# Effect of Insulin on Fat Metabolism During and After Normal Pregnancy

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**Whereas development of resistance to the action of insulin on glucose metabolism during gestation has been recognized, it is presently not known whether there is also resistance to the action of insulin on lipid metabolism. We have, therefore, examined the effect of physiological hyperinsulinemia (during euglycemic-hyperinsulinemic clamping) on free fatty acid (FFA) turnover in seven nondiabetic overweight or obese women during and after pregnancy. Basal rates of FFA release, oxidation, and reesterification and basal plasma FFA concentrations were not significantly different from each other during the 2nd and 3rd trimester of pregnancy and postpartum. During euglycemic-hyperinsulinemic (~500 pmol/l) clamping, however, lipolysis was significantly less inhibited during the 3rd trimester (from 7.0 ± 0.9 to 4.9 ± 0.9 μmol · kg$^{-1}$ · min$^{-1}$, –30%) than during the 2nd trimester (from 8.4 ± 0.6 to 4.1 ± 0.9 μmol · kg$^{-1}$ · min$^{-1}$, –51%) and postpartum (from 8.5 ± 1.1 to 4.2 ± 0.6 μmol · kg$^{-1}$ · min$^{-1}$, –51%). Similarly, fat oxidation was not inhibited at all (from 3.5 ± 0.3 to 3.8 ± 0.5 μmol · kg$^{-1}$ · min$^{-1}$) during the 3rd trimester but was suppressed by 51% (from 3.9 ± 0.2 to 1.9 ± 0.3 μmol · kg$^{-1}$ · min$^{-1}$) during the 2nd trimester and by 38% (from 2.6 ± 0.7 to 1.6 ± 0.5 μmol · kg$^{-1}$ · min$^{-1}$) postpartum. These data demonstrated that resistance to the action of insulin on lipolysis and on fat oxidation developed during late gestation and disappeared postpartum.** ***Diabetes*** **48:834–838, 1999**

Late pregnancy in healthy women is characterized by resistance to the action of insulin on glucose uptake and utilization (1–4). The development of insulin resistance after mid-pregnancy has been interpreted as a physiological adaptation of the mother to preserve carbohydrate (CHO) for the rapidly growing fetus (5), whose glucose utilization during the 3rd trimester has been estimated to reach ~33 μmol · kg$^{-1}$ · min$^{-1}$ (6,7). Because total caloric expenditure rises during late pregnancy (8), it follows that any reduction in maternal CHO utilization, resulting from insulin resistance, must be accompanied by an increase in utilization of another fuel, presumably fat. The increase in fat utilization could result from resistance to the action of insulin on free fatty acid (FFA) release. Insulin strongly suppresses lipolysis (via inhibition of hormone-sensitive adipose tissue lipase); hence, insulin resistance could be expected to increase release of FFA, resulting in increased plasma FFA concentrations and fat oxidation. Surprisingly, there is currently no published information on the effects of insulin on FFA turnover during pregnancy. It was, therefore the purpose of this study to examine the effects of insulin on release, oxidation, and reesterification of FFA throughout pregnancy and during the postpartum period in normal overweight and obese women.

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CHO, carbohydrate; FFA, free fatty acid; HPL, human placental lactogen; npRQ, nonprotein respiratory quotient; $R_a$, rate of appearance.

## RESEARCH DESIGN AND METHODS

**Subjects.** We studied seven healthy pregnant glucose-tolerant women. Of these women, three were obese (BMI ≥30) (9) and four were overweight (BMI 25–29.9) (9). The women's age, weight, height, and body composition are shown in Table 1. None of the women had a family history of diabetes or other endocrine disorders and none were taking any medications. All were seen by a dietitian before the studies, and their food intake was standardized to contain a minimum of 250 g of CHO for at least 2 days before the studies. Of the seven women, five had a normal 1-h diabetes screening test (blood glucose <130 mg/dl) and two had a normal 3-h oral glucose tolerance test according to the criteria of Carpenter and Coustan (10). The studies were approved by the Temple University Hospital Institutional Review Board, and informed consent was obtained from each subject before the study.

