CAT, GSH, GR, GPx and therefore, obesity should be treated with the same attention. Further studies using antioxidant enzymes, vitamin supplementations alone and in combinations, lifestyle and dietary modification might be beneficial to reduce oxidative stress in obese individuals.

Abbreviations: CVDs: Cardiovascular disease; DM: Diabetes Mellitus; MDA: Malondialdehyde; LPO: lipid peroxidation; SOD: Superoxide dismutase; CAT: Catalase; GSH: Reduced Glutathione; GR: Glutathione Reductase; GPx: Glutathione Peroxidase; TAA: Total Antioxidant Activity; Vit.-E: Vitamin-E; Vit.-C: Vitamin-C; H_2O: Water; O_2: Oxygen; H_2O_2: Hydrogen Peroxide; O_2^{-}: Superoxide anion.

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