Editorial

Macronutrient intake, insulin secretion, oxidative stress & inflammation: Clinico-pathological implications

The landmark observation that adipose tissue in the insulin-resistant ob/ob mouse expresses an excess of the proinflammatory cytokine, tumour necrosis factor-alpha (TNF-α) and that the infusion of soluble TNF-α receptor reverses the insulin-resistant state through neutralization of TNF-α was a conceptual leap and created a veritable intellectual explosion[1]. This was followed by the demonstration that obese humans not only had elevated concentrations of TNF-α which fell after dietary restriction and weight loss[2] but also had increased levels of oxidative stress which also fell rapidly after caloric restriction and weight loss[3]. It was further shown that TNF-α interfered with insulin signal transduction at the insulin receptor substrate-1 (IRS-1) level[4]. Further exploration revealed that several serine kinases induced by inflammatory mediators caused serine phosphorylation of IRS-1 to interfere with insulin signaling[5]. These included protein kinase C-β (PKC-β), mammalian target of rapamycin (m-TOR), S-6-K, c-Jun N-terminal kinase-1 (JNK-1) and p38 mitogen-activated protein kinase (p38MAPK). In addition, suppressor of cytokine signalling-3 (SOCS-3) causes ubiquitylation and proteolysis of IRS-1[6] while protein-tyrosine phosphatase 1B (PTP-1B) dephosphorylates tyrosine residues on the beta subunit of the insulin receptor[7]. Thus it became clear that obesity was associated with insulin resistance through inflammatory mechanisms[8].

The question that arises, therefore, is what induces chronic inflammation in the obese? The answer lies in the simple observation that the ingestion of glucose administered as a glucose tolerance test in normal controls induces a marked increase in reactive oxygen species (ROS) generation by mononuclear cell (MNC) and polymorphonuclear leucocyte (PMNL) acutely with a concomitant increase in lipid peroxidation[9]. Since ROS generation is proinflammatory through the activation of the proinflammatory transcription factor, nuclear factor kappa B (NFkB)[10], further investigations were directed at inflammatory mechanisms. Glucose intake induces an increase in key proinflammatory transcription factors, NFkB, activator protein-1 (AP-1), early growth response gene-1 (EGR1) and the inflammatory genes modulated by them: monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), matrix metalloproteinase-2 (MMP-2), MMP-9, tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1)[11,12]. Furthermore, it was shown that saturated fat, taken as cream, also induced oxidative stress[13]. An infusion of triglycerides with heparin to induce an acute increase in free fatty acids (FFAs) also led to an increase in ROS generation, NFkB binding, proinflammatory cytokine, macrophage migration inhibitory factor (MIF), in addition to inducing impaired flow-mediated dilation of the brachial artery[14]. Such increases in FFAs are known to lead to insulin resistance as well[15,16]. Thus, oxidative stress, inflammation and insulin resistance are closely linked. These observations led us to investigate whether a high-fat high-calorie (HFHC) meal would induce oxidative stress and inflammation. A 900 calorie fast food meal was shown to induce oxidative and inflammatory stress at the cellular and molecular level, involving the key transcription factors and genes[17,18]. Further investigations along these lines

---

revealed that this fatty meal induced endotoxaemia and the expression of its receptor, toll-like receptor-4 (TLR-4) and its co-receptor CD-14[19]. This meal also induced an increase in the expression of TNF-α and interleukin-1β (IL-1β). As stated above, TNF-α mediates insulin resistance; IL-1β, on the other hand, is a key cytokine mediating damage to the β-cell. The administration of IL-1 receptor antagonist has been shown to benefit type 2 diabetics through improved β cell function and greater insulinogenesis[20-22].

The acute induction of a proinflammatory milieu and several mediators of insulin resistance by the HFHC meal led to a search for non-inflammatory and anti-inflammatory meals. When the effects of the HFHC meal were compared to those of a high fruit and fibre meal, the latter was shown not to induce oxidative and inflammatory stress. Orange juice was the first food shown to be non-inflammatory in spite of all its calories being due to glucose, fructose and sucrose. This was attributed to the antioxidant and anti-inflammatory effect of flavonoids, hesperidin and naringenin, which give it its orange colour[23]. Further investigation demonstrated that the intake of fresh orange juice with an HFHC meal prevented oxidative and inflammatory stress, thereby increasing insulinogenesis and reducing postprandial hyperglycaemia[24]. Similar anti-inflammatory effects were observed with a preparation of resveratrol and grape polyphenols when administered with the HFHC meal[24]. Most recently, the administration of insoluble fibre with the HFHC meal has been shown to exert a similar effect on oxidative and inflammatory stress, glycaemia and insulinogenesis (unpublished observation).

