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For Debate

The anti-inflammatory and potential anti-atherogenic effect of insulin: a new paradigm

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Hyperinsulinaemia has been known to be associated with an increase in coronary heart disease and atherosclerosis for sometime [1–4]. However, this association has not been shown in all studies nor has it been shown to occur in women. In a large study involving elderly patients, this association was absent [5]. In the absence of an obvious explanation for the increased occurrence of heart attacks and strokes in hyperinsulinaemic patients, it was proposed that insulin was an atherogenic hormone. Data based on in vitro experiments was proposed as the mechanism underlying this putative effect of insulin. For example, MAP kinase-dependent mechanisms, which are stimulated by high insulin concentrations and trigger mitosis and increased expression of plasminogen activator inhibitor-1 (PAI-1) in vascular smooth muscle cells, were suggested as the mechanisms underlying hyperinsulinaemia-associated atherogenicity [6, 7]. Since atherosclerosis involves mitosis and an increase in the cellularity of the arterial wall, the effect of insulin on p21 Ras signalling was investigated. p21 Ras signalling is considered important for the cellular effects of growth factors and their synergism with insulin. Insulin at pharmacological concentrations increases farnesyltransferase activity and thus the farnesylation of p21 Ras in the cytosol in VSMC. Moreover, farnesylated p21 Ras translocates to the membrane and is ready for activation by other growth factors like PDGF [8]. p21 Ras activation is also promoted by GTP-loading and inhibited by hyperphosphorylation, processes which are appropriately modulated by insulin [9]. p21 Ras activation in turn stimulates MAP kinase and the mitogenic stimulation dependent on this pathway in 3T3-L1 fibroblasts. It was suggested that insulin could exert a pro-atherogenic effect through these growth promoting actions. These effects were observed in vitro at concentrations of 10 to 100 nmol/l of insulin (= 1400–14000 μU/ml) [10–12]. Such concentrations of insulin have not been observed in vivo, even in patients with obesity and insulin resistance and have been rarely observed in patients with extreme insulin resistance and severe acanthosis nigricans [13]. Furthermore, chronic hyperinsulinaemia associated with insulinoma is not associated with atherosclerosis [14]. The state of human growth hormone (HGH) deficiency is associated with low insulin concentrations and an increased risk of atherosclerosis [15–17]. Though this increased risk is considered an effect of HGH deficiency, it is possible that low insulin concentrations contribute to it. Of interest, the increased concentrations of inflammatory mediators, c-reactive protein (CRP) and interleukin-6 (IL-6) decrease with HGH therapy while insulin concentration increases [18].

According to the evolving concept of inflammation at the cellular and molecular levels we now know that proinflammatory stimuli like lipopolysac-

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Abbreviations: MAPK, Mitogen-activated protein kinase; PAI-I, plasminogen activator inhibitor-1; VSMC, vascular smooth muscle cells; PDGF, platelet-derived growth factor; HGH, human growth hormone; CRP, c-reactive protein; IL-6, interleukin-6; LPS, lipopolysaccharide; *I*κB, inhibitor κB; *NF*-κB, nuclear factor; *IL-1β*, interleukin-1*β*; ICAM-1, intercellular adhesion molecule-1; sICAM-1, soluble ICAM-1; VCAM-1, vascular cell adhesion molecule-1; MCP-1, monocyte chemoattractant protein-1; MNC, mononuclear cells; HAEC, human aortic endothelial cells; NOS, nitric oxide synthase; L-NNA, N(G)-nitro-L-arginine; AP-1, activator protein-1; MMPs, matric metalloproteinases; Egr-1, early growth response gene-1; TF, tissue factor; *IKKα*, IκB Kinase α; *IKKβ*, IκB Kinase *β*; ROS, reactive oxygen species; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA

charide (LPS) and pro-inflammatory cytokines, like tumour necrosis factor-α (TNFα) and IL-6, cause phosphorylation of inhibitor κB (IκB) and subsequent translocation of nuclear factor κB (NF-κB) to the nucleus. Intranuclear NF-κB induces the transcription of proinflammatory genes like TNFα, IL-6, interleukin-1β (IL-1β), adhesion molecules like intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), chemokines like monocyte chemoattractant protein-1 (MCP-1) and CRP [19–23]. Using these principles we recently showed that hydrocortisone, a glucocorticoid, induces IκB and suppresses intranuclear NF-κB in mononuclear cells (MNC), in vivo [24]. The use of the circulating MNC allowed us to investigate pro-inflammatory and anti-inflammatory actions of several drugs and/or agents in vivo [24–27].

