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Review

Biomarkers of stress-mediated metabolic deregulation in diabetes mellitus

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ABSTRACT

This review illustrates the relationship of oxidative and nitrative stress to diabetes mellitus and its complications. This is of considerable interest because diabetes mellitus is a lifetime systemic metabolic disease that may have childhood or adult onset and affects not only a triad of pancreatic islet cell insulin, pituitary insulin-like growth hormone, and liver steatosis, it has a long-term association with adiposity, atherosclerosis, coronary vascular disease, kidney disease of the nature afferent arteriolar sclerosis and nodular glomerulosclerosis, cerebrovascular disease, and amyloid deposition in the pancreas and kidney. Only at the end of the 20th century do we gain insight into oxidative and nitrative stress and their consequences. Of special interest here is the fact that reactive oxygen and nitrogen radicals are with us generated throughout the life cycle, and the roles for glutathione and Fe^{3+} are key elements in the metabolic picture, which brings into the picture dietary factors. More research is required to demonstrate the clinical relivance of naturally-occuring whole-food antioxidants in ameliorating human diabetic complications *in vivo*.

Contents

Abbreviations: eNOS, endothelial nitric oxide synthase; NOS3, nitric oxide synthase 3; cNOS, constitutive nitric oxide synthase; nNOS, neuronal nitric oxide synthase; iNOS, induced nitric oxide synthase; kDa, kilodalton; NADPH, nicotinamide adenine dinucleotide phosphate; MDA, malondialdehyde; α-T, α-tocopherol; GSH, glutathione; SOD, superoxide dismutase; CAT, catalase; SeCys, selenocysteine; FAD, flavin adenine dinucleotide; -SH, thiol; mTOR, mammalian target of rapamycin; TLR4, toll-like receptor 4; SIRT, sirtuin; O-GlcNAc, N-acetylglucosaminyltransferase; CRTC2, CREB regulated transcription coactivator 2; PPARγ, peroxisome proliferator-activated receptor gamma; RXR, retinoid X receptor alpha; TTR, plasma transthyretin; OLETF, Otsuka Long Evans Tokushima Fatty rats; hCMEC, human cerebral microvascular endothelial cell line; FR-OH, free retinol; TBARS, thiobarbituric acid reactive substrate

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1. Oxidative stress in diabetes mellitus

Oxidative stress is a pathogenetic mechanism in diabetic complications [1]. Chronic oxidative stress caused by prolonged hyperglycaemia worsens the diabetic state [2]. Evidence for upregulated oxidative stress responses in diabetes includes observations of decreased plasma antioxidant concentrations in both diabetic subjects [3] and animal models [4].

1.1. *Free radicals generating systems*

The handling of ROS in the plasma is related to the action of nitric oxide synthase (NOS) isoenzymes: eNOS, iNOS and nNOS. Endothelial eNOS, also known as nitric oxide synthase 3 (NOS3) or constitutive NOS (cNOS), in humans is encoded by the NOS3 gene. This may have relevance to the relationship of diabetes to atherosclerosis risk. inducible NOS in humans is