The observations described above have shown that the intake of a single 900 calorie high fatty meal is a potent stimulator of oxidative stress and inflammation. In addition, it induces SOCS-3, PTP-1B and a series of serine kinases, which interfere with insulin signalling at IRS-1 level. Thus, it is clear that the cumulative effect of such meals consumed habitually is likely to result in insulin resistance and caloric overload[25]. In addition, since SOCS-3 interferes with leptin signalling, such meals could potentially result in lack of satiety and an increase in caloric intake[26,27]. Progressive weight gain with insulin resistance would lead to hypertension and hypertriglyceridaemia and thus to metabolic syndrome. The data also show that a high fruit and fibre meal does not induce such changes[19]. Furthermore, the intake of orange juice and other fruit products results in the prevention of such changes[24]. Finally, the intake of

fibre not only prevents oxidative stress, inflammation, endotoxaemia and the induction of TLR-4, TNF-α, IL-1β, SOCS-3 and PTP-1B but also increases insulinogenesis and reduces glycaemic excursion (unpublished observation). These observations provide the basis of strategies for a healthy lifestyle, consistent with a Mediterranean diet, known to be preventive for diabetes and atherosclerosis[28,29].

Insulin exerts vasodilatory and anti-inflammatory actions in addition to its profound functions as a metabolic hormone[30-33]. It is also relevant that insulin is a vasodilator at the macrovascular and microvascular level. This action serves to distribute glucose and other macronutrients to tissues effectively in the postprandial phase. In addition, insulin also induces the expression of endothelial nitric oxide synthase (eNOS) and the secretion of nitric oxide (NO) from the endothelium[34]. While it regulates the distribution, uptake and storage of macronutrients postprandially, it is also probably crucial in modulating postprandial oxidative and inflammatory stress. The concentrations at which insulin exerts the antioxidant and anti-inflammatory effects are comparable to those observed postprandially[31]. Thus, postprandial insulin concentrations are sufficient to prevent oxidative and inflammatory stress after an AHA meal but not an isocaloric HFHC meal since the magnitude of ROS generation, induction of inflammation and the increase in endotoxin and the expression of its receptor TLR-4 and co-receptor CD-14 are far greater[19].

Insulin suppresses ROS generation, p47[phox] expression, intra-nuclear NFkB binding, TNF and IL-1β expression as well as MCP-1, ICAM-1, MMP-9, TF and PAI-1 concentrations when infused intravenously[31,32]. It suppresses the expression of a series of TLRs namely, TLR-1, TLR-2, TLR-4, TLR-7, TLR-9 and the transcription factor PU.1 which regulates their transcription[35]. It also suppresses high mobility group box-1 (HMGB-1) expression and plasma concentrations in the obese and patients with types 2 and 1 diabetes[36-38]. When secreted by a damaged cell, HMGB-1 functions as a proinflammatory cytokine through its binding to advanced glycation end-product (AGE) receptor. Insulin suppresses the plasma concentration of chemokines and the expression of their receptors[39] and oxidative, nitrosative and inflammatory stress induced by an injection of endotoxin in normal controls[36]. Insulin also suppresses the proinflammatory

serine kinases, IκB kinase, JNK-1 and p38MAPkinase, which cause serine phosphorylation of IRS-1 and interfere with insulin signaling[37]. In addition, insulin suppresses plasma FFA concentrations through the inhibition of lipolysis[40,41]. Thus, insulin is a potential insulin sensitizer.

These comprehensive anti-inflammatory actions of insulin have led to the investigation of its potential benefits in inflammatory states. The infusion of a low dose of insulin in patients with acute myocardial infarction demonstrated that it had a suppressive effect on plasma concentrations of C-reactive protein (CRP), serum amyloid A (SAA), PAI-1, oxidative stress, creatine kinase (CK) and creatine kinase-MB (CKMB)[42]. Thus, in this acute clinical setting, it was anti-inflammatory, antioxidant, profibrinolytic and cardioprotective. The infusion of insulin has also been shown to suppress the expression of asthma-related genes, IL-4, CCR-2, CCR-5, transforming growth factor-β (TGF-β), LIGHT and lymphotoxin-β receptor in MNC; in addition, plasma concentrations of chemokines MCP-1 and eotaxin also fall[43]. Genes related to Alzheimer's disease, as expressed in MNC, have also been shown to be reduced following an insulin infusion. The genes suppressed include amyloid precursor protein, presenilins 1 and 2 and glycogen synthase kinase 3β[44].

