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Effect of *N. sativa* Oil on Impaired Glucose Tolerance and Insulin Insensitivity Induced by High-Fat-Diet and Turpentine-Induced Trauma

Mohammed A. Alsaif
Department of Community Health Sciences, College of Applied Medical Sciences,
P.O. Box 10219, King Saud University, Riyadh-11433, Saudi Arabia

Abstract: The aim of this study was to investigate the effect of *N. sativa* oil on impaired glucose tolerance and insulin insensitivity induced by high-fat diet and trauma. Three dietary groups were used in this study; Rat-Chow (RC), *N. sativa* oil diet (Combination 4% *N. sativa* oil and 16% butter oil) (NSOD) and 20% Butter Oil Diet (BOD). Each group was subdivided in two groups; control and trauma. Diets were supplemented for five consecutive weeks body weight increase per week was calculated. At end of the dietary treatments, single dose (2 mL kg^{-1} body weight) of turpentine was injected in the dorso-lumber region. Intravenous glucose tolerance test (iv GTT) was performed, insulinogenic index and insulin sensitivity was measured. The results showed butter oil diet significantly increased the body weights and visceral fats compared other two groups, respectively. Fasting glucose levels did not change in trauma induced rats while insulin levels increased significantly and it found highest in butter oil diet fed animals. Impaired glucose tolerance was found sever in BOD fed traumatized rats. *N. sativa* oil diet protected impaired glucose tolerance and insulin insensitivity induced either via saturated fatty acids or injury. In conclusion, *N. sativa* oil may be used in post surgery diabetic patients to prevent the long going adverse effects from surgical trauma.

Key words: *N. sativa* oil, butter oil, glucose tolerance, insulin sensitivity, turpentine

INTRODUCTION

Insulin resistance is a complex metabolic defect that most likely has several etiologies dependent on the pathophysiologic state. In humans, there is a genetic component to decreased insulin sensitivity in patients with type-2 diabetes mellitus (T2DM) and it has been suggested capacity to oxidize fatty acids is a contributory factor (Petersen *et al*., 2004). In addition obesity is a major cause of insulin resistance in T2DM and recent information demonstrates that chronic, low-grade inflammation associated with obesity is an important etiologic mechanism in decreasing insulin signaling (De Luca and Olefsky, 2008). Experimentally and clinically demonstrated that, consuming High-Fat (HF) diets causes as increase in body fat deposition and a decrease in insulin sensitivity, which ultimately leads to an increased risk of developing T2DM (Lovejoy *et al*., 2001; Storlien *et al*., 1991, 1993). It has also seen in our earlier studies that, saturated fat diet fed rats induced impaired glucose tolerance and decreased the insulin sensitivity compared to control animals (Alsaif, 2004; Alsaif and Duwaihy, 2004).

Hyperglycemia associated with insulin resistance is common alter trauma and surgical procedures (Frayn, 1986). Both reduced tissue insulin sensitivity and alterations in insulin release may contribute to the impaired glucose homeostasis. For example, insulin-mediated glucose disposal is impaired both in humans after injury, surgery, burns and sepsis (Strommer *et al*., 2002; Black *et al*., 1982; Thorell *et al*., 1994) and in laboratory animals with injury and sepsis (Barton and Passingham, 1980; Virkamaki and Yki-Jarvinen, 1994). Insulin has been shown to have a specific effect limiting net protein catabolism after trauma, independent of the source or number of calories provided. While more then 80% of the fuel required meeting the increased metabolic rate is derived from fat stores (Kinney, 1977), the superiority of glucose over fat as a protein sparing calorie source in catabolic states is probably related to its ability to excite an insulin response (Woolfson *et al*., 1977). Clinical and experimental studies have demonstrated than hyperglycaemia persisted for at least 24 h after severe injury (Davies, 1982), at which point to body's glycogen reserves are already exhausted indicating a state of impaired glucose tolerance. Furthermore, a state of hypermetabolic stress prevailed which caused these alterations in carbohydrate metabolism viz., enhanced peripheral glucose uptake and utilization, hyperlactatemia, gluconeogenesis, depressed glycogenesis, glucose intolerance and insulin resistance (Mizock, 1995). Experimental studies have used diverse methods to

induce trauma and to elicit inflammatory responses in animals. Chemical induction of tissue damage is a simpler alternative of inducing injury which can be applied quickly and reproducibly (Wusteman *et al*., 1990). Several studies have made use of subcutaneous injections of mineral turpentine to induce trauma in rats. Woloski and Jamieson (1987) used this model to study hormonal changes after injury. Wusteman *et al*. (1990) showed that subcutaneous injection of turpentine induced discrete aseptic abscess in rats without detectable injury to other tissues.

