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Hypothesis Paper

HYPERINSULINEMIA: THE MISSING LINK AMONG OXIDATIVE STRESS AND AGE-RELATED DISEASES?

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Abstract—Mounting evidence supports Harman's hypothesis that aging is caused by free radicals and oxidative stress. Although it is known that oxidant species are produced during metabolic reactions, it is largely unknown which factor(s), of physiological or pathophysiological significance, modulate their production in vivo. In this hypothesis paper, it is postulated that hyperinsulinemia may have such function and therefore promote aging, independently of elevations of glycemia. Hyperinsulinemia is secondary to impaired insulin stimulated glucose metabolism at the level of skeletal muscle (insulin resistance) and is seen in about one third of glucose tolerant humans following dietary carbohydrate intake. If other insulin-stimulated (or inhibited) pathways retain normal sensitivity to the hormone, hyperinsulinemia could, by its effects on antioxidative enzymes and on free radical generators, enhance oxidative stress. Other proaging effects of insulin involve the inhibition of proteasome and the stimulation of polyunsaturated fatty acid (PUFA) synthesis and of nitric oxide (NO). The hypothesis that hyperinsulinemia accelerates aging also offers a metabolic explanation for the life-prolonging effect of calorie restriction and of mutations decreasing the overall activity of insulin-like receptors in the nematode *Caenorhabditis elegans.*

Keywords—Aging, Insulin, Glucose, Oxidative stress, Free radicals

INTRODUCTION

As the human organism ages, a variety of chronic diseases (namely atherosclerosis, diabetes, hypertension, cardiac and renal failure, cancer) become increasingly prevalent, determining organ failure and limiting lifespan [1]. In 1956, Harman proposed that free (oxygen-derived) radicals determine rate of aging [2]. The same author also suggested that the biological clock is set by mitochondrial production of free radicals [3], leading to oxidation, damage and loss of mitochondria with consequent dysfunction, and death of nonmitotic cells. Gradual loss and replacement of such cells by fat or extracellular matrix would determine progressive decline of organ function and the characteristic associated anatomical changes typical of aging organisms [4,5].

Over the past 25 years considerable evidence has accumulated in support of the free radical theory of aging [6]. The theory gained strength particularly from studies demonstrating the failure of aging cells to repair mitochondrial damage [7]; the accumulation, in aging cells and organisms, of oxidized dysfunctional proteins [8,9]; the decreased longevity [10] of nematodes in hyperoxic conditions (hyperoxia increases free radical production); the increased longevity of nematodes [11] phenotypically overexpressing mitochondrial superoxide dismutase (manganese-SOD: the enzyme that eliminates superoxide ion); the frequency [12] of age-related illnesses such as cancer following irradiation (ionizing radiations produce free radicals); and the radiation-like effect of ad libitum feeding versus restriction of calories but not of essential nutrients in rodents [13–15].

In the latter circumstance, it is interesting to note that 40% calorie-restricted rodents had a 40% increase in lifespan and that this effect was associated with lower plasma glucose and insulin levels and with enhancement of insulin sensitivity [16,17]. In these studies, calorie restriction was usually achieved by a 40% reduction of both carbohydrates (CHO) and fat while amount of pro-

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tein and mineral was kept constant. Since composition of the ad lib diet was 21% protein, ~60% CHO, ~10% fat [13], calorie restriction mainly entailed an absolute reduction of CHO (rather than fat) intake. For example, if ad lib fed rats had a dietary intake of 10 g/d, quantitative proportions of CHO, fat, and protein were, respectively, 6, 1.2, and 2 g/d; with 40% calorie restriction, reduction in CHO intake was therefore ~5-fold greater (from 6 to 3.6 g/d: $\Delta = 2.4$ g), than that of fat (from 1.2 to 0.7 g: $\Delta = 0.5$ g).

Therefore, in rodents, a 40% greater CHO intake had a radiation-like effect with development of premature cancer, heart and kidney failure, and consequent shortening of lifespan. Moreover, insulin sensitivity was enhanced by calorie restriction with consequent lowering of day-long plasma glucose and insulin levels. It is noteworthy to mention that comparable effects on blood glucose and insulin levels were recently published from an ongoing primate calorie-restriction longevity study as well [18]. Since considerable evidence also exists linking insulin resistance/hyperinsulinemia to a variety of human age-related diseases (ARD), most notably diabetes [19–21] and atherosclerosis [22–24], but also renal failure [25,26], hypertension [27], and cancer [28–30], there is the distinct possibility that insulin resistance and/or hyperinsulinemia are essential mediators of the aging process.

