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Short communication

# $F_2$-isoprostane evidence of oxidant stress in the insulin resistant, obese Zucker rat: effects of vitamin E

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**Abstract**

We have concurrently investigated oxidant stress, glucose tolerance and glucose-stimulated insulin responses in the obese Zucker rat, a widely used model of insulin resistance. The plasma level of the lipid peroxidation product 8-epi-prostaglandin $F_{2\alpha}$, a sensitive in vivo marker of oxidant stress, was elevated approximately 5-fold in 13-week old obese relative to age-matched, insulin-sensitive lean Zucker rats. Supplementation of the diet with vitamin E (as (±)-α-tocopherol acetate, 0.5% w/w) for 4 weeks, reduced plasma 8-epi-prostaglandin $F_{2\alpha}$ and concomitantly reversed glucose-stimulated hyperinsulinaemia in the obese Zucker rat without worsening glucose tolerance. We therefore provide evidence of oxidant stress, measured as elevated plasma 8-epi-prostaglandin $F_{2\alpha}$, for the first time in the obese Zucker rat which now provides a rationale for the beneficial effects of antioxidants on insulin action previously reported in this model of insulin resistance. 

*Keywords:* Oxidant stress; Insulin resistance; Obese, Zucker rat; Diabetes mellitus

## 1. Introduction

The obese Zucker rat exhibits many features of metabolic Syndrome X and non-insulin-dependent diabetes mellitus, including impaired glucose tolerance, insulin resistance and hyperinsulinaemia (Zucker and Antoniades, 1972; Reaven, 1995). Oxidant stress associated with insulin resistance and non-insulin-dependent diabetes mellitus (Gopaul et al., 1995; Nourooz-Zadeh et al., 1995) contributes to poor insulin action (Paolisso et al., 1994; Faure et al., 1997; Rudich et al., 1997) and, in this respect, the obese Zucker rat may provide a suitable paradigm for studying the role of oxidative damage in insulin resistance.

However, oxidant stress remains to be demonstrated in this important model of insulin resistance. Furthermore, the role of reduced oxidant stress in putative antioxidant actions to improve glucose disposal/insulin action, so far reported in the obese Zucker rat (Fujiwara et al., 1988; Jacob et al., 1996; Henriksen et al., 1997; Streeper et al., 1997), has not been verified. Our primary aim was therefore to assess oxidant stress using a sensitive in vivo marker, the lipid peroxidation product 8-epi-prostaglandin $F_{2\alpha}$ (Roberts and Morrow, 1994; Gopaul et al., 1995; Pratico et al., 1997), in the obese Zucker rat relative to its lean, insulin-sensitive littermate. 8-Epi-prostaglandin $F_{2\alpha}$ is a member of a series of prostaglandin $F_2$-like compounds or $F_2$-isoprostanes, formed in situ during the reactive oxygen species-dependent peroxidation of lipid-esterified arachidonic acid (Morrow et al., 1990) and is regarded as an accurate measure of oxidant stress in vivo (Morrow and Roberts, 1991). It was of additional interest to evaluate the effects of an antioxidant, vitamin E added to the diet, on this marker of oxidant stress in obese animals together with glucose tolerance and glucose-stimulated insulin responses in vivo.

## 2. Materials and methods

### 2.1. Dietary protocol

Male, 9-week old obese/lean Zucker rats (Harlan, Blackthorn, Bicester, UK) were maintained for 4 weeks on

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standard chow (Special Diet Services, Witham, Essex, UK) with or without enrichment with vitamin E ((±)-α-tocopherol acetate added at 0.5% w/w). Food and water were allowed ad libitum.

### 2.2. Determination of plasma 8-epi-prostaglandin $F_{2\alpha}$

Thirteen-week old Zucker rats were anaesthetised with thiopentone sodium (120 mg/kg i.p.) and 4.5 ml arterial blood collected in 0.5 ml trisodium citrate (3.8% w/v) treated with indomethacin (15 μM final) and butylated hydroxytoluene (20 μM final) to prevent cyclooxygenase activity and ex vivo oxidation, respectively. Blood/citrate was then centrifuged to derive plasma which was treated with butylated hydroxytoluene (20 μM final) and stored at −80°C before the measurement of 8-epi-prostaglandin $F_{2\alpha}$ levels by gas chromatography–mass spectrometry as described by Gopaul et al. (1995).