Nigella sativa Linn. (Ranunculaceae), commonly known as black cumin or black seed is an erect herbaceous annual plant. Its seeds have traditionally been used in Middle Eastern folk medicine as natural remedy for various diseases as well as a spice for over 2000 years. It has been to shown to contain more than 30% (w/w) of a fixed oil with 85% of total unsaturated fatty acid (Houghton *et al*., 1995). The seeds of *N. sativa* have been subjected to a range of pharmacological, phytochemical and nutritional investigations in recent years (Ali and Blunden, 2003; El-Dakhakhny *et al*., 2002a). The effect of *N. sativa* oil on some of the complications of experimental (alloxan-induced and streptozotocin-induced) diabetes mellitus in experimental animals has been investigated by a number of workers (Al-Hader *et al*., 1993; El-Dakhakhny *et al*., 2002b; Fararh *et al*., 2002, 2004). Al-Hader *et al*. (1993) reported that intraperitoneal administration of the volatile oil of *N. sativa* seeds (50 mL kg^{-1}) significantly reduced the fasting blood glucose concentration in normo- and hyperglycaemic rabbits. El-Dakhakhny *et al*. (2002b) reported that, treatment with *N. sativa* oil for 2, 4 and 6 weeks to the experimentally induced diabetic rats, significantly reduced glucose concentrations, this hypoglycemic effect may be mediated by extrapancreatic actions rather than by stimulated insulin release. Fararh *et al*. (2002) reported that *N. sativa* oil has a stimulatory effect on β cell function with consequent increase in serum insulin level and has insulinotropic properties in type 2-like model. The present study was designed to investigate the possible insulinotropic properties of *N. sativa* oil and whether it could correct the insulin resistance model induced by high-fat (20% butter oil) diet supplemented traumatized rats.

MATERIALS AND METHODS

The objective of this study was to investigate the effects of *Nigella sativa* seeds oil (predominantly n-6 polyunsaturated fatty acids) and butter oil (saturated medium chain fatty acids, low in n-6 precursors) on glucose tolerance and insulin sensitivity in normal and in rats induced clinical trauma by injecting single dose of turpentine. The present study has been performed during January to Jun, 2007.

Animals: A total of 36 male Wistar rats of similar age group, weighing 150-170 g were obtained from Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh. The animals were maintained under the standard conditions of temperature (23±1°C), humidity (50-55%) and light (12 h light and 12 h dark cycle). The rats were housed in separate cage (two rats/cage) and fed regular rat chow and had free access to drinking water. After the adjustment period, the rats were divided into 3 groups (12 rats each) and fed regular Rat Chow (RC), Butter Oil Diet (BOD) and *N. sativa* oil diet (NSOD) for 5 consecutive weeks. The composition of the diets is given in (Table 1). Diets were prepared every week. Control group of animals were received regular rat chow.

Body weights were recorded daily and mean weight increases were calculated for every week. At the end of the 5 week feeding period, 6 rats from each diet group were randomly separated and turpentine (0.2 mL/100 g body weight) was subcutaneously injected in the dorso-lumber area. The remaining 6 rats from each dietary group were used as normal (non-trauma) groups. Collection of timed glucose samples for glucose tolerance measurements were performed exactly 24 h after the turpentine injection.

