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COMMENTARY

Oxidative stress and glucose metabolism—is there a need to revisit effects of insulin treatment?

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Abbreviations

8-Iso-PGF2α	8-Isoprostaglandin $F_{2\alpha}$
MAGE	Mean amplitude of glycaemic excursions
OHA	Oral hypoglycaemic agent

Oxidative stress induced by hyperglycaemia and subsequent cellular damage is thought to be one of the major pathophysiological factors causing late complications in diabetes [1, 2]. Hyperglycaemia-dependent superoxide overproduction by the mitochondrial electron transport chain plays a key role in the cellular activation leading to endothelial damage. The generation of reactive oxygen species also involves an increased polyol and hexosamine pathway flux as well as activation of protein kinase C and excess formation of advanced glycation end-products [1]. These pathways lead to pro-inflammatory cell activation and subsequent endothelial dysfunction in diabetes [1, 3].

Even in healthy individuals, glucose challenges have been shown to increase formation of cellular oxidative stress [4] and trigger proinflammatory cellular activation [5]. In addition to hyperglycaemia itself, Monnier and colleagues previously defined glucose fluctuations as determinants of the amount of oxidative stress generated in type 2 diabetes patients [6]; they showed that the mean amplitude of glycaemic excursions (MAGE) from continuous glucose monitoring data correlated positively and independently with urinary 8-iso prostaglandin $F_{2\alpha}$ (8-iso-PGF2α) excretion as an indicator of oxidative stress [6] in patients with poorly controlled diabetes on oral hypoglycaemic agents (OHA). In this issue of *Diabetologia*, Monnier et al. report an influence of insulin therapy on urinary 8-iso-PGF2α excretion in a cross-sectional setting and provide data from small groups of type 2 diabetes patients started on either insulin therapy or receiving additional OHA [7]. The results from this study are particularly interesting, as they imply an independent effect of insulin therapy on oxidative stress and could help explain some recent observations on glucose variability and the development of late complications in clinical trials [8–10].

In the cross-sectional study, type 2 diabetes patients on OHA were shown to have a ~60% increased urinary 8-iso-PGF2α excretion compared with type 1 or type 2 diabetes patients treated with insulin. 8-Iso-PGF2α excretion in the latter two groups did not differ significantly compared with non-diabetic controls. As reported in the previous publication [6], 8-iso-PGF2α excretion was independently associated with MAGE and 24 h mean glucose levels in type 2 diabetes patients treated with OHA only. In addition, in the current study, 8-iso-PGF2α excretion was independently associated with long-term glucose control reflected by HbA_{1c} [7]. However, these associations were not found in the insulin-treated type 1 and type 2 diabetes patients, further suggesting that insulin treatment itself inhibits oxidative stress in type 1 and type 2 diabetes, reducing it to levels observed in non-diabetic individuals [7]. Additional evidence for an antioxidant effect of insulin was

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added by longitudinal observations in type 2 diabetes patients. Initiation of insulin therapy significantly decreased 8-iso-PGF2α excretion, while no significant change in HbA_{1c}, MAGE and mean 24 h glucose levels was seen. In contrast, patients given add-on OHA did not show any differences in 8-iso-PGF2α excretion, despite a significant decrease of glucose variability and better long-term glucose control [7].