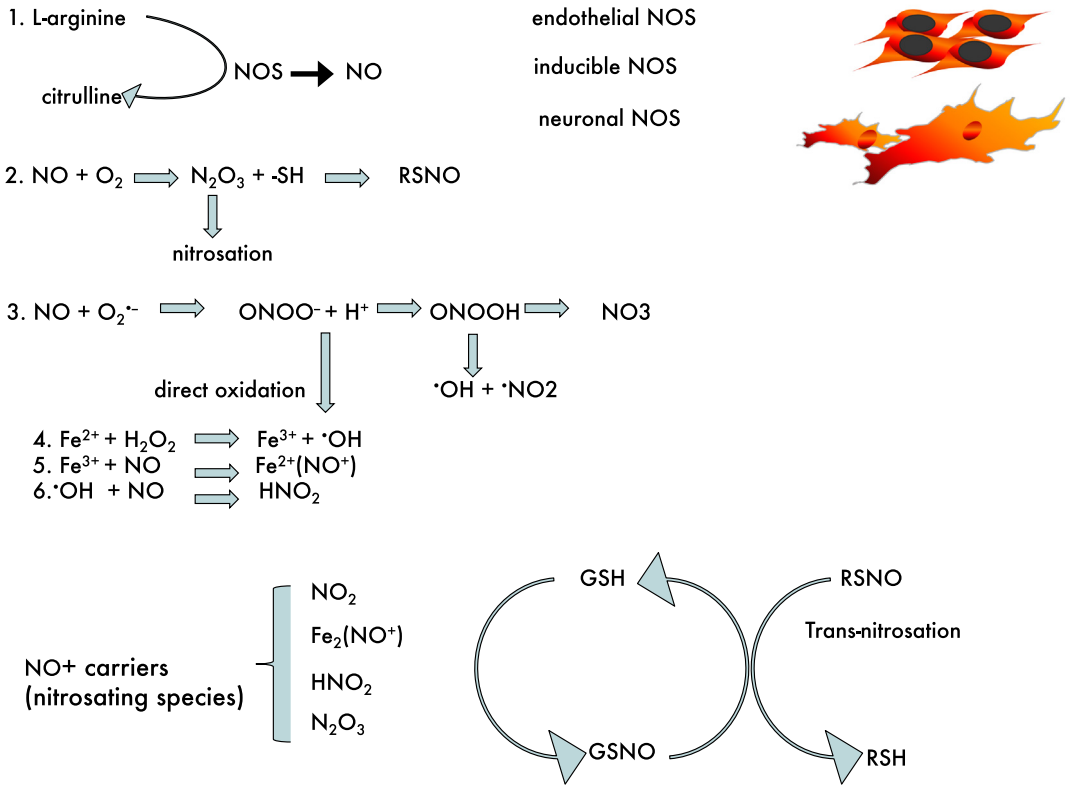

Illustration 1 – The graphic shows the mechanism of oxidative and nitrosative stress in two parts, divided into direct enzyme mediated oxidation and nitrosation requiring the activities of cell mediated isoenzymes: (1) endothelial, constitutive (in macrophage), and nNOS. These convert L-Arg to citrulline. (2) Is the reaction that after conversion of $NO^{\cdot}$ + O_2 to N_2O_3 which immediately reacts with a -SH group, or in (3) by nitrosation forms $ONOO^{\cdot-}$. The $ONOO^{\cdot-}$ is itself not stable and undergoes direct oxidation. These reactions are very fast. In the lower panel of the first part, four nitrosating species form GSNO from GSH, and the GSNO is transnitrosated. The second part of the graphical summary shows non-lipid peroxidation. This involves three steps: (1) initiation; (2) propagation; (3) termination. The rapidity of these reactions is critical in the metabolic scheme of life systems. It has nothing resembling the translation of genetic code.

encoded by the NOS2 gene and may have relevance to hepatosteatosis.

3-nitrotyrosine, a protein oxidation product, has been identified in various inflamed tissues indicating the activity of peroxynitrite $ONOO^{\cdot-}$. Further, nitrite NO_2^-, the primary metabolic end product of $NO^{\cdot}$, can be oxidized by the heme peroxidases horseradish peroxidase, myeloperoxidase (MPO), and lactoperoxidase (LPO), in the presence of H_2O_2, to most likely form $NO_2^{\cdot}$, which can also contribute to tyrosine nitration during inflammatory processes. Formation of NO_2^- via peroxidase-catalyzed oxidation of $NO^{\cdot}$ may provide an additional pathway contributing to cytotoxicity or host defence associated with increased $NO^{\cdot}$ production [5], Illustration 1.

Mechanisms that contribute to increased oxidative stress in diabetes include increased non-enzymatic- and autooxidative glycosylation, variations in the level of inflammatory mediators, alteration in sorbitol pathway activity, alteration in antioxidant defence systems and energy metabolism [1].

1.2. *Damaging effects mediated by oxygen free radicals*

Glycation end products (GEP) are stable covalent adducts that form of glucose reaction with proteins *in vivo*. The aldehyde group in glucose is condensed with ε-amino groups in protein via Shiff's base linkage, and this aldimine product can rearrange to the corresponding reactive ketoamine and amadori products [6]. Advanced glycation end products can occur on free amine-containing lipids and DNA and proceeds via a complex series of chemical rearrangements to generate OFR during autooxidation [7].

Amadori adducts are source of $O_2^{\cdot-}$ and H_2O_2 radicals. The formation of GEP of protein enhances the potential exposure of cell membranes to oxidation damage and alters its structure and function [1]. Modification of long-lived extracellular proteins is associated with the development of complications in diabetes [8]. Also, glycation in diabetes modifies lipids, lipoproteins as in the case of apo B and low-density lipoprotein (LDL) [9], and increases the susceptibility of LDL to oxidation [29]. Methylglyoxal hydroimidazolones are quantitatively major advanced glycation end products (AGEs) of human lens proteins [8]. The AGE distribution in the elderly group suggests that the advanced glycation is implicated in cataract formation, which in diabetic patients occurs vigorously as compared with nondiabetic cataract patients [10].

Lipid peroxidation is an oxidative stress-relevant sequential reaction that is regulated either enzymatically, or non-enzymatically. This latter form is associated mostly with cellular damage [11]. The primary targets for attack by OFR are the polyunsaturated fatty acids (PUFAs) of membrane phospholipids. However, attack of LDL-PUFAs should also be considered [12]. The reaction then proceeds through three stages as depicted in [13] and in Illustration 1.

The enzymes lipoxygenase, cyclooxygenase and peroxidase promote the controlled peroxidation of fatty acids to generate hydroperoxides and endoperoxides. Hydroperoxides degrade to various secondary products including hydroxy-fatty acids, epoxides and scission products such as aldehydes (including malondialdehydes (MDA)), ketons, and lactones, many of which are toxic [14]. Other products are alkenals and hydroxy alkenals such as 4-hydroxynonenal [15] and hydrocarbon genes as pentane [40]. As well as