Glucose tolerance test: Intravenous glucose tolerance test was performed with little modification as described by Davidson and Garvey (1993). After an overnight fast, the rats were anesthetized with urethane (20% w/v, 0.5 mL/100 g body weight, administered intra-peritoneally) and placed on a warming operating table. Through a ventral midline neck incision, the left carotid artery was catheterized. After a baseline sample (400 μL) was taken, heparin (1000 IU kg^{-1}) and glucose (50 mg/100 g body weight) were rapidly loaded through the same catheter. Further blood samples were collected at 3, 6, 9, 12 and 15 min in microcentrifuge tubes and centrifuged at 5000 rpm for 10 min. The plasma samples were stored at -20°C until analysis for glucose and insulin levels. All

Table 1: Composition of the diets (g/100 g diet)

Ingredients	Butter oil diet	*N. sativa* seed oil
Casein	18.0	18.0
Corn starch	23.5	23.5
Sucrose	23.5	23.5
Cellulose	10.0	10.0
Butter oil	20.0	16.0
Black seed oil	-	4.0
Standard mineral and vitamin mix	5.0	5.0

animals were killed after the timed blood samples were drawn and dissected. Visceral fat pads (epididymal, mesenteric and retroperitoneal) were excised weighed and calculated in grams per 100 g of body weight.

Plasma samples were analyzed for glucose concentrations by using a diagnostic kit (Human Diagnostics, Hamburg, Germany). Glucose disappearance rat ($K_{glucose}$Value) was calculated as described by Davidson and Garvey (1993). Plasma insulin levels were measured by immunoenzymatic calorimetric method based on ELISA. The protocol used was according to the methods described for the kit (DIA. METRA, Italy). The insulin area under the curve (AUC) at 15 min, insulinogenic index (insulin/glucose) and insulin sensitivity ($K_{glucose}$ Value/AUC x 10^3) were calculated using the computer software program Mini Stat (USA).

Statistical analysis: Results were assessed for statistical significance on-way analysis of variance and Student's t-test applied to individual groups. Values of $p<0.05$ were considered to be significant.

RESULTS

Mean body weights of rats supplemented with BOD was significantly ($p<0.001$) increased compare to RC and NSOD fed rats from the 1st week of experiment, respectively. NSOD feeding also increased ($p<0.001$) the body weights of rats but significance found after 3 weeks from starting date compared to controls. Mean weight of visceral fat pads also significantly increased in BOD supplemented rats compared to control and NSOD fed animals (Table 2).

Fasting plasma glucose concentrations did not change in all trauma groups compared to their respective controls (Table 3). Glucose tolerance curves 24 h post trauma exhibited a glucose peak at 3 min after the intra-arterial glucose load (50 mg/100 g body weight). Among injured groups BOD fed rats reached the highest value (25.99±0.48) followed by RC (25.70±0.64) and NSOD (22.33±0.45) fed rats. The decay of glucose started after 3 min and the values declined steadily till the 15 min (the last sample) in all diets with and without trauma (Fig. 1). However, in all injured rats glucose values found significantly higher than their controls at 15 min after the glucose load. The lowest value was seen in NSOD fed group and that significantly less than BOD and RC trauma groups. Glucose disappearance rate ($K_{glucose}$-value)

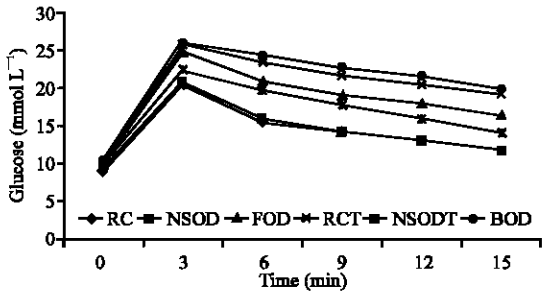

Fig. 1: Glucose tolerance curves 24 h after induction of clinical trauma at minutes 0, 3, 6, 9, 12 and 15 of rats fed rat chow, butter oil and *N. sativa* oil diets for a period of 5 consecutive weeks. RC (rat chow), NSOD (*N. sativa oil*), BOD (butter oil), RCT (RC + Trauma), NSODT (NSOD + Trauma) and BODT (BOD + Trauma)

Table 2: Weekly mean body weight increased and means visceral fat pads weights of rats supplemented with RT, BOD and NSOD for 5 consecutive weeks

	Body weight increased (g) (Mean ± SE)					
Dietary treatments	1st week	2nd week	3rd week	4th week	5th week	Visceral fat pads (g/100 g body weight)
RC	28.15±1.29	47.45±2.02	65.20±2.21	76.40±2.05	96.70±2.27	1.54±0.12
BOD	46.10±2.14^{ac}	80.25±2.89^{ac}	114.35±2.57^{ac}	134.90±3.58^{ac}	165.50±4.83^{ac}	2.24±0.13^{ac}
NSOD	33.25±1.94	58.95±2.28	85.25±2.61^{ab}	100.90±2.47^{ab}	128.40±2.67^{ab}	1.75±0.17^{ab}

Statistically significance was determined by one-way ANOVA and Student's t-test at $p<0.05$ level. a: Compared with RC, b: Compared with BOD, c: Compared with NSOD. 12 rats were used in each group.