The fact that macronutrient intake is proinflammatory, in addition to inducing metabolic changes, is probably due to the fact that macronutrients are 'external', and when these change internal milieu significantly, inflammation ensues. However, insulin which is secreted in response to caloric intake has a major role as a metabolic regulator. The recently recognized anti-inflammatory role of insulin is linked to its metabolic role since macronutrient intake of the wrong kind and in excessive amounts can result in inflammation. This new paradigm provides the basis for the link between macronutrient intake obesity, insulin resistance and eventually, diabetes and atherosclerotic disease.

**Paresh Dandona*, Husam Ghanim,
Ajay Chaudhuri & Priya Mohanty**
Division of Endocrinology, Diabetes &
Metabolism, State University of
New York at Buffalo, Buffalo, NY 14215, USA
*For correspondence:
pdandona@kaleidahealth.org

References

1. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science* 1993; *259* : 87-91.

2. Dandona P, Weinstock R, Thusu K, Abdel-Rahman E, Aljada A, Wadden T. Tumor necrosis factor-alpha in sera of obese patients: fall with weight loss. *J Clin Endocrinol Metab* 1998; *83* : 2907-10.

3. Dandona P, Mohanty P, Ghanim H, Aljada A, Browne R, Hamouda W, *et al.* The suppressive effect of dietary restriction and weight loss in the obese on the generation of reactive oxygen species by leukocytes, lipid peroxidation, and protein carbonylation. *J Clin Endocrinol Metab* 2001; *86* : 355-62.

4. Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science* 1996; *271* : 665-8.

5. Hotamisligil GS. Inflammation and metabolic disorders. *Nature* 2006; *444* : 860-7.

6. Rui L, Yuan M, Frantz D, Shoelson S, White MF. SOCS-1 and SOCS-3 block insulin signaling by ubiquitin-mediated degradation of IRS1 and IRS2. *J Biol Chem* 2002; *277* : 42394-8.

7. Byon JC, Kusari AB, Kusari J. Protein-tyrosine phosphatase-1B acts as a negative regulator of insulin signal transduction. *Mol Cell Biochem* 1998; *182* : 101-8.

8. Ghanim H, Aljada A, Hofmeyer D, Syed T, Mohanty P, Dandona P. Circulating mononuclear cells in the obese are in a proinflammatory state. *Circulation* 2004; *110* : 1564-71.

9. Mohanty P, Hamouda W, Garg R, Aljada A, Ghanim H, Dandona P. Glucose challenge stimulates reactive oxygen species (ROS) generation by leucocytes. *J Clin Endocrinol Metab* 2000; *85* : 2970-3.

10. Barnes PJ, Karin M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 1997; *336* : 1066-71.

11. Aljada A, Friedman J, Ghanim H, Mohanty P, Hofmeyer D, Chaudhuri A, *et al.* Glucose ingestion induces an increase in intranuclear nuclear factor kappaB, a fall in cellular inhibitor kappaB, and an increase in tumor necrosis factor alpha messenger RNA by mononuclear cells in healthy human subjects. *Metabolism* 2006; *55* : 1177-85.

12. Aljada A, Ghanim H, Mohanty P, Syed T, Bandyopadhyay A, Dandona P. Glucose intake induces an increase in activator protein 1 and early growth response 1 binding activities, in the expression of tissue factor and matrix metalloproteinase in mononuclear cells, and in plasma tissue factor and matrix metalloproteinase concentrations. *Am J Clin Nutr* 2004; *80* : 51-7.

13. Mohanty P, Ghanim H, Hamouda W, Aljada A, Garg R, Dandona P. Both lipid and protein intakes stimulate increased generation of reactive oxygen species by polymorphonuclear leukocytes and mononuclear cells. *Am J Clin Nutr* 2002; *75* : 767-72.