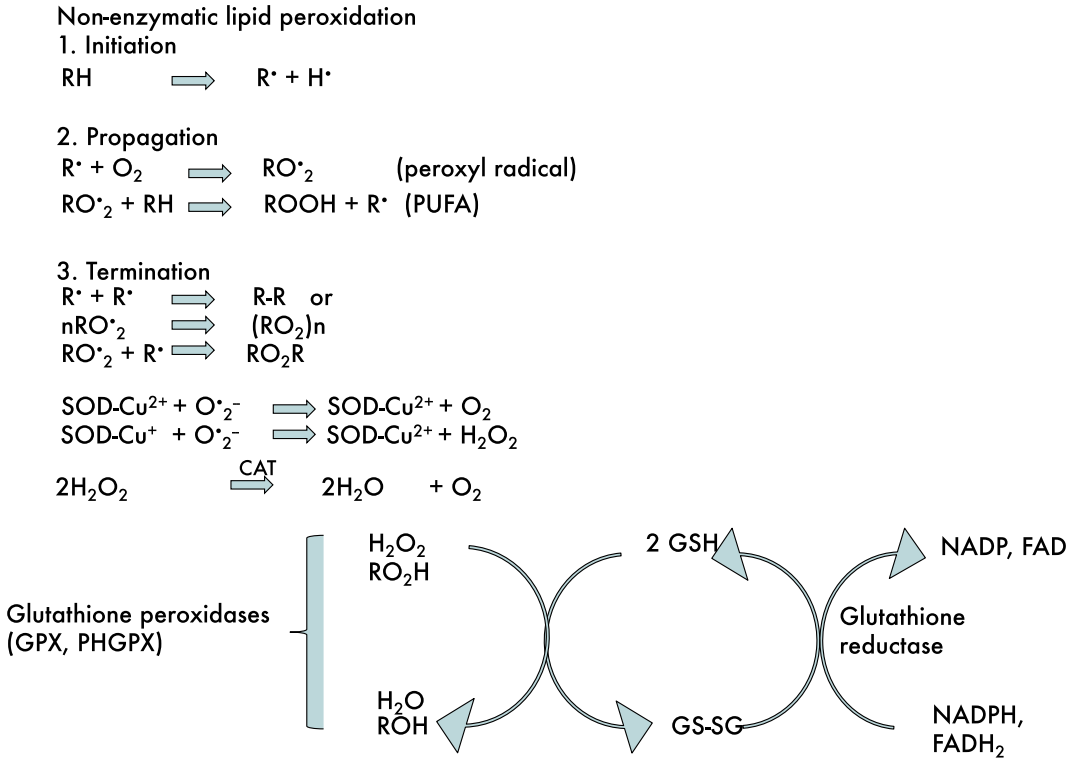

Illustration 1. *(continued)*

8-isoprostaglandin F2α [16]. Peroxidation of membrane lipids subsequently causes PUFAs depletion, decreased lipid fluidity, alterations in membrane permeability and membrane-bound enzymes, ion transport, substance release from the subcellular compartment and the release of cytotoxic metabolites of lipid hydroperoxides [17]. MDA is a widely used marker of lipid peroxidation that uprises in erythroctyes of type 2 diabetic patients [18]. In addition, lipid peroxidation data presented by thiobarbituric acid reactive substrates (TBARS) in plasma were found to be elevated by 80% in the early stages of diabetes in human, with time-dependent progressive increases [19].

2. Antioxidant defence system

The antioxidant defence system comprises a series of enzymatic and non-enzymatic components listed in [20] and include methionine or lipotropes for choline biosynthesis. Selenium is an essential trace element with known antioxidant properties. Cytosolic thioredoxin reductase from mammalian cells is a dimeric flavin enzyme comprising a glutathione reductase-like equivalent elongated with 16 residues including the conserved carboxy-terminal sequence, Gly-Cys-SeCys-Gly, where SeCys is selenocysteine. Selenium is essential for the activity of thioredoxin reductase, explaining why this trace element is required for cell proliferation by effects on thioredoxin-dependent control of the intracellular redox state, ribonucleotide reductase production of deoxyribonucleotides, or activation of transcription factors. The selenazol drug Ebselen mimic GSH peroxidase mimic with antioxidant properties. The hydrogen peroxide reductase activity of human thioredoxin reductase was stimulated 15-fold by 2 μM Ebselen. Glutaredoxins protect against oxidative stress by catalyzing the reduction of protein mixed disulfides with GSH. The mechanism of glutaredoxins as efficient general GSH-mixed disulfide oxidoreductases may protect proteins from inactivation as well as play a significant role in general redox signaling [21].

2.1. *Superoxide dismutase, catalase, glutathione peroxidase*

The most important antioxidant enzymes include SOD, CAT, and GSH peroxidase acting cooperatively at different sites in the metabolic cascade of free radicals. Relatively high concentrations of Cu-Zn SOD are present in β-cells of human islets as it specifically correlates with maintenance of β-cell function and homeostasis [22]. SOD is ubiquitous, especially in tissues with high oxygen utilization [23]. Both Cu-Zn SOD and Mn-SOD counteract the deleterious effect of $O_2^{\cdot-}$ by catalyzing the reaction described in [22]. In tissues, CAT is mitochondria- and peroxisomes-bound, whereas it exists in a soluble state in red blood cells [24]. CAT mediates the detoxification of H_2O_2 and thus counteracts the deleterious effects of $OH^{\cdot}$ radical [12,25]. A negative correlation between the course of diabetes and CAT exists ($r = -0.72$) [26]. The cytosolic compartment in mammalian cells is also protected from oxidative damage caused by H_2O_2 by the selenoenzyme GSH peroxidase [12]. Importantly, GSH peroxidase's role is importantly concerned with the reduction of lipid hydroperoxide [19], and soluble peroxides to the corresponding alcohols, at the expense of GSH which is oxidized to GSSG [20].