**Experimental design.** All women were studied at the General Clinical Research Center at Temple University Hospital. Six women were studied during the 2nd trimester of pregnancy (22.5 ± 2.0 weeks' gestation). These six plus one additional woman were also studied in the 3rd trimester (35.3 ± 2.8 weeks' gestation) and again 15.6 ± 1.4 weeks postpartum when none of the women were lactating. During the studies, the women were reclining in bed. A short polyethylene catheter was inserted into an antecubital vein for infusion of test substances. Another catheter was placed into a contralateral forearm vein for blood sampling. This arm was kept at ~70°C with a heating blanket to arterialize venous blood (11). At 6:00 P.M., all subjects received their last meal consisting of ~55% CHO, 30% fat, and 15% protein. After an overnight fast (at ~8:00 A.M.), a 4-h euglycemic-hyperinsulinemic clamp was performed in combination with infusion of stable isotopes (for measurement of rates of lipolysis) and indirect calorimetry (for estimation of rates of fat oxidation).

### Methods and procedures

***Euglycemic-hyperinsulinemic clamp.*** Regular human insulin (Humulin R; Eli Lilly, Indianapolis, IN) was infused intravenously at a rate of 7 pmol · kg$^{-1}$ · min$^{-1}$ for 4 h, starting at 0 min. Glucose concentrations were maintained at 4.7 ± 0.08 mmol/l by a variable rate infusion with 20% glucose, as previously described (12). Blood glucose concentrations were determined every 10–15 min with a Beckman glucose analyzer (Palo Alto, CA), and glucose infusions were adjusted accordingly.

***Indirect calorimetry.*** Respiratory gas exchange rates were determined at 30-min intervals with a metabolic measurement cart as previously described (13). Rates of protein oxidation were estimated from the urinary nitrogen excretion after correction for changes in urea nitrogen pool size (14). Rates of protein oxidation were used to determine the nonprotein respiratory quotient (npRQ). Rates of fat oxidation were determined with the tables of Lusk, which are based on an npRQ of 0.707 for 100% fat oxidation and 1.00 for 100% CHO oxidation.

***Glycerol turnover.*** [$^2H_5$]glycerol (98 atom percent deuterium; Tracer Technologies, Somerville, MA) dissolved in normal saline was infused for 5.5 h (–90 to 240 min) starting with a priming dose of 1.6 μmol/kg followed by a continuous infusion of 0.11 μmol · kg$^{-1}$ · min$^{-1}$. [$^2H_5$]glycerol enrichment reached steady state 60 min after the start of the infusion (at –30 min), as shown in Table 2. Blood for determination of [$^2H_5$]glycerol was collected at 30-min intervals from the start (–90 min) until the end (240 min) of the infusions. Plasma was immediately separated

TABLE 1
Study subjects

| | 2nd trimester (22.5 ± 2.0 weeks) | 3rd trimester (35.3 ± 2.8 weeks) | Postpartum (15.6 ± 1.4 weeks) |
|---|---|---|---|
| *n* | 6 | 7 | 7 |
| Age (years) | 27 ± 4.0 | 27 ± 3.0 | 27 ± 3.0 |
| Height (cm) | 159 ± 1.7 | 163 ± 3.9 | 163 ± 4.0 |
| Weight (kg) | 76.6 ± 6.2 | 89.4 ± 8.7 | 80.9 ± 10 |
| BMI (kg/m$^2$) | 30.4 ± 2.8 | 33.4 ± 2.6 | 30.2 ± 3.1 |
| % Fat | 37.4 ± 2.5 | 38.7 ± 3.5 | 37 ± 2.8 |

Data are means ± SE.

at 4°C and stored at –20°C until analyzed. The trimethylsilyl derivative of glycerol was prepared as described elsewhere (15). [$^2H_5$]glycerol enrichment was determined by gas chromatography–mass spectrometry (model 4610-B; Finnigan-Matt, San Jose, CA) with the use of electron impact ionization and monitoring of ions at m/e 205 and 208. Plasma FFAs were measured using an enzymatic calorimetric method (Wako Chemicals, Richmond, VA).

***Body composition.*** Body composition was assessed by bioimpedance analysis (RJL Systems, Clinton Township, MO). Total body water was calculated using the equation of Lukaski et al. (16), which was derived from data obtained from pregnant women and validated against the deuterium-labeled water technique.