Glucose-tolerant humans who exhibit selective resistance to insulin-stimulated muscle glucose disposal tend to be mildly hyperglycemic and, unless overt diabetes ensues, very hyperinsulinemic [31,32]. In fact, the greater the amount of CHO insulin-resistant individuals consume, the greater the degree of insulin hypersecretion and hyperinsulinemia needed to achieve glucose balance (e.g., to normalize glycemia). This "compensatory" hyperinsulinemia can be substantial: for example, after 75 g oral glucose loading, sluggish (muscle) glucose disposal may elevate glycemia only 20–50%, but this mild elevation is sufficient to determine sustained insulin secretion leading to plasma venous insulin concentrations easily 3–20-fold greater than the ones seen in individuals where muscle is most sensitive to insulin stimulation of glucose metabolism [24,32]. This metabolic defect is not rare as it was demonstrated in up to a third of nonobese, apparently healthy adult Americans [24,32].

HYPOTHESIS

Hyperglycemia is an important aging factor via enhanced nonenzymatic glycosilation of proteins [33]. Hyperglycemia is a particularly relevant aging factor in diabetes, where blood glucose levels are 1.5–3.0-fold higher than normal, on average, often despite optimization of treatment.

However, healthy, nondiabetic insulin-resistant individuals are only mildly hyperglycemic following CHO intake, and the key question here is whether exposure of their body cells to extreme degrees of hyperinsulinemia may have pro-aging effects as well.

The following is a review of the published evidence showing that this, in fact, seems to be the case. One of the fundamental manifestations of aging is the accumulation of damaged, oxidized, dysfunctional proteins both intra- and extracellularly [8,9]. This phenomenon seems to result from both an age-related increase in the rate of oxygen free radical-mediated damage and a loss in the ability to degrade oxidized proteins [8]. Oxidized protein degradation is catalyzed by an enzyme called proteasome [34]. Relative but progressive inactivity of the proteasome has been observed in primate and human aging brain while more complete inhibition of such enzyme causes programmed cell death [35–37]. In this context, it is established that insulin's major effect on cellular protein turnover is inhibition of protein degradation [38]. Recent studies have clearly demonstrated that the major effect of insulin on cellular protein degradation is in fact due to inhibition of proteasome [39,40]. Therefore, the higher the insulin levels, the lower the proteasome activity and, presumably, the faster the accumulation of oxidized proteins. Although speculative, this conclusion is supported by both an extrapolation of results from the above in vitro studies and by the fact that insulin's efficacy in inhibiting protein degradation in humans [41] seems preserved [42] in individuals who are selectively resistant to insulin-stimulated glucose uptake.

As evidence exists suggesting that hyperinsulinemia favors accumulation of oxidized protein by reducing its degradation, there is also evidence that insulin may facilitate protein oxidation by increasing steady-state levels of oxidative stress, independently from hyperglycemia.

Xu and Badr [43] have in fact demonstrated, in Sprague-Dawley rats, that a 6-fold increase in serum insulin concentrations, maintained for 1 week, inhibited both peroxisomal oxidation of fatty acids and catalase activity (catalase is the enzyme that degrades hydrogen peroxide). However, inhibition of catalase activity was much more pronounced, leading to a mismatch between hydrogen peroxide (H_2O_2) production and clearance. In the same study, the authors found a significant negative linear correlation ($R^2 = 0.86$) among serum insulin levels and liver catalase activity (i.e., the higher the insulin level the greater the decrease of catalase activity). Even if the molecular mechanism(s) may differ, insulin also stimulated H_2O_2 generation in cultured human fat cells [44]. A foreseeable consequence of catalase activity inhibition and of stimulation of H_2O_2 production is an increase in steady-state levels of H_2O_2. Superoxide anion

generation was also 50–100% greater following both acute and chronic exposure of aortic endothelial cells to hyperinsulinemia [45]. Hydroxyl radical generation via the Haber-Weiss reaction [46] occurs slowly at physiological pH and temperature, unless catalytic iron is available [47]. Since hyperinsulinemia appears to also be interacting with size of body iron stores [48], there are multiple mechanisms by which, either directly or indirectly, it might accelerate the Haber-Weiss reaction and generation of hydroxyl radical.