2.2. *Glutathione*

GSH is the major intracellular redox buffer in almost all cell types [27]. A relatively high concentration of GSH is present in β-cells [28]. A decrease in the levels of both plasma and intracellular antioxidants such as GSH, tocopherol, -SH and ascorbate has been demonstrated in both types of diabetes [29]. Blood GSH was significantly reduced by 25% at the first two years of diagnosis of type 2 diabetes, and by 50% in plasma [30]. Such deficiency is associated with the increased peroxidation [19].

2.3. *Glutamine*

Glutamine and its metabolites are relevant nutrients to consider in the context of lipid-induced macrophages dysfunction. Pathologic inflammation driven by macrophages is recognized as a contributing factor to the impaired tissue repair responses observed in obese and diabetic patients [31]. This occurs because glutamine deficiency reduces lipid-induced lysosome dysfunction, inflammasome activation, and cell death. Under glutamine deficient conditions mTOR activation is decreased and autophagy enhanced. Glutamine deficiency prevented the suppressive effect of the saturated fat palmitate on mitochondrial respiration and this phenotype was associated with protection from macrophage cell death. Together, the findings reveal that crosstalk between activation-induced metabolic reprogramming and the nutrient microenvironment can dramatically alter macrophage responses to inflammatory stimuli. This is related to toll-like receptor 4 (TLR4) activation; an inflammatory receptor expressed at high levels on macrophages in response to bacterial infection and/or sterile tissue damage. Moreover, activation of macrophage TLR4 in a lipid-rich environment triggers lysosome damage, which contributes to NLRP3 inflammasome activation and macrophage cell death.

2.4. *Sirtuins*

Sirtuin enzymes regulate metabolic enzymes, with a particular role of lysine acetylation in mitochondria. Recent advances have highlighted the role of sirtuins in type 2 diabetes. Significant insights reviewed by Osborne et al. [32] are: (i) SIRT3, SIRT4 and SIRT5 modulate acyl modifications such as acetylation and succinylation; (ii) mitochondrial SIRTs are implicated in metabolism, mitochondrial fidelity, and cell stress. SIRT3 can deacetylate and thereby activate central metabolic regulators in the mitochondrial matrix, glutamate dehydrogenase and isocitrate dehydrogenase 2, as those regenerate antioxidants. Expression of SIRT3 is selectively activated during fasting and calorie restriction. This is not surprising because SIRT3 regulates the acetylation level and enzymatic activity of key metabolic enzymes, such as acetyl-CoA synthetase, long-chain acyl-CoA dehydrogenase, and 3-hydroxy-3-methylglutaryl-CoA synthase 2, and enhances fat metabolism during fasting. SIRT5, in contrast to SIRT3, deacetylates none of the mitochondrial matrix

proteins tested. Instead, it can deacetylate cytochrome c, a protein of the mitochondrial intermembrane space with a central function in oxidative metabolism, as well as initiating apoptosis. SIRT5 exhibits demalonylase/desuccinylase activity, and lysine succinylation and malonylation are abundant mitochondrial protein modifications. However, Newman et al. [33] have reported that transcriptional activity of SIRT1 is increased by phosphorylation, while glycosylation decreases it. The list of SIRT1 substrates is continuously growing and includes numerous transcription factors. During aging and diabetes, addition of N-acetylglucosaminyltransferase (O-GlcNAc) attenuates the activity of SIRT1 on the function of related proteins. SIRT4 in addition to being a deacetylase, has ADP-ribosylase, and a lipoamidase function, as well as key roles in lipid and glutamine metabolism. This raises many new questions regarding the role of mitochondrial sirtuins in the regulation of energy metabolism.

2.5. PPARγ

We have shown that PPARγ/Retinoid X receptor alpha (RXR) promotes fat storage in the body by a combination of direct induction of molecules involved in Triglycerids accumulation and suppression of leptin gene expression as well as inactivation of PPARα signaling pathways. In times of fasting, this PPARγ/leptin/PPARα network maximizes energy storage, which is quite advantageous for survival. In times of feast, which are the norm in industrialized nations nowadays, however, this network causes excessive adiposity, insulin resistance, obesity and diabetes. Thus appropriate antagonism of PPARγ/RXR, which simultaneously leads to appropriate agonism of leptin and PPARα, may be a logical approach to protection against obesity and type 2 diabetes. Treating subjects with the wild-type PPARγ (Pro 12 allele) with higher activity with PPARγ/RXR inhibitors is an example for "personalized treatment" of subjects genetically susceptible to obesity and diabetes. In contrast, treating subjects with the variant PPARγ (Ala 12 allele) with lower PPARγ activity with PPARγ/RXR inhibitors may worsen their insulin resistance [34].

3. Endothelial dysfunction in diabetes mellitus

Vascular injury is the principal complication of all forms of diabetes. Multiple studies in patients and *in vitro* have revealed that hyperglycemia alters endothelial metabolism and function in such a way that could lead to vascular disease [35].