14. Tripathy D, Mohanty P, Dhindsa S, Syed T, Ghanim H, Aljada A, *et al.* Elevation of free fatty acids induces inflammation and impairs vascular reactivity in healthy subjects. *Diabetes* 2003; *52* : 2882-7.

15. Boden G, Lebed B, Schatz M, Homko C, Lemieux S. Effects of acute changes of plasma free fatty acids on intramyocellular fat content and insulin resistance in healthy subjects. *Diabetes* 2001; *50* : 1612-7.

16. Homko CJ, Cheung P, Boden G. Effects of free fatty acids on glucose uptake and utilization in healthy women. *Diabetes* 2003; *52* : 487-91.

17. Aljada A, Mohanty P, Ghanim H, Abdo T, Tripathy D, Chaudhuri A, *et al.* Increase in intranuclear nuclear factor kappaB and decrease in inhibitor kappaB in mononuclear cells after a mixed meal: evidence for a proinflammatory effect. *Am J Clin Nutr* 2004; *79* : 682-90.

18. Patel C, Ghanim H, Ravishankar S, Sia CL, Viswanathan P, Mohanty P, *et al.* Prolonged reactive oxygen species generation and nuclear factor-kappaB activation after a high-fat, high-carbohydrate meal in the obese. *J Clin Endocrinol Metab* 2007; *92* : 4476-9.

19. Ghanim H, Abuaysheh S, Sia CL, Korzeniewski K, Chaudhuri A, Fernandez-Real JM, *et al.* Increase in plasma endotoxin concentrations and the expression of toll-like receptors and suppressor of cytokine signaling-3 in mononuclear cells after a high-fat, high-carbohydrate meal: implications for insulin resistance. *Diabetes Care* 2009; *32* : 2281-7.

20. Larsen CM, Faulenbach M, Vaag A, Vølund A, Ehses JA, Seifert B, *et al.* Interleukin-1-receptor antagonist in type 2 diabetes mellitus. *N Engl J Med* 2007; *356* : 1517-26.

21. Larsen CM, Faulenbach M, Vaag A, Ehses JA, Donath MY, Mandrup-Poulsen T. Sustained effects of interleukin-1 receptor antagonist treatment in type 2 diabetes. *Diabetes Care* 2009; *32* : 1663-8.

22. van Poppel PC, van Asseldonk EJ, Holst JJ, Vilsbøll T, Netea MG, Tack CJ. The interleukin-1 receptor antagonist anakinra improves first-phase insulin secretion and insulinogenic index in subjects with impaired glucose tolerance. *Diabetes Obes Metab* 2014; *16* : 1269-73.

23. Ghanim H, Mohanty P, Pathak R, Chaudhuri A, Sia CL, Dandona P. Orange juice or fructose intake does not induce oxidative and inflammatory response. *Diabetes Care* 2007; *30* : 1406-11.

24. Ghanim H, Sia CL, Upadhyay M, Korzeniewski K, Viswanathan P, Abuaysheh S, *et al.* Orange juice neutralizes the proinflammatory effect of a high-fat, high-carbohydrate meal and prevents endotoxin increase and Toll-like receptor expression. *Am J Clin Nutr* 2010; *91* : 940-9.

25. Lim S, Won H, Kim Y, Jang M, Jyothi KR, Kim Y, *et al.* Antioxidant enzymes induced by repeated intake of excess energy in the form of high-fat, high-carbohydrate meals are not sufficient to block oxidative stress in healthy lean individuals. *Br J Nutr* 2011; *106* : 1544-51.

26. Lubis AR, Widia F, Soegondo S, Setiawati A. The role of SOCS-3 protein in leptin resistance and obesity. *Acta Med Indones* 2008; *40* : 89-95.

27. McEwen HJ, Inglis MA, Quennell JH, Grattan DR, Anderson GM. Deletion of Suppressor of cytokine signaling 3 from forebrain neurons delays infertility and onset of hypothalamic leptin resistance in response to a high caloric diet. *J Neurosci* 2016; *36* : 7142-53.

28. O'Keefe JH, Gheewala NM, O'Keefe JO. Dietary strategies for improving post-prandial glucose, lipids, inflammation, and cardiovascular health. *J Am Coll Cardiol* 2008; *51* : 249-55.

29. Shen J, Wilmot KA, Ghasemzadeh N, Molloy DL, Burkman G, Mekonnen G, *et al.* Mediterranean dietary patterns and cardiovascular health. *Annu Rev Nutr* 2015; *35* : 425-49.