### 2.3. Intravenous glucose tolerance test

Thirteen-week old Zucker rats were fasted overnight and anaesthetised with thiopentone sodium (120 mg/kg i.p.) to allow an intravenous glucose tolerance test. After 30 min stabilisation, a blood sample was collected immediately before the glucose load at $t = 0$, to determine basal values of plasma glucose and insulin. D-Glucose was then rapidly injected intravenously (0.5 g/kg) and venous blood samples collected in ethylenediaminetetraacetic acid anticoagulant at $t = 1$, 3, 6, 12, and 24 min.

### 2.4. Determination of plasma glucose and insulin

Samples were kept on ice before centrifugation to derive plasma (within 30 min) which was stored at −20°C prior to analysis. D-Glucose and insulin were determined using a photometric method (Trinder assay, Sigma, Poole, Dorset, UK) and by radioimmunoassay (Linco Research, St. Charles, MO, USA), respectively.

### 2.5. Statistical analysis

Data are expressed as mean ± S.E.M. Glucose tolerance and glucose-stimulated hyperinsulinaemia were determined as the area under the curve for stimulated, i.e., basal value subtracted, glucose and insulin levels, respectively, between $t = 0$ and 24 min. The difference between two means was assessed by Student's unpaired, two-tailed *t*-test or the Welch test in the case of heterogeneous sample variances. A multicomparison of means was conducted using One-way analysis of variance followed by Dunnett's test. Statistical significance was accepted at the 5% level.

## 3. Results

### 3.1. Animal body weight

The body weight of 13-week old obese animals (435.7 ± 18.4 g, $n = 4$) was greater relative to age-matched lean animals (268.2 ± 11.9 g, $n = 5$) ($P < 0.01$) and was unaffected by dietary vitamin E (453.0 ± 7.1 g, $n = 6$) ($P > 0.05$).

### 3.2. Effects of dietary vitamin E on plasma 8-epi-prostaglandin $F_{2\alpha}$

The obese plasma level of 8-epi-prostaglandin $F_{2\alpha}$ was increased (approximately 5-fold) relative to the lean value and this elevation could be reversed by dietary vitamin E (Fig. 1). Dietary supplementation with vitamin E raised obese plasma levels of α-tocopherol from 1.0 ± 0.1 ($n = 4$) to 5.3 ± 0.9 μg/ml ($n = 6$) ($P < 0.01$).

### 3.3. Effects of dietary vitamin E on glycaemia and insulinaemia

Fasting plasma glycaemia was elevated in obese (7.7 ± 0.7 mM, $n = 4$) relative to lean (4.1 ± 0.5 mM, $n = 5$) ($P < 0.05$) animals and showed a tendency to become reduced in obese animals following dietary vitamin E (6.1 ± 0.9 mM, $n = 6$) ($P > 0.05$). Similarly, fasting plasma insulinaemia was significantly elevated in obese (23.8 ± 6.4 ng/ml, $n = 4$) relative to lean (0.8 ± 0.2 ng/ml, $n = 5$) ($P < 0.05$) animals and was reduced in obese animals by dietary vitamin E (16.5 ± 7.6 ng/ml, $n = 6$), although statistical significance was not attained. Both plasma glycaemia and insulinaemia following the i.v. glucose load (0.5 g/kg) were significantly greater in obese

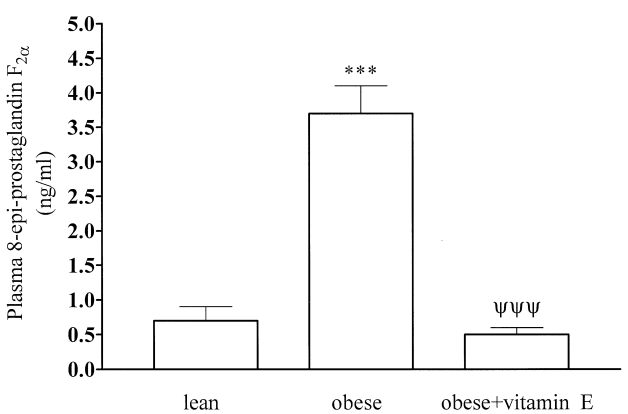


Fig. 1. Plasma levels of 8-epi-prostaglandin $F_{2\alpha}$ in 13-week old lean ($n = 5$) and obese Zucker rats ($n = 4$–6). Vitamin E was administered to obese animals by supplementing the diet with (±)-α-tocopherol acetate (0.5% w/w) for 4 weeks. Values are means ± S.E.M. $^{***}P < 0.01$ with respect to lean control group; $\psi\psi\psi P < 0.01$ with respect to obese control group.