3.1. Transthyretin

Plasma transthyretin (TTR) is a plasma protein secreted by the liver that circulates bound to retinol-binding protein 4 (RBP4) and its retinol ligand. TTR is the sole plasma protein that reveals from birth to old age evolutionary patterns that are closely superimposable to those of lean body mass (LBM) and thus works as the best surrogate analyte of LBM (30). Any alteration in energy-to-protein balance impairs the accretion of LBM reserves and causes early depression of TTR production. In acute inflammatory states, cytokines induce urinary leakage of nitrogenous catabolites, deplete LBM stores, and cause an abrupt decrease in TTR/RBP4 values. As a result, thyroxine and retinol ligands are released in free form, creating a second frontline that strengthens that primarily initiated by cytokines. Malnutrition and inflammation thus keep in check TTR and RBP4 secretion by using distinct and unrelated physiologic pathways, but they operate in concert to downregulate LBM stores. The biomarker complex integrates these opposite mechanisms at any time and thereby constitutes an ideally suited tool to determine residual LBM resources still available for metabolic responses, hence predicting outcomes of the most interwoven disease conditions [36].

Finally, unrelated to the above, as the preventive effects against diabetes have been demonstrated using several functional food factors including α-lipoic acid [37], fermented-grain food [38], vitamin E [39], and S-allyl cysteine [40], a recent study of experimental diabetes showed that chronic treatment with partially hydrolyzed guar gum (PHHG) improved insulin resistance, delayed the onset of diabetes, and inhibited the development of diabetic complications, as well as identified cysteinylated transthyretin as a predictive biomarker of treatment response to PHGG in Otsuka Long Evans Tokushima Fatty (OLETF) rats [41]. This study is unique in that it demonstrated repressed steatosis in OLETF rats by PHGG, a water-soluble dietary fiber. Perhaps dietary interventions by PHGG may offer potential for nontoxic and physiological methods for altering the intestinal environment, leading to beneficial effects in the liver. The study also identified cys-TTR as a predictive biomarker of the response to treatment with PHGG in OLETF rats.

Moreover, a recent study showed transthyretin (TTR) binds Aβ peptide, preventing its deposition and toxicity [42]. TTR is decreased in Alzheimer's disease (AD) patients [43]. Additionally, AD transgenic mice with only one copy of the TTR gene show increased brain and plasma Aβ levels when compared to AD mice with both copies of the gene, suggesting TTR involvement in brain Aβ efflux and/or peripheral clearance. Here we showed that TTR promotes Aβ internalization and efflux in a human cerebral microvascular endothelial cell line, hCMEC/D3. TTR also stimulated brain-to-blood but not blood-to-brain Aβ permeability in hCMEC/D3, suggesting that TTR interacts directly with Aβ at the blood-brain-barrier [42].

Healthy subjects do not excrete detectable amounts of retinol in the urine, indicating that stress-associated retinoluria, which has been shown to be correlated with the duration and severity of injury, results from an expanded extracellular free pool in which its unmetabolized fractions undergo, like FT4, kidney overflow. Holo-RBP is, in contrast with TTR, the sole conveyor of retinol, for which the normal concentration in adult humans (60 mg/L) ensures the carriage of ~500 mg R-OH/L with a free fraction (free retinol (FR-OH)) measured at 1 mg/L, yielding a normal free to bound ratio of 1:500 [37]. In the case of acute stress of medium severity, decreasing holo-RBP values by 40%, an estimated proportion of 200 mg retinol is released as FR-OH, which diffuses uniformly in a larger distribution space (18 L) than that of FT4 and causes an augmented FR-OH concentration estimated

at 10–12 mg/L or ~10 times the normal free value [37]. The delivery of retinol targets a variety of cell-surface receptors that are unevenly distributed in body tissues, with high concentrations found in the epithelial cells of the choroid plexus (CP) and in organs belonging to the visceral compartment (liver, intestinal mucosa, bone marrow) [28,44,38]. Cell surface receptors for holo-RBP operate the transmembrane uptake of the ligand, a process followed by cytosolic internalization [38,39] although nonspecific intracellular transfer of FR-OH was also documented [45].

4. How might this be related to the process involved in reactive oxygen species generation and control in diabetes mellitus, also referred to as a chronic metabolic syndrome?

Retinoid-induced reactions principally modulate cytokine activities, immune responses, and cellular components implicated in growth and repair processes [46]. Despite what has just been described, there is not an observed weight loss in any large sample of the population with adult onset diabetes. This might not be the case in early onset type 1 diabetes. The patients have hyperglycemia and glucosuria in any acute episode, and unlike type 2 patients, they are not overweight or obese. Type 1 and type 2 diabetes have different genome-phenomic pathologic expression. In Type 1 diabetes, there has now been identified an association with the C4 (complement) gene [47]. One would expect the impaired glucose entry into the cell in an acute episode of type 2 diabetes to resemble the acute inflammatory response. This would be accompanied by a decreased hepatic production of TTR, RBP, and albumin, all of which are transporters of thyroxin, and increase in production of cytokines. More important, the hyperglycaemia and glucosuria have to be accompanied with the breakdown of plasma and lean body mass to generate gluconeogenic precursors for energy production in the acute phase [36].