Table 3: Plasma glucose concentrations and its disappearance rate ($K_{glucose}$value) until 15 minutes after the glucose load (50 mL kg^{-1} body weight iv) of rats supplemented with RC, BOD and NSOD for five consecutive weeks and traumatized by a single injection of turpentine

Dietary treatments		0 min fasting glucose (mMol L^{-1})	Glucose levels at 3 min after the glucose load	Glucose levels at 15 min after the glucose load	$K_{glucose}$-Value at 15 min after the glucose load
RC	Control	9.64±0.31	20.43±0.40^{b}	11.67±0.28^{b}	4.33±0.11^{b}
	Trauma	10.81±0.46	25.70±0.64^{*,3}	19.22±0.69^{*,3}	2.41±0.19^{*,3}
BOD	Control	9.85±0.26	24.83±0.85^{ac}	16.49±0.50^{ac}	3.23±0.08^{ac}
	Trauma	10.81±0.41	25.99±0.48	19.80±0.28^{*,3}	2.21±0.12^{*3}
NSOD	Control	9.87±0.27	20.77±0.31^{b}	11.74±0.45^{b}	4.48±0.26^{b}
	Trauma	10.11±0.29	22.33±0.45^{*,2}	14.06±0.13^{*,12}	3.79±0.17^{*,12}

$^{*}p<0.05$ vs respective controls (Student's t-test), $^{abc}p<0.05$ Non trauma dietary groups compared to each other, ^{a}vs RC, ^{b}BOD and ^{c}NSOD, $^{123}p<0.05$ Trauma dietary groups compared to each other, ^{a}vs RC, ^{b} BOD and ^{c} NSOD, Six rats were used in each group

Table 4: Plasma insulin ($\mu U\ mL^{-1}$) concentrations and area under the curve until 15 minutes after the glucose load (50 mL kg^{-1} body weight iv) of rats supplemented with rat chow, butter oil and *N. sativa* oil diets for five consecutive weeks and traumatized by a single injection of turpentine

Dietary treatments		Insulin levels before the glucose load (Fasting insulin)	Insulin levels at 3 min after the glucose load	Insulin levels at 15 min after the glucose load	Insulin area under the curve (IAUC)
RC	Control	31.15±1.18[b]	74.86±3.25	38.06±1.74[b]	813.12±33.35[b]
	Trauma	50.33±4.75*,2	99.38±4.04*	100.67±2.90*,2	1237.76±27.22*,23
BOD	Control	39.89±3.05[ac]	83.03±4.33[c]	50.31±3.21[ac]	942.53±34.61[ac]
	Trauma	64.65±3.84*,13	112.37±3.91*,3	116.59±4.34*,13	1394.00±46.43*,13
NSOD	Control	32.05±2.26[b]	67.40±2.86[b]	34.52±0.32[b]	732.35±20.62[b]
	Trauma	52.30±2.49*,2	83.02±5.88*,2	83.12±2.78*,2	1005.52±58.65*,12

*p<0.05 vs respective controls (Student's t-test), [abc] p<0.05 Non trauma dietary groups compared to each other, [a]vs RC, [b] BOD and [c]NSOD, [123]p<0.05 Trauma dietary groups compared to each other, [a]vs RC, [b]BOD and [c]NSOD, Six rats were used in each group

Table 5: Insulinogenic index (Insulin/glucose ratio) and insulin sensitivity ($K_{glucose}/IAUC \times 10^3$) of normal and traumatized rats (24 h after induction of clinical trauma) of rats supplemented with RC, BOD and NSOD for five consecutive weeks