We have also shown that insulin has a potent anti-inflammatory effect. This effect was observed in human aortic endothelial cells (HAEC) in vitro [28, 29], and in MNC, in vivo [30]. Thus, insulin at physiologically relevant concentrations causes a suppression of intranuclear NF-κB, ICAM-1 and MCP-1 in HAEC in vitro [28, 29]. These effects could be related to the ability of insulin to induce the release of nitric oxide [31] and to enhance the expression of constitutive nitric oxide synthase (NOS) [32] because the ICAM-1 suppressive effect of insulin can be prevented by inhibiting NOS by N(G)-nitro-L-arginine (L-NNA) [29]. There is considerable evidence that nitric oxide has an anti-atherosclerotic effect and that its inhibition is pro-atherogenic [33, 34]. The infusion of a low dose of insulin to achieve concentrations of 24–30 μU/ml into obese subjects, led to the suppression of intranuclear NF-κB, a decrease in the $p47^{phox}$ subunit of NADPH oxidase (the enzyme which converts molecular O_2 to superoxide, $O_2^{\cdot-}$), radical and a decrease in CRP, sICAM-1 and MCP-1. There was also an increase in cellular IκB, which binds NF-κB and prevents its translocation into the nucleus, thus inhibiting the transcriptional action of NF-κB [21]. These effects of insulin are rapid, profoundly anti-inflammatory and probably anti-atherogenic in the long-term. Atherosclerosis is now recognised as an inflammation of the arterial wall and thus the action of NF-κB is considered central to atherosclerosis because it induces the transcription of pro-inflammatory molecules. NF-κB expression in atherosclerotic plaques is known to be consistent [35].

Thus insulin has a suppressive effect on NF-κB with a corresponding reduction in the expression of pro-inflammatory genes. It also suppresses activator protein–1 (AP-1) [36], the transcription factor which modulates matrix metalloproteinases (MMPs), expressed in the atherosclerotic plaque and could be responsible for plaque rupture [37, 38]. In addition, it suppresses early growth response gene–1 (Egr-1) [39], the transcription factor which modulates tissue factor (TF) which, in turn activates thrombin generation. These effects indicate that insulin could have a key inhibitory role in the regulation of factors which are central to atherogenesis, plaque rupture and thrombosis, the final events which precipitate acute myocardial or cerebral ischemia and infarction. The successful use of insulin in acute myocardial infarction, with and without the use of thrombolytics in diabetic patients and non-diabetic subjects in improving clinical outcomes [40], might reflect the profound anti-inflammatory and potential anti-thrombotic properties of insulin.

Potentiation by pre-incubation with insulin on angiotensin II, AGE and glucose induced increases in NF-κB expression in VSMC was reported, but no data have been provided [41] on the direct effect of insulin on NF-κB. It is possible that pre-incubation with insulin reduced the NF-κB in these cells and thus the NF-κB stimulatory effect of the pro-inflammatory agents was amplified because the results were expressed as percentage increase. The direct action of insulin on NF-κB in VSMC requires careful reassessment. Furthermore, insulin has an inhibitory effect (NF-κB suppression) on the two cells which initiate atherosclerotic inflammation, endothelial cells [28] and the MNC [30]. Furthermore, the NF-κB suppressive effect of insulin has been shown in humans in vivo, at physiologically relevant concentrations. Recent work has also shown that insulin reduces the adhesion of inflammatory cells to the endothelium in vivo in experimental animals [42].