The burden of cytokines on protein metabolism is correlated with the severity and duration of initial impact. The decrease in TTR/RBP4 is an obligatory process lasting some days, which is poorly responsive to dietetic manipulations and associated with culminating FT4 and FR-OH plasma concentrations causing superactivated inflammatory responses. Patients afflicted with a combination of 2 distinct diseases or conditions exhibit accelerated LBM downsizing identified by plasma TTR concentrations far worse than those recorded with a single disease [48]. The maintenance of lowered TTR concentrations during days or weeks [49] indicates that catabolic and anabolic processes neutralize each other.

On the other hand, Type 2 diabetes is often accompanied by obesity, and it may develop in the child or in the adult, and extends beyond 65 years age. Most important, both the TTR and the lean body mass of both men and women increase linearly with age to a peak in the late 20 s, becomes steady to the late 50 s, and declines thereafter [50,36]. It is of some interest that in the period of late decline, the production of TTR by the choroid plexus declines more markedly than the decline of TTR in the plasma, which is a problem for the delivery of retinol across the blood brain barrier.

5. Why have we explored the importance of TTR at this point in the discussion?

The potential tie in of TTR and metabolic syndrome is here only surmised in relation to anabolic/catabolic status. However, there is also another consideration that has to do with the quality of protein intake. The consumption of a vegan diet compared with that which includes meat imposes a reduction by half of sulfur in the form of methionine content because of the difference in (sulfur/nitrogen) S/N ratio between the two food types [51]. The role of S has already been discussed with respect to glutathione, GS, GSSG, cysteine, and so forth in the regulation of reactive oxygen species. Methionine has a relationship to energy metabolism through the intermediate S-adenosyl methionine. A deficiency in S is associated with an elevation of homocysteine in the circulation, which is a biomarker for the development of cardiovascular disease [52], which has been proved for the vegan subject [50,53,54].

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REFERENCES

[1] Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991;40(4):405–12. PubMed PMID: 2010041.
[2] Wolff SP, Jiang ZY, Hunt JV. Protein glycation and oxidative stress in diabetes mellitus and ageing. Free Radical Biol Med 1991;10(5):339–52. PubMed PMID: 1855674.
[3] Karpen CW, Cataland S, O'Dorisio TM, Panganamala RV. Production of 12-hydroxyeicosatetraenoic acid and vitamin E status in platelets from type I human diabetic subjects. Diabetes 1985;34(6):526–31. PubMed PMID: 3924690.
[4] McLennan S, Yue DK, Fisher E, Capogreco C, Heffernan S, Ross GR, et al. Deficiency of ascorbic acid in experimental diabetes. Relationship with collagen and polyol pathway abnormalities. Diabetes 1988;37(3):359–61. PubMed PMID: 2836250.
[5] van der Vliet A, Eiserich JP, Halliwell B, Cross CE. Formation of reactive nitrogen species during peroxidase-catalyzed oxidation of nitrite. A potential additional mechanism of nitric oxide-dependent toxicity. J Biol Chem 1997;272 (12):7617–25. PubMed PMID: 9065416.
[6] Stitt AW, Vlassara H. Advanced glycation end-products: impact on diabetic complications. In: Betteridge DJ, editor. Diabetes, current perspectives. UK: Martin Dunitz Ltd; 2000.
[7] Mullarkey CJ, Edelstein D, Brownlee M. Free radical generation by early glycation products: a mechanism for accelerated atherogenesis in diabetes. Biochem Biophys Res Commun 1990;173(3):932–9. PubMed PMID: 2176495.
[8] Ahmed N, Thornalley PJ, Dawczynski J, Franke S, Strobel J, Stein G, et al. Methylglyoxal-derived hydroimidazolone advanced glycation end-products of human lens proteins. Invest Ophthalmol Vis Sci 2003;44(12):5287–92. PubMed PMID: 14638728.