***Calculations.*** Rate of appearance ($R_a$) of glycerol was calculated with the non–steady-state equation of Steele, corrected for the amount of exogenously infused stable isotope (17,18). The glycerol $R_a \times 3$ was assumed to reflect rates of whole-body lipolysis. The validity of this assumption is supported by the following data: *1*) Glycerol is only produced by lipolysis (15,19,20); *2*) All glycerol released by lipolysis should appear in the plasma, since there is virtually no α-glycerol kinase in human adipose and muscle tissue (21); *3*) Because splanchnic release of glycerol under basal and hyperinsulinemic conditions is minimal, underestimation of glycerol $R_a$ because of first-pass hepatic clearance is not likely (22); and *4*) The underestimation of lipolysis because of partial hydrolysis of triacylglycerol is minimal (21,23).

***FFA reesterification.*** Under steady-state conditions, the difference between the rate of lipolysis (glycerol $R_a \times 3$) and the rate of FFA oxidation provides an index of the rate of FFA reesterification, because recycling is ultimately the fate of all nonoxidized FFAs (15). Therefore, FFA reesterification = glycerol $R_a \times 3$ – FFA oxidation.

***Fetal assessment.*** Fetal well-being was assessed every 30 min throughout the insulin infusions by fetal heart rate determinations. Assessment of amniotic fluid volume, fetal body tone, movement, and breathing (biophysical profile) was done by ultrasound on admission and before discharge. All the women participating in this study delivered a healthy baby.

***Analytical procedures.*** Plasma glucose was measured with a glucose analyzer with the glucose oxidase method. Serum-free insulin was determined by radioimmunoassay with a specific antibody that cross-reacts only minimally (<0.2%) with proinsulin (Linco, St. Charles, MO). Total plasma fatty acids were determined enzymatically in chilled plasma containing EDTA with a kit from Wako (Richmond, VA). Glycerol was measured enzymatically.

***Statistical analysis.*** All data are expressed as means ± SE. Statistical significance was assessed using two-way repeated measures analysis of variance and two-tailed Student's $t$ test.

## RESULTS

**Insulin, glucose, FFAs, and glycerol.** Insulin infusion raised serum insulin concentrations from 81 ± 10 to 457 ± 52 pmol/l in the 2nd trimester, from 103 ± 20 to 531 ± 86 pmol/l in the 3rd trimester, and from 93 ± 16 to 530 ± 41 pmol/l in the postpartum period (Fig. 1). Neither the basal nor the clamped insulin concentrations were significantly different from each other during the three studies.

Basal glucose concentrations were 4.4 ± 0.1 mmol/l (2nd trimester), 4.5 ± 0.2 mmol/l (3rd trimester), and 5.0 ± 0.3 mmol/l (postpartum). Plasma glucose was clamped at 4.7 ± 0.08 mmol/l in all three studies. The association of modest elevations of basal insulin with normal glucose levels suggested a mild degree of insulin resistance, which was consistent with the degree of obesity present in these women.

Basal FFA concentrations were slightly higher in the 2nd and 3rd trimesters (624 ± 39 and 609 ± 41 μmol/l) than in the postpartum period (505 ± 62 μmol/l). These differences, however, were not statistically significant ($P = 0.14$), probably as result of the relatively small number of subjects. Insulin reduced FFA concentrations by 59% (from 624 ± 39 to 254 ± 35 μmol/l, $P < 0.01$) during the 2nd trimester, by 57% (from 609 ± 44 to 263 ± 41 μmol/l, $P < 0.01$) in the 3rd trimester, and by 74% (from 505 ± 62 to 129 ± 10 μmol/l, $P < 0.01$) during the postpartum period. Plasma glycerol levels fell during hyperinsulinemia by 50% (from 0.06 ± 0.001 to 0.03 ± 0.001 mmol/l) during the 2nd trimester, by 43% (from 0.07 ± 0.007 to 0.04 ± 0.008 mmol/l) during the 3rd trimester, and by 50% (from 0.08 ± 0.015 to 0.04 ± 0.008 mmol/l) postpartum. There were no significant differences in basal or in clamp glycerol levels between the three groups.