The reported data could be of great importance, since previous clinical endpoint studies have focused on the indirect metabolic effects of insulin therapy geared primarily to achieving pre-defined HbA_{1c} levels and reducing postprandial glucose excursions [9, 11]. The possible direct effects of insulin on oxidative stress and vascular function have remained out of the focus. There is, however, some clinical and experimental evidence supporting this possibility. In an experimental setting it has been shown that intracellular oxidative stress as reflected by the glutathione: glutathione disulfide ratio is diminished by insulin in type 2 diabetes patients undergoing a euglycaemic–hyperinsulinaemic clamp after only 60 min [12]. In vitro, in human aortic endothelial cells, insulin suppressed intercellular adhesion molecule-1 and monocyte chemoattractant protein-1 production, as well as NF-κB binding, which are known to be central mediators of inflammation and diabetic complications [3, 13, 14]; inhibition of NF-κB and of oxidative stress have also been shown to be downregulated by infusion of insulin in obese patients [15]. In patients with acute myocardial infarction, insulin infusion was shown to elicit anti-inflammatory and pro-fibrinolytic effects [16]. Consequently, insulin improved arterial vasodilation, elasticity and limb perfusion by means of nitric oxide-dependent and -independent mechanisms in healthy controls as well as in type 2 diabetes patients [17–20]. In addition to these effects on inflammation and vascular function, insulin potentially influences vascular regeneration by stimulating and mobilising endothelial progenitor cells in vitro and in vivo [21–23]. Yet, insulin effects on cellular homeostasis could also depend on insulin concentrations, since supra-physiological doses of insulin have been shown to induce generation of reactive oxygen species in vitro [24]. Taken together, these data and the current results of Monnier and colleagues [7] imply that insulin itself could have protective effects on the vasculature. However, so far these glucose-independent effects of insulin have not been studied in patients. Clinical trials comparing early insulin therapy and escalation of OHA in type 2 diabetes would be needed to address this question.

Glucose variability and postprandial glucose excursions are thought to influence the development of micro- and macrovascular late complications. Previous data and the current study by Monnier and colleagues show that MAGE is significantly associated with 8-iso-PGF2α excretion in patients with type 2 diabetes [6, 7]. It seems plausible that in these patients glucose variability increases oxidative stress, thereby influencing progression of the disease. Targeting of postprandial glucose values in the STOP-NIDDM trial did indeed reduce the development of diabetes, hypertension and cardiovascular events [25, 26]. Yet, glucose variability was not identified as a risk factor for microvascular disease or neuropathy in the DCCT study of type 1 diabetes patients treated with insulin [8, 10], while the HEART-2D trial failed to show that prandial insulin therapy is superior to a basal regimen in the prevention of secondary cardiovascular events [9]. These considerations and the observation that insulin therapy improves oxidative stress to levels observed in healthy non-diabetic patients [7] prompts speculation that glucose variability does not contribute significantly to the progression of vascular complications in diabetes patients on insulin treatment in whom insulin itself could elicit beneficial effects on the vessel. Most interestingly in this context, recent data from the Pittsburgh Epidemiology of Diabetes Complications study in type 1 diabetes showed a glucose-independent association between lower insulin dose at baseline and the development of non-fatal myocardial infarction [27]. However, these considerations remain speculative and should be followed up in controlled clinical trials.

The observational design of the study by Monnier and colleagues [7] means that data should be interpreted with caution. Thus the cross-sectional patient groups are unbalanced and do not match well, especially with regard to age, vascular risk factors and prevalent late complications [7]. In addition, urinary 8-iso-PGF2α excretion has not so far been established as a marker for prediction of complications in diabetes and vascular disease, while the concept that urinary 8-iso-PGF2α excretion resembles oxidative burden is widely accepted [28]. Future studies will have to show whether other direct and indirect markers of oxidative stress and inflammation support the data obtained in this study. The exact mechanisms of insulin's vascular effects and its anti-oxidative capacity need to be clarified, since the current study by Monnier and colleagues [7] cannot discriminate direct and indirect effects of insulin.

It is time to revisit insulin effects. Especially in type 2 diabetes, different treatment strategies need to be studied with a focus on macrovascular endpoints and myocardial function. Associations between hyperinsulinaemia and vascular events seem to be largely explained by confounding factors [29], while recent data from type 1 diabetes patients suggest that future interventional and epidemiological studies should adjust for the possibility that insulin dosage along with markers of glucose control could influence outcomes [27]. The question of whether insulin therapy should be initiated early in the course of type 2 diabetes and which regimen should be applied cannot be

answered at this point. However, insulin therapy might be superior to a polypharmacological approach as long as effects of novel substances on vascular late complications remain unclear.

Duality of interest The author declares that there is no duality of interest associated with this manuscript.

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