Dietary treatments		Insulinogenic index (insulin/glucose ratio) 0 min	3 min	15 min	Insulin sensitivity ($K_{glucose}$/IAUC x 10^3)
RC	Control	4.37±0.22[c]	3.68±0.21	3.27±0.16	5.38±0.28[c]
	Trauma	4.25±0.33[3]	3.93±0.20	5.27±0.26*	1.95±0.17*,2
BOD	Control	4.06±0.35	3.36±0.22	3.07±0.25	3.45±0.13[c]
	Trauma	4.12±0.42[3]	4.33±0.18*	5.88±0.19*	1.61±0.13*,13
NSOD	Control	3.39±0.19[a]	3.35±0.07	2.96±0.13	6.16±0.45[ab]
	Trauma	2.99±0.28[12]	3.74±0.31	5.91±0.21*	3.84±0.28*,2

*p<0.05 vs respective controls (Student's t-test), [abc]p<0.05 Non trauma dietary groups compared to each other, [a]vs RC, [b]BOD and [c]NSOD, [123]p<0.05 Trauma dietary groups compared to each other, [a]vs RC, [b]BOD and [c]NSOD, Six rats were used in each group

significantly decreased in traumatized rats compared to their respective controls. In normal rats saturated fat reduced the $K_{glucose}$-value, in contrast *N. sativa* oil diet fed animals found similar as controls (RC fed rats) and it also reduced the effect of trauma on $K_{glucose}$ compared to BOD trauma group (Table 3).

Mean plasma fasting insulin levels were significantly elevated in trauma groups as compared to their respective controls. The highest increased was found in BOD fed animals and it is found significantly more than RC and NSOD groups. Insulin levels showed peaks at 3 min after the glucose load in all control and trauma groups. Injury caused significant elevation at the peak values compared to their respective controls. However, the highest peak was seen in BOD trauma group that is significantly more than NSOD trauma group. Insulin decay was started at 3 min and continued till 15 min (last sample) in normal rats but in trauma groups, the decay was continued only till 9 min then started increase till end (Fig. 2). In last sample (15 min after the glucose load), insulin levels were found significantly less in NSOD fed traumatized rats compared to trauma BOD group of rats. Insulin area under the curve (IAUC) found highest in BOD fed normal animals. Turpentine-induced injury caused significant increase the IAUC values in all dietary groups compared to their respective controls. In comparison to BOD trauma group NSOD trauma showed significantly less values of IAUC (Table 4).

Insulinogenic index (insulin/glucose ratio) showed no significant change between the normal and trauma groups at 3 min after the glucose load. While at 15 min after the glucose load the ratio found significantly different than the respective control in all dietary groups. Although, in 0 min values the ratio was significantly less in NSOD group compared to normal or trauma of dietary groups (Table 5). Insulin sensitivity calculated ($K_{glucose}$value/IAUC x 10^3) found significantly low in BOD fed normal rats. *N. sativa* oil supplementation significantly enhanced the insulin sensitivity compared to RC and BOD fed animals. However, injury caused significant increase insulin sensitivity in all dietary groups compared to their respective controls. Saturated fatty acid diet worsens the insulin sensitivity after trauma and that found significantly lower than NSOD trauma group (Table 5).

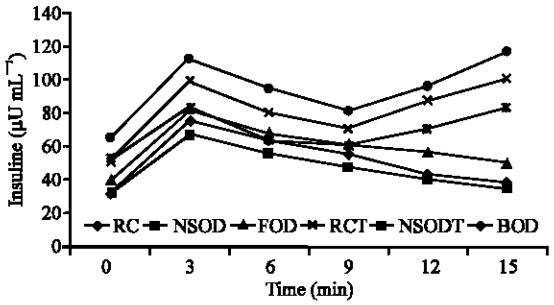

Fig. 2: Mean insulin levels 24 h after induction of clinical trauma at minutes 0, 3, 6, 9, 12 and 15 of rats fed rat chow, butter oil and *N. sativa* oil diets for a period of 5 consecutive weeks. RC (rat chow), NSOD (N. sativa oil), BOD (butter oil), RCT (RC + Trauma), NSODT (NSOD + Trauma) and BODT (BOD + Trauma)

DISCUSSION

Mean body weights of rats supplemented with 20% saturated fat (butter oil) diet were significantly ($p<0.001$) increased compare to controls group of rats from 1st week. These results are in agreement with our earlier studies that, 20% butter oil diet significantly increased the body weights of rats compared to control animals (Alsaif, 2004; Alsaif and Duwaihy, 2004). While 4% *N. sativa* oil combined with 16% butter oil diet, delayed the body weights increase for 3 weeks as compared to control group of rats. The delay in growth of rats supplemented *N. sativa* oil diet in the present study may attributes its sliming property that has confirmed with significant reduction in visceral fats compared to BOD fed animals. Zaoui *et al.* (2002) reported that, *N. sativa* oil (2 mL kg^{-1} body weight) treatment to mice for six weeks significantly reduced the body weights compared to normal mice.