**FFA release, oxidation, and reesterification.** Basal rates of lipolysis (8.4 ± 0.6, 7.0 ± 0.9, and 8.5 ± 1.1 μmol · kg$^{-1}$ · min$^{-1}$), fat oxidation (3.9 ± 0.2, 3.5 ± 0.3, and 2.6 ± 0.7 μmol · kg$^{-1}$ · min$^{-1}$), and FFA reesterification (4.8 ± 0.6, 3.5 ± 0.9, and 5.8 ± 1.2 μmol · kg$^{-1}$ · min$^{-1}$) as well as metabolic rates (1.09 ± 0.07, 1.20 ± 0.10, and 0.97 ± 0.11 kcal/min) and respiratory quotients (0.77 ± 0.02, 0.76 ± 0.02, and 0.78 ± 0.02) were not significantly different during the 2nd and 3rd trimesters of pregnancy and postpartum.

In response to 240 min of hyperinsulinemia, the rate of lipolysis declined by 51% (from 8.4 ± 0.6 to 4.1 ± 0.9 and from 8.5 ±

TABLE 2
[$^2H_5$]glycerol enrichment at baseline (3rd trimester pregnancy)

| Subject | Time | APE |
|---|---|---|
| 1 | –30 | 6.73 |
| | 0 | 6.32 |
| 2 | –30 | 5.55 |
| | 0 | 6.02 |
| 3 | –30 | 3.4 |
| | 0 | 3.6 |
| 4 | –30 | 3.46 |
| | 0 | 3.09 |
| 5 | –30 | 3.56 |
| | 0 | 3.38 |
| 6 | –30 | 4.95 |
| | 0 | 4.94 |

APE, atoms percent excess.

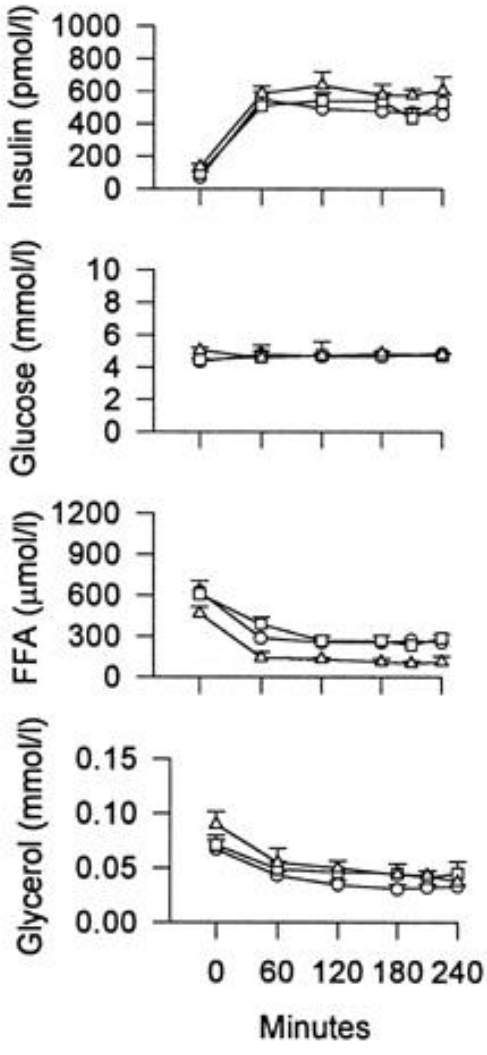


**FIG. 1. Plasma glucose, insulin, FFAs, and glycerol concentrations during the 2nd trimester (22.5 ± 2.0 weeks), the 3rd trimester (35.3 ± 2.8 weeks), and postpartum (15.6 ± 1.4 weeks) in healthy women before and during 4 h of euglycemic-hyperinsulinemic clamping. ○, 2nd trimester ($n$ = 6); □, 3rd trimester ($n$ = 7); △, postpartum ($n$ = 7). Statistical analysis: basal values (0 min) were significantly different ($P$ < 0.001) from values at 60–120 min for insulin, FFAs, and glycerol concentrations.**