Our series of observations on insulin sensitizers of the thiazolidinedione class are consistent with our observation on the anti-inflammatory effects of insulin: both troglitazone and rosiglitazone are profoundly anti-inflammatory with inhibitory effects on the transcription factor NF-κB. Troglitazone has also been shown to inhibit AP-1 and Egr-1 and the respective pro-inflammatory matrix dissolving (MMPs) and pro-thrombotic (TF) genes which they modulate [25, 27, 43, 44]. Thus, thiazolidinediones suppress the plasma concentrations of TNFα, sICAM-1, MCP-1, CRP, MMP-2, MMP-9 and TF. Troglitazone also caused a small but significant increase in plasma interleukin-10 (IL-10) [25], an anti-inflammatory cytokine secreted by Th_2 cells [45, 46]. This enhancing effect by IL-10 is also observed with glucocorticoids. It is intriguing that the decrease in plasma insulin concentrations following troglitazone in the obese is rapid and it parallels the impressive decrease in intranuclear NF-κB concentration and reactive oxygen species (ROS) generation by MNC. It is possible that insulin resistance and inflammation are closely and possibly causally related. The rapid, profound and consistent ROS suppressive anti-inflammatory effects of troglitazone [25, 43, 47] and rosiglitazone [27] allow us to anticipate also their anti-atherogenic action in the long-term. Indeed, short-term treatment

(3 to 6 months) with troglitazone and pioglitazone have been shown to reduce the intimal medial thickness of the internal carotid artery [48], [49]. Troglitazone and rosiglitazone have also been shown to improve post-ischemic endothelium mediated vasodilatation [27, 47] and to improve vasospastic angina [50]. The ability of insulin to induce an acute release of nitric oxide [31] from endothelial cells and to increase the expression of eNOS [32] in these cells is associated with an acute vasodilatory effect. This effect has been observed as an increase in the blood flow of lower [51] and upper limbs [52] and the dilation of the internal carotid artery after systemic insulin infusion [53]. It has also been shown in the veins of the hand and the wrist where the vasodilatory response occurs within 3 to 5 min at the site of infusion, indicating that insulin has a direct action on the vessel walls in vivo [54, 55]. It is, therefore, not surprising that insulin resistant states of obesity and Type II (non-insulin-dependent) diabetes mellitus are associated with impaired vasodilatory responses [56–59]. Consistent with this concept, insulin sensitizers like troglitazone and rosiglitazone, have profound beneficial effects on vascular reactivity while they reverse insulin resistance and actually lower plasma insulin concentrations [27, 47, 50]. Whether TZDs increase insulin-induced nitric oxide release or have a direct action on nitric oxide release from endothelial cells has yet to be shown. It is also of interest that pro-inflammatory cytokines like TNFα, increased in the obese, might not only promote inflammation and atherogenesis but could reduce the vasodilatory effect of insulin through the inhibition of insulin induced eNOS expression, insulin receptor tyrosine phosphorylation and actual reduction in insulin content [60]. Of note, troglitazone and rosiglitazone treatments reduce plasma TNFα concentrations.

In contrast to these actions of insulin and insulin sensitizers, we now know that macronutrient intake in the pure form as carbohydrates, fats, proteins, and mixed meals cause oxidative stress and lead to NF-κB increase in the nucleus and in a pro-inflammatory state [61–63]. Glucose intake (75 g) and a mixed meal intake, result in an increase in the expression of IκB kinase-α (IKKα), which phosphorylates IκB, results in its ubiquitination and causes its content to decrease. Indeed, the ratio of phosphorylated to non-phosphorylated IκB increases after glucose challenge. This allows NF-κB to translocate into the nucleus to induce the transcription of pro-inflammatory cytokines, adhesion molecules, chemokines and enzymes generating ROS. Evidence of an increase in lipid peroxidation after glucose or mixed meal intake has been shown [64]. We have also shown that obesity is associated with a marked increase in oxidative stress and an increase in inflammatory mechanisms (increased plasma TNFα) [65]. CRP has also been shown to be increased [66]. An assessment of the NF-κB/IκB status in the obese has yet to be done.