Table 1
Glucose tolerance and glucose-stimulated insulinaemia following an i.v. glucose load (0.5 g/kg) at $t = 0$ min in anaesthetised, 13-week old lean and obese Zucker rats
Glucose and insulin area: area under glucose and insulin curves, respectively, between $t = 0$ and $t = 24$ min. Vitamin E was administered to obese animals by supplementing the diet with (±)-α-tocopherol acetate (0.5% w/w) for 4 weeks. Values are mean ± S.E.M.

| | Lean ($n = 5$) | Obese ($n = 4$) | Obese + vitamin E ($n = 6$) |
|---|---|---|---|
| Glucose area (mM min) | 140.8 ± 17.9 | 221.6 ± 15.3*** | 183.7 ± 21.3 |
| Insulin area (ng min/ml) | 28.4 ± 2.6 | 113.5 ± 30.6* | 30.4 ± 3.6† |

*$P < 0.05$.
***$P < 0.01$ with respect to corresponding lean value.
†$P < 0.05$ with respect to obese value following standard diet.

relative to lean animals (Table 1). This glucose-stimulated hyperinsulinaemia in the obese Zucker rat could be significantly and markedly reduced by dietary vitamin E which did not significantly affect glucose-stimulated plasma glycaemia (Table 1).

## 4. Discussion

The elevation in plasma levels of 8-epi-prostaglandin $F_{2\alpha}$ determined in the obese Zucker rat, agrees well with a report of raised plasma levels of this $F_2$-isoprostane in non-insulin-dependent diabetes mellitus patients (Gopaul et al., 1995) and indicates enhanced lipid peroxidation contingent on oxidant stress (Morrow and Roberts, 1991; Roberts and Morrow, 1994; Pratico et al., 1997). Since our measurements of plasma 8-epi-prostaglandin $F_{2\alpha}$ reflect the esterified form, which is independent of cylcooxygenase activity, observed elevations in this isoprostane in the obese Zucker rat can only result from the increased oxidative modification of arachidonic acid in situ (see Bachi et al., 1997). This interpretation is supported by the ability of an antioxidant, vitamin E added to the diet, to abolish this elevation in plasma 8-epi-prostaglandin $F_{2\alpha}$ in the obese Zucker rat. Furthermore, our data now support the suggestion that the greater antioxidant defence system reported in the obese relative to the lean Zucker rat (Keen et al., 1992; Gunnarsson et al., 1998), may represent an adaptation to oxidant stress.

In addition to reducing a marker of oxidant stress, dietary vitamin E in the obese Zucker rat was able to reverse glucose-stimulated hyperinsulinaemia in the absence of any deterioration in i.v. glucose tolerance. Although we did not specifically measure insulin sensitivity, which was beyond the scope of the present study, our observations are therefore qualitatively consistent with reports that antioxidants such as vitamin E improve insulin action in both man and animals (Paolisso et al., 1994; Faure et al., 1997; Rudich et al., 1997). Indeed, our data with vitamin E in the obese Zucker rat in vivo, are in good agreement with the ability of a lipophilic antioxidant to enhance insulin action in vitro at the level of skeletal muscle in this model of insulin resistance (Jacob et al., 1996; Henriksen et al., 1997; Streeper et al., 1997). In the present study, the approximate 30% decrease in fasting insulinaemia seen in the obese Zucker rat following dietary vitamin E, although its statistical significance was probably precluded by the small number of animals employed, is also consistent with an antioxidant-mediated reduction in in vivo insulin resistance (see Reaven, 1995). Overall, our metabolic observations are therefore in good agreement with a previous report that vitamin E reduces fasting hyperinsulinaemia without affecting fasting plasma glycaemia in the obese Zucker rat (Vormann et al., 1997).

## 5. Conclusion

Our study presents clear evidence of oxidant stress in the obese Zucker rat in vivo and therefore endorses the use of this animal as a suitable insulin-insensitive model in which to investigate the role of oxidant stress in obesity-related pathologies, including insulin resistance in man. Moreover, our data now substantiate the suggestion that antioxidant-mediated improvements in glucose metabolism/insulin action in the obese Zucker rat, previously reported in the literature (see Fujiwara et al., 1988; Jacob et al., 1996; Henriksen et al., 1997; Streeper et al., 1997), may be mediated by a reduction in oxidant stress.

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