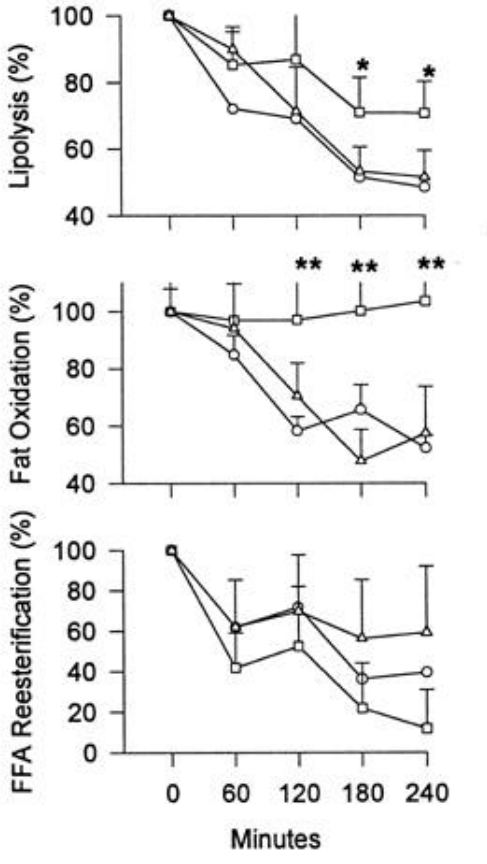


**FIG. 2. Changes in rates of lipolysis, fat oxidation, and FFA reesterification before and during 4 h of euglycemic-hyperinsulinemic clamping during pregnancy (2nd and 3rd trimesters) and postpartum in healthy women. ○, 2nd trimester ($n$ = 6); □, 3rd trimester ($n$ = 7); △, postpartum ($n$ = 7). *$P$ < 0.05, **$P$ < 0.01 comparing postpartum vs. 2nd and 3rd trimesters.**

1.1 to 4.2 ± 0.6 µmol · kg$^{-1}$ · min$^{-1}$) in the 2nd trimester and postpartum but only by 30% (from 7.0 ± 0.9 to 4.9 ± 0.9 µmol · kg$^{-1}$ · min$^{-1}$) in the 3rd trimester (Fig. 2). Hence, the insulin-mediated inhibition of lipolysis was significantly reduced during the 3rd trimester when compared with either the postpartum period or with the 2nd trimester ($P$ < 0.03). These differences remained statistically significant ($P$ < 0.05) when comparisons between the 2nd and 3rd trimesters were performed with the six women who participated in all three studies.

In response to hyperinsulinemia, rates of fat oxidation decreased by 51% (from 3.9 ± 0.2 to 1.9 ± 0.3 µmol · kg$^{-1}$ · min$^{-1}$) and by 38% (from 2.6 ± 0.7 to 1.6 ± 0.5 µmol · kg$^{-1}$ · min$^{-1}$) in the 2nd trimester and postpartum periods, respectively. During the 3rd trimester, however, fat oxidation did not decline (from 3.5 ± 0.3 to 3.5 ± 0.5 µmol · kg$^{-1}$ · min$^{-1}$) and remained significantly higher than it did during the 2nd trimester or the postpartum period ($P$ < 0.01) (Fig. 2). These differences could not be explained by accumulation in the blood of partially oxidized metabolites, since there was no differential accumulation of lactate or β-hydroxybutyrate, the two major metabolites of CHO and fat oxidation, in either of the study groups during the clamps (Table 3).

The decline in the rate of FFA reesterification was 66% (from 3.5 ± 0.9 to 1.2 ± 1.1 µmol · kg$^{-1}$ · min$^{-1}$) during the 3rd trimester, 54% during the 2nd trimester (from 4.8 ± 0.6 to 2.2 ± 1.0 µmol · kg$^{-1}$ · min$^{-1}$), and 55% during the postpartum period (from 5.8 ± 1.2 to 2.6 ± 0.8 µmol · kg$^{-1}$ · min$^{-1}$). These differences did not reach statistical significance.

## DISCUSSION

In the present study, we have examined the effects of physiological hyperinsulinemia on the release, oxidation, and reesterification of fatty acids in nondiabetic overweight and obese women throughout pregnancy and during the postpartum period. We found that insulin actions on lipolysis and on fat oxidation were significantly impaired during late pregnancy compared with early pregnancy and the postpartum period. These data indicated that insulin sensitivity (with respect to lipid metabolism) remained essentially normal during early pregnancy but that marked insulin resistance developed during late pregnancy, presumably in adipose tissue (increased lipolysis), liver, and muscle (increased fat oxidation).