Since insulin is secreted in response to macronutrient intake, the subsequent action of insulin should be looked at not merely in terms of its ability to normalize glucose, lipid, and amino acid concentrations (the classic paradigm); therefore we have conceptualised a new paradigm in which macronutrients and food are potentially pro-inflammatory and whereby insulin is anti-inflammatory and is secreted in response to these agents. The epitome of excessive food intake, the state of obesity, is characterized by pro-oxidant stress and inflammation which cannot be controlled by insulin in spite of marked increases in its plasma concentrations both in the fasting and the post-prandial state. This is the state of insulin resistance in terms of metabolism and inflammation. It is remarkable that 4 weeks of dietary restriction in the obese results in a marked reduction in oxidative stress, including ROS generation by leucocytes, lipid peroxidation and protein carbonylation without the use of antioxidants [65]. Plasma TNFα concentrations are also increased in the obese and tend to decrease with long-term weight loss [67]. Whether there is a single metabolic step at which the nutrition-induced pro-inflammatory state converges with that of metabolic insulin resistance is not known. Current studies have focused on IKKβ, the enzyme that phosphorylates IκB as a possible site where the pro-inflammatory and metabolic cascades could converge; this hypothesis is supported by the fact that aspirin inhibits this enzyme and also causes a reduction in plasma glucose concentrations in the rat models of obesity and diabetes. Furthermore, targeted disruption of IKKβ protects animals from insulin resistance [68]. In addition, salicylates protect animals from fat-induced insulin resistance [69]. It has been suggested that Type II diabetes itself could be a proinflammatory state [70].

Thus, insulin is an anti-inflammatory hormone and insulin resistant states, including obesity and polycystic ovary syndrome (plasma TNFα concentrations are increased), are pro-inflammatory. While these states are pro-atherogenic, insulin is anti-inflammatory and probably anti-atherogenic in the long-term. The novel ROS suppressive and anti-inflammatory effect of insulin should be compared with that of glucocorticoids, the classic anti-inflammatory drugs or hormones. Our observations suggest that 100 mg of hydrocortisone has a ROS suppressive and NF-κB suppressive effect, comparable to that of 2 IU/h of insulin (25–30 μU/ml) and similar to that of 4 mg of dexamethasone [24, 71, 72]. Hydrocortisone also suppresses AP-1 [73], another pro-inflammatory transcription factor, as well as suppressing plasma MMP-2 concentration. AP-1 induces an increase in the expression of MMP-9 and MMP-2 genes. Insulin inhibits AP-1, MMP-2 and MMP-9 in a similar way to hydrocortisone [36]. Thus, insulin is anti-inflammatory