30. Chaudhuri A, Kanjwal Y, Mohanty P, Rao S, Sung BH, Wilson MF, *et al.* Insulin-induced vasodilatation of internal carotid artery. *Metabolism* 1999; *48* : 1470-3.

31. Dandona P, Aljada A, Mohanty P, Ghanim H, Hamouda W, Assian E, *et al.* Insulin inhibits intranuclear nuclear factor kappaB and stimulates IkappaB in mononuclear cells in obese subjects: evidence for an anti-inflammatory effect? *J Clin Endocrinol Metab* 2001; *86* : 3257-65.

32. Aljada A, Ghanim H, Mohanty P, Kapur N, Dandona P. Insulin inhibits the pro-inflammatory transcription factor early growth response gene-1 (*Egr*)-1 expression in mononuclear cells (MNC) and reduces plasma tissue factor (TF) and plasminogen activator inhibitor-1 (PAI-1) concentrations. *J Clin Endocrinol Metab* 2002; *87* : 1419-22.

33. Dandona P, Aljada A, Mohanty P, Ghanim H, Bandyopadhyay A, Chaudhuri A. Insulin suppresses plasma concentration of vascular endothelial growth factor and matrix metalloproteinase-9. *Diabetes Care* 2003; *26* : 3310-4.

34. Aljada A, Saadeh R, Assian E, Ghanim H, Dandona P. Insulin inhibits the expression of intercellular adhesion molecule-1 by human aortic endothelial cells through stimulation of nitric oxide. *J Clin Endocrinol Metab* 2000; *85* : 2572-5.

35. Ghanim H, Mohanty P, Deopurkar R, Sia CL, Korzeniewski K, Abuaysheh S, *et al.* Acute modulation of toll-like receptors by insulin. *Diabetes Care* 2008; *31* : 1827-31.

36. Dandona P, Ghanim H, Bandyopadhyay A, Korzeniewski K, Ling Sia C, Dhindsa S, *et al.* Insulin suppresses endotoxin-induced oxidative, nitrosative, and inflammatory stress in humans. *Diabetes Care* 2010; *33* : 2416-23.

37. Dandona P, Ghanim H, Green K, Sia CL, Abuaysheh S, Kuhadiya N, *et al.* Insulin infusion suppresses while glucose infusion induces toll-like receptors and high-mobility group-B1 protein expression in mononuclear cells of type 1 diabetes patients. *Am J Physiol Endocrinol Metab* 2013; *304* : E810-8.

38. Dandona P, Ghanim H, Sia CL, Green K, Abuaysheh S, Dhindsa S, *et al.* A mixed anti-inflammatory and pro-inflammatory response associated with a high dose of corticosteroids. *Curr Mol Med* 2014; *14* : 793-801.

39. Ghanim H, Korzeniewski K, Sia CL, Abuaysheh S, Lohano T, Chaudhuri A, *et al.* Suppressive effect of insulin infusion on chemokines and chemokine receptors. *Diabetes Care* 2010; *33* : 1103-8.

40. Jensen MD, Caruso M, Heiling V, Miles JM. Insulin regulation of lipolysis in nondiabetic and IDDM subjects. *Diabetes* 1989; *38* : 1595-601.

41. Moskowitz J, Fain JN. Hormonal regulation of lipolysis and phosphorylase activity in human fat cells. *J Clin Invest* 1969; *48* : 1802-8.

42. Chaudhuri A, Janicke D, Wilson MF, Tripathy D, Garg R, Bandyopadhyay A, *et al.* Anti-inflammatory and profibrinolytic

effect of insulin in acute ST-segment-elevation myocardial infarction. *Circulation* 2004; *109* : 849-54.

43. Ghanim H, Green K, Abuaysheh S, Batra M, Kuhadiya ND, Patel R, *et al.* Suppressive effect of insulin on the gene expression and plasma concentrations of mediators of asthmatic inflammation. *J Diabetes Res* 2015; *2015* : 202406.

44. Dandona P, Mohamed I, Ghanim H, Sia CL, Dhindsa S, Dandona S, *et al.* Insulin suppresses the expression of amyloid precursor protein, presenilins, and glycogen synthase kinase-3beta in peripheral blood mononuclear cells. *J Clin Endocrinol Metab* 2011; *96* : 1783-8.