The stability of plasma glucose is a reflection of the balance between rates of whole body glucose production and glucose utilization. Each of these processes is tightly regulated by the levels of hormones and substrates in blood. It is the liver, therefore, that is responsible for providing glucose to insulin sensitive tissues such as skeletal muscle and fat. The primary feedback loops involved in the regulation of glucose production by the liver *in vivo*. When plasma glucose increases, glucose production by the liver falls and vice versa. In insulin resistance, under normal physiological conditions, the increase in glucose disposal rate lags behind the increase in circulating insulin levels and another important aspect of insulin resistance is decreased kinetics of insulin action including impaired glucose tolerance (Bloomgarden, 1999).

Insulin resistance is common after trauma and surgical procedures (Frayn, 1986). Both reduced tissue insulin sensitivity and alterations in insulin release may contribute to the impaired glucose homeostasis. For example, insulin-mediated glucose disposal is impaired both in humans after injury, surgery, burns and sepsis (Strommer *et al.*, 2002; Black *et al.*, 1982; Thorell *et al.*, 1994) and in laboratory animals with injury and sepsis (Barton and Passingham, 1980; Virkamaki and Yki-Jarvinen, 1994). In the present study, diet as well as trauma exerted its influence on glucose tolerance following a glucose i.v. load (50 mg/100 g body weight). Glucose tolerance was impaired in trauma considerably compared to controls. With saturated fat (butter oil) even though the intolerance was visible in uninjured rats it was markedly worsened by trauma. Saturated fatty acid rich diets are known to cause impaired glucose tolerance and increased insulin resistance compared to polyunsaturated fatty acids (Pan *et al.*, 1994). The possible mechanism is that in trauma the fatty acids in the diet would influence membrane long chain polyunsaturated fatty acids and consequent reactivity of the eicosanoids formed which in turn would affect the sensitivity of insulin and glucose uptake, to the extent that these compounds participate in the regulation of insulin action and glucose uptake. The insulin area under the curve in the present study also supports this hypothesis. An overall insulin resistance was observed in traumatized rats on all diets but the effect of diet in minimizing this effect was prominent in *N. sativa* oil diet fed rats after injury. Feeding period and the level of fat has also been reported to influence the glucose tolerance in rats. Feeding a 40% corn oil diet caused a lower rate of glucose disappearance in rats compared to a glucose diet or a chow diet (Ramirez *et al.*, 1990) whilst a longer feeding period resulted in a gradual deterioration of glucose tolerance in rats consuming 30% corn oil (Wiersma *et al.*, 1993).

N. sativa oil have been used for treatment of experimentally induced diabetes in animals based on its combined hypoglycaemic and immunopotentiatig effects that help in ameliorating the impaired immunity and infections associated with diabetes (Al-Hader *et al.*, 1993). Kaleem *et al.* (2006) confirmed the *N. sativa* seeds extract antidiabetic activity through its antioxidant properties. El-Dakhakhny *et al.* (2002a) reported that, hypoglycemic effect of *N. sativa* oil may be mediated by extrapacreatic actions rather than by stimulated insulin release. Whereas, Richid *et al.* (2004) reported that, *N. sativa* seed extract enhance glucose-induced insulin release from rat-isolated Langerhans islets. Kanter *et al.* (2003) also reported that, hypoglycaemic action of *N. sativa* due to amelioration in the beta-cells of pancreatic islets causing an increase in insulin secretion. Present study revealed the antidiabetic effect of *N. sativa* oil by enhancing the insulin sensitivity in traumatized rats.

The present results concluded that *N. sativa* oil effect against glucose intolerance-induced by saturated fat or injury and the effect attributed its antidiabetic property through increasing the insulin sensitivity. More studies are needed to demonstrate the exact mechanism of action of *N. sativa* oil on ameliorated insulin resistance.

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