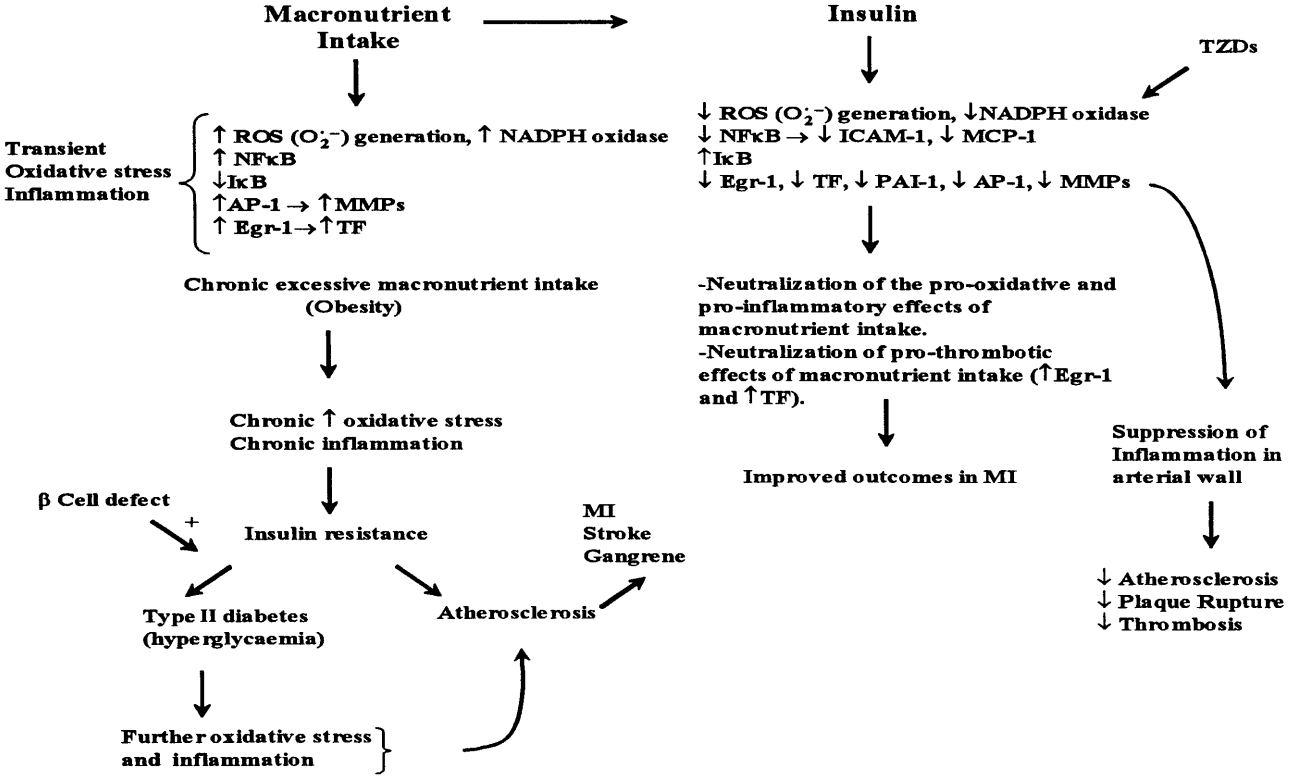

Fig. 1. The pro-oxidant and the pro-inflammatory effects of macronutrient intake, the relation to chronic overnutrition (obesity) and insulin resistance; the anti-inflammatory effects of insulin and TZDs. In addition to NF-κB regulated pro-inflammatory genes, glucose stimulates and insulin and TZDs inhibit AP-1, MMPs, and Egr-1 and TF, the major regulators of atherosclerotic plaque rupture and thrombosis

at physiological concentrations and could exert an anti-inflammatory effect, especially in the post-prandial period as a physiological function. The basic difference in the action of insulin when compared with that of glucocorticoids probably lies in the anabolic effect of insulin: increasing glycogen, protein and lipid synthesis and storage, while decreasing hepatic glucose production through the inhibition of gluconeogenesis and glycogenolysis. In contrast, glucocorticoids are catabolic: they increase gluconeogenesis, causing protein breakdown and lipolysis.

The chronic caloric overload in obesity thus results in a state of chronic oxidative stress and pro-inflammatory changes. These changes contribute to or directly cause insulin resistance which then leads to atherosclerosis and Type II diabetes particularly if there is a concomitant defect in beta-cell function (Fig. 1).

In the Women's Health study, the increase of plasma IL-6 and/or CRP concentrations was associated with the future occurrence of Type II diabetes in obese women over a 4-year follow-up. The multivariate analysis confirmed that the association between increased CRP and IL-6 was independent of obesity, hypertension, hyperlipidaemia, smoking and a family history of diabetes mellitus [74]. This would be consistent with the pre-diabetic insulin resistant state being proinflammatory. Similarly, a reduction in the incidence of Type II diabetes in patients treated with ramipril [75, 76], an ACE-inhibitor, and pravastatin [77], a 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase inhibitor, are consistent with Type II diabetes being an inflammatory state since ACE inhibitors and HMG-CoA reductase inhibitors could have anti-inflammatory effects.

This novel action of insulin has found clinical application in the DIGAMI [78] and ECLA [79] studies, which have shown that low dose insulin infusions benefit the clinical outcomes of both diabetic and non-diabetic patients with acute myocardial infarction. A recent study in ventilated patients in a surgical intensive care unit showed remarkable improvements in the mortality and morbidity after low dose infusions of insulin [80].

In conclusion, insulin is not just an anabolic hormone which lowers blood glucose concentrations, it exerts a potent anti-inflammatory effect which could contribute to an anti-atherogenic effect in the long-term. Similarly, macronutrients induce oxidative stress and are potentially pro-inflammatory. It is thus, rational to assert that insulin, a hormone secreted in response to nutritional intake, is anti-inflammatory.

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