[9] Hunt JV, Smith CC, Wolff SP. Autoxidative glycosylation and possible involvement of peroxides and free radicals in LDL modification by glucose. Diabetes 1990;39(11):1420–4. PubMed PMID: 2227114.
[10] Gardiner TA, Bucala R, Archer DB, Vlassara H. Advanced glycation end products (AGEs) colocalize with AGE receptors in the retinal vasculature of diabetic and of AGE-infused rats. Am J Path 1997;15(2):523–31.
[11] Romero FJ, Bosch-Morell F, Romero MJ, Jareno EJ, Romero B, Marin N, et al. Lipid peroxidation products and antioxidants in human disease. Environ Health Perspect 1998;106(Suppl 5):1229–34. PubMed PMID: 9788902; PubMed Central PMCID: PMCPMC1533372.
[12] Diplock AT. Antioxidants and free radical scavengers. In: Rice-Evans CAaBR, editor. Netherlands: Elsevier; 1994.
[13] Slater TF. Free-radical mechanisms in tissue injury. Biochem J 1984;222(1):1–15. PubMed PMID: 6383353; PubMed Central PMCID: PMCPMC1144137.
[14] Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 2nd ed. Oxford: Clarendon press; 1995.
[15] Esterbauer HPG. Free radicals in liver injury. Oxford: IRL press; 1985.
[16] Laight DW, Desai KM, Gopaul NK, Anggard EE, Carrier MJ. F2-isoprostane evidence of oxidant stress in the insulin resistant, obese Zucker rat: effects of vitamin E. Eur J Pharmacol 1999;377(1):89–92. PubMed PMID: 10448931.
[17] Rice-Evans CA. Formation of free radicals and mechanisms of action in normal biochemical processes and pathological states. In: Rice-Evans CAaB RH, editor, New comprehensive biochemistry: free radical damage and its control, 1st ed. Netherlands: Elsevier; 1994.
[18] Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radical Biol Med 1990;9 (6):515–40. PubMed PMID: 2079232.
[19] Sundaram RK, Bhaskar A, Vijayalingam S, Viswanathan M, Mohan R, Shanmugasundaram KR. Antioxidant status and lipid peroxidation in type II diabetes mellitus with and without complications. Clin Sci 1996;90(4):255–60. PubMed PMID: 8777831.
[20] Parke DV. Nutritional antioxidants and disease prevention: mechanisms of action. In: Basu TK TNaGM, editor, Antioxidants in human health and disease, 1st ed. Wallingford, UK: CABI; 1999.
[21] Holmgren A. Antioxidant function of thioredoxin and glutaredoxin systems. Antioxid Redox Signal 2008;2 (4):811–20.
[22] Gandy SE, Buse MG, Crouch RK. Protective role of superoxide dismutase against diabetogenic drugs. J Clin Investig 1982;70 (3):650–8. PubMed PMID: 6213639; PubMed Central PMCID: PMCPMC370268.
[23] Sinclair AJ, Barnett AH, Lunec J. Free radicals and antioxidant systems in health and disease. Br J Hosp Med 1990;43 (5):334–44. PubMed PMID: 2194616.
[24] Aebi HE. Oxidoreductases acting on groups other than CHOH. In: Bergmeyer HUBJ, Graß1 M, editors. Methods of enzymatic analysis. Weinheim VCH verlagsgesellschaft mbH; 1987.
[25] Yadav P, Sarkar S, Bhatnagar D. Lipid peroxidation and antioxidant enzymes in erythrocytes and tissues in aged diabetic rats. Indian J Exp Biol 1997;35(4):389–92. PubMed PMID: 9315241.
[26] Wohaieb SA, Godin DV. Alterations in free radical tissue-defense mechanisms in streptozocin-induced diabetes in rat: effects of insulin treatment. Diabetes 1987;36(9):1014–8. PubMed PMID: 3301471.
[27] Meister A. The biochemistry of glutathione. In: Taniguchi N, Higashi T, Sakamoto Y, Meister A, editors. Glutathione centennial: molecular perspectives and clinical implications. New York: Academic press; 1998.
[28] Grankvist K, Marklund S, Sehlin J, Taljedal IB. Superoxide dismutase, catalase and scavengers of hydroxyl radical protect against the toxic action of alloxan on pancreatic islet cells in vitro. Biochem J 1979;182(1):17–25. PubMed PMID: 40548; PubMed Central PMCID: PMCPMC1161229.
[29] Collier A, Wilson R, Bradley H, Thomson JA, Small M. Free radical activity in type 2 diabetes. Diabetic Med: J Brit Diabetic Assoc 1990;7(1):27–30. PubMed PMID: 2137060.
[30] Zaltzberg H, Kanter Y, Aviram M, Levy Y. Increased plasma oxidizability and decreased erythrocyte and plasma antioxidative capacity in patients with NIDDM. Israel Med Assoc J: IMAJ 1999;1(4):228–31. PubMed PMID: 10731349.
[31] Mirza R, Koh TJ. Dysregulation of monocyte/macrophage phenotype in wounds of diabetic mice. Cytokine 2011;56 (2):256–64. http://dx.doi.org/10.1016/j.cyto.2011.06.016. PubMed PMID: 21803601.
[32] Osborne B, Cooney GJ, Turner N. Are sirtuin deacylase enzymes important modulators of mitochondrial energy metabolism? Biochim Biophys Acta 2014;1840(4):1295–302. http://dx.doi.org/10.1016/j.bbagen.2013.08.016. PubMed PMID: 23994496.
[33] Newman JC, He W, Verdin E. Mitochondrial protein acylation and intermediary metabolism: regulation by sirtuins and implications for metabolic disease. J Biol Chem 2012;287 (51):42436–43. http://dx.doi.org/10.1074/jbc.R112.404863. PubMed PMID: 23086951; PubMed Central PMCID: PMCPMC3522244.
[34] Barroso I, Gurnell M, Crowley VE, Agostini M, Schwabe JW, Soos MA, et al. Dominant negative mutations in human PPARgamma associated with severe insulin resistance, diabetes mellitus and hypertension. Nature 1999;402 (6764):880–3. http://dx.doi.org/10.1038/47254. PubMed PMID: 10622252.
[35] McVeigh GE, Brennan GM, Johnston GD, McDermott BJ, McGrath LT, Henry WR, et al. Impaired endothelium-dependent and independent vasodilation in patients with type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 1992;35(8):771–6. PubMed PMID: 1511805.
[36] Ingenbleek Y, Bernstein LH. Plasma transthyretin as a biomarker of lean body mass and catabolic states. Adv Nutr 2015;6(5):572–80.
[37] Song KH et al. Alpha-Lipoic acid prevents diabetes mellitus in diabetes-prone obese rats. Biochem Biophys Res Commun 2005;326:197–202.
[38] Minamiyama Y, Takemura S, Tsukioka T, Shinkawa H, Kobayashi F, Nishikawa Y, et al. Effect of AOB, a fermented-grain food supplement, on oxidative stress in type 2 diabetic rats. BioFactors 2007;30(2):91–104. PubMed PMID: 18356581.
[39] Minamiyama Y, Takemura S, Bito Y, Shinkawa H, Tsukioka T, Nakahira A, et al. Supplementation of alpha-tocopherol improves cardiovascular risk factors via the insulin signalling pathway and reduction of mitochondrial reactive oxygen species in type II diabetic rats. Free Radical Res 2008;42 (3):261–71. http://dx.doi.org/10.1080/10715760801898820. PubMed PMID: 18344121.
[40] Takemura S, Minamiyama Y, Kodai S, Shinkawa H, Tsukioka T, Okada S, et al. S-Allyl cysteine improves nonalcoholic fatty liver disease in type 2 diabetes Otsuka Long-Evans Tokushima Fatty rats via regulation of hepatic lipogenesis and glucose metabolism. J Clin Biochem Nutr 2013;53(2):94–101. http://dx.doi.org/10.3164/jcbn.13-1. PubMed PMID: 24062606; PubMed Central PMCID: PMCPMC3774923.
[41] Naito Y, Ichikawa H, Akagiri S, Uchiyama K, Takagi T, Handa O, et al. Identification of cysteinylated transthyretin, a