TABLE 3
β-OHB and lactate

| | 2nd trimester | 3rd trimester | Postpartum |
|---|---|---|---|
| *n* | 6 | 7 | 7 |
| Lactate (mmol/l) | | | |
| 0 min | 0.74 ± 0.06 | 0.84 ± 0.15 | 0.80 ± 0.15 |
| 240 min | 0.77 ± 0.06 | 0.92 ± 0.12 | 1.08 ± 0.19 |
| *P* | NS | NS | NS |
| β-OHB (mmol/l) | | | |
| 0 min | 0.17 ± 0.02 | 0.32 ± 0.05 | 0.13 ± 0.04 |
| 240 min | 0.07 ± 0.04 | 0.11 ± 0.01 | 0.07 ± 0.02 |
| *P* | <0.004 | <0.006 | <0.018 |

Data are means ± SE. β-OHB, β-hydroxybutyrate.

It appears unlikely that a significant part of the differences in fat metabolism observed during the 3rd trimester can be attributed to changes in fetal metabolism for the following reason. At the end of the 3rd trimester, the fetal weight was ~4% of the weight of the mother (3.6 vs. 89 kg). Even if one assumes that all the fetal weight was accumulated between the 2nd and 3rd trimesters and that fetal tissues were completely unresponsive to insulin, the fetus could have only contributed a trivial amount (perhaps ~5%) to the increase in insulin resistance that developed between the 2nd and 3rd trimesters.

There were no significant differences in basal plasma glucose, insulin, or FFA levels or in basal rates of fat oxidation or lipolysis during pregnancy. This is not surprising, however, since changes in basal parameters are usually late consequences of insulin resistance, comparable to a rise in fasting blood glucose in type 2 diabetes, which occurs, if at all, long after development of insulin resistance.

While there are no previously published data on insulin sensitivity during pregnancy with respect to lipid metabolism, we have recently reported that the same women had a similar course of events with respect to insulin action on CHO metabolism, i.e., they developed insulin resistance during late pregnancy followed by normalization postpartum (4). Specifically, insulin-stimulated glucose uptake decreased between the 2nd and 3rd trimesters (–36%, $P < 0.05$) and normalized between the 3rd trimester and the postpartum period (+44%, $P < 0.05$). CHO oxidation decreased between the 2nd and 3rd trimesters (–80%, $P < 0.05$) and normalized between the 3rd trimester and the postpartum period (+640%, $P < 0.05$) (4). The consequence of the insulin resistance in everyday life would be that after a CHO-rich meal (when serum insulin levels rise), FFA levels would be higher than usual and a larger percentage of these FFAs would be oxidized.

The cause for the insulin resistance during late pregnancy was not specifically investigated. The parallel development of insulin resistance and increases in blood levels of human placental lactogen (HPL) and placental growth hormone, both hormones with strong lipolytic and anti-insulin action (24–27), suggested that placental growth hormone, HPL, and perhaps other diabetogenic hormones, including cortisol, progesterone, and estrogens, may be responsible for much of the observed insulin resistance (28–32). Moreover, it was noteworthy that during the 3rd trimester, hyperinsulinemia lowered plasma FFA levels by 53% but did not decrease fat oxidation. The unchanged rate of fat oxidation at lower plasma FFA concentrations indicated an increase in fractional oxidation of FFAs. This could have been due to a fall in intracellular malonyl-CoA concentrations, occurring, perhaps, as result of impaired insulin stimulation of acetyl-CoA carboxylase in addition to decreased glucose uptake and utilization during late gestation (2,4). Malonyl-CoA is known to control FFA oxidation by inhibiting carnitine palmitoyl transferase 1, the rate-limiting enzyme for transport of FFAs across mitochondrial membranes (33).

**Significance.** Late pregnancy is characterized by fetal growth and maternal responses to the increasing fetal needs for nutrients. Of particular importance is the preservation of CHO, which is needed as fuel for the fetal and maternal central nervous systems. Our data are consistent with the concept that the development of insulin resistance during late pregnancy is a physiological adaptation to the special requirements of the developing fetus and the mother (5). Resistance to the action of insulin on glucose uptake and oxidation preserves glucose by reducing its utilization in insulin-sensitive tissues, such as muscle and adipose tissue, while resistance to the action of insulin on lipolysis and fat oxidation provides fatty acids as an alternate fuel for oxidation.

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