predictive biomarker of treatment response to partially hydrolyzed guar gum in type 2 diabetes rats, by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry. J Clin Biochem Nutr 2016;58(1):23–33. http://dx.doi.org/10.3164/jcbn.15-98. PubMed PMID: 26798194; PubMed Central PMCID: PMCPMC4706097.

[42] Bloomgarden ZT. Antioxidants and diabetes. Diabetes Care 1997;20(4):670–3. PubMed PMID: 9097001.

[43] He L, Weber KJ, Schilling JD. Glutamine modulates macrophage lipotoxicity. Nutrients 2016;8:4. http://dx.doi.org/10.3390/nu8040215. PubMed PMID: 27077881; PubMed Central PMCID: PMCPMC4848684.

[44] Smeland S, Bjerknes T, Malaba L, Eskild W, Norum KR, Blomhoff R. Tissue distribution of the receptor for plasma retinol-binding protein. Biochem J 1995;305(Pt 2):419–24. PubMed PMID: 7832754; PubMed Central PMCID: PMCPMC1136378.

[45] Hodam JR, Creek KE. Comparison of the metabolism of retinol delivered to human keratinocytes either bound to serum retinol-binding protein or added directly to the culture medium. Exp Cell Res 1998;238(1):257–64. http://dx.doi.org/10.1006/excr.1997.3857. PubMed PMID: 9457079.

[46] Ingenbleek Y, Bernstein LH. The stressful condition as a nutritionally dependent adaptive dichotomy. Nutrition 1999;15:305–20.

[47] Mason MJ, Speake C, Gersuk VH, Nguyen QA, O'Brien KK, Odegard JM, et al. Low HERV-K(C4) copy number is associated with type 1 diabetes. Diabetes 2014;63(5):1789–95. http://dx.doi.org/10.2337/db13-1382. PubMed PMID: 24430436.

[48] Niyongabo T, Henzel D, Idi M, Nimubona S, Gikoro E, Melchior JC, et al. Tuberculosis, human immunodeficiency virus infection, and malnutrition in Burundi. Nutrition 1999;15(4):289–93. PubMed PMID: 10319361.

[49] Dalrymple LS, Johansen KL, Chertow GM, Grimes B, Anand S, McCulloch CE, et al. Longitudinal measures of serum albumin and prealbumin concentrations in incident dialysis patients: the comprehensive dialysis study. J Ren Nutr 2013;23(2):91–7. http://dx.doi.org/10.1053/j.jrn.2012.03.001. PubMed PMID: 22633987; PubMed Central PMCID: PMCPMC3434280.

[50] Yves Ingenbleek LHB. Downsizing of lean body mass is a key determinant of alzheimer's disease. J Alzheimer's Dis JAD; 2014.

[51] Ingenbleek Y, Young VR. The essentiality of sulfur is closely related to nitrogen metabolism: a clue to hyperhomocysteinaemia. Nutr Res Rev 2004;17(2):135–51. http://dx.doi.org/10.1079/NRR200489. PubMed PMID: 19079922.

[52] Nehler MR, Taylor Jr LM, Porter JM. Homocysteinemia as a risk factor for atherosclerosis: a review. Cardiovasc Surg 1997;5(6):559–67. PubMed PMID: 9423939.

[53] Ingenbleek Y, McCully KS. Vegetarianism produces subclinical malnutrition, hyperhomocysteinemia and atherogenesis. Nutrition 2012;28(2):148–53. http://dx.doi.org/10.1016/j.nut.2011.04.009. PubMed PMID: 21872435.

[54] McCully KS. Homocysteine, vitamins, and vascular disease prevention. Am J Clin Nutr 2007;86(5). 1563S-8S PubMed PMID: 17991676.