Insulin also causes NO-mediated vasodilation in skeletal muscle by stimulating nitric oxide synthase (NOS), as such effect is blunted by NOS inhibitors [49,50]. NO, in micromolar amounts, inhibits respiration [51,52] and by rapid reaction with superoxide anion, generates peroxynitrite [53]. Peroxynitrite has a half-life of under a second, allowing it to diffuse to critical cellular targets and, for example, inhibit the mitochondrial MnSOD by nitration [54] while NO inhibits catalase by binding to its heme group [55]. In addition, both NO and peroxynitrite inhibit multiple enzymes of the mitochondrial respiratory chain (METC) such as complex I, II, and ATP-synthase [51]. NO inhibition of ATP-synthase would increase reduction of METC, which is known to enhance superoxide anion production [56], particularly in an energy-repleted state [52,56,57]. Following CHO intake, energy repletion is expected to be long-lasting, particularly in insulin-resistant individuals in whom muscle glycogen synthesis is impaired [58] and the sluggish disposal of glucose occurs via other pathways such as nonenzymic glycosilation, fatty acid synthesis and, most likely, mitochondrial one electron reduction of oxygen to superoxide anion. The final stoichiometry, in terms of electrons donated in the process of oxygen consumption, is identical whether oxygen is reduced to superoxide ion or to water (as during oxidative phosphorilation), perhaps explaining why rates of oxidative glucose disposal have been found normal or only mildly depressed in insulin-resistant individuals [59]. All of the above mechanisms may play an important role in mitochondrial damage and loss, with resultant cytotoxicity and loss of nonmitotic cells.

Finally, it should be mentioned that insulin upregulates liver $\Delta 6$, $\Delta 5$-desaturase activity [60]. This is a rate-limiting step in the synthesis and secretion of n-6 long-chain polyunsaturated fatty acids. After transport via VLDL-lipoproteins to cellular and subcellular membranes, such fatty acids increase membrane fluidity, thereby favoring substrate transmembrane flux.

The utility of such function might, however, be offset by the greater susceptibility of n-6 long-chain polyunsaturated fatty acids to peroxidation [61]. In fact, mitochondrial susceptibility to peroxidation was found to be increased by greater desaturation of membrane fatty acids and postulated to play a role in explaining differences in longevity of long- versus short-lived mammals with similar basal metabolic rates [62].

In addition to greater mitochondrial peroxidation, lipoprotein peroxidation plays a key role in the early steps of atherogenesis [63], perhaps via byproducts such as 4-hydroxynonenal and singlet oxygen, that are highly genotoxic [64,65] and cytotoxic [64]. Lipoprotein peroxidation is expected to be enhanced by increasing degrees of desaturation as well.

As expected from these in vitro studies, several studies have indeed demonstrated greater markers of lipid peroxidation in NIDDM, a state where virtually 100% of the subjects are insulin-resistant [66–70]. Furthermore, plasma concentrations of lipid hydroperoxides are higher, and of antioxidant vitamins lower in individuals who are resistant to insulin-stimulated glucose disposal but otherwise glucose tolerant, nonobese, and normotensive [71]. This finding indicates that enhanced oxidative stress is present before diabetes ensues and therefore cannot simply be explained by overt hyperglycemia.

Thus, there is substantial evidence supporting the hypothesis that selective resistance to insulin-stimulated (muscle) glucose disposal and the consequential compensatory hyperinsulinemia trigger a variety of metabolic effects, likely resulting in accelerated oxidative stress and aging. Also consistent with this view are findings, in the soil nematode *Caenorhabditis elegans*, that mutations reducing overall insulin signalling decrease accumulation of oxidized proteins, increase Mn-SOD activity, decrease oxidative stress, and extend lifespan [72–74].

CONCLUSIONS

Individuals who have selective impairment of insulin-stimulated glucose disposal at the level of skeletal muscle develop compensatory hyperinsulinemia following CHO-containing meals. Several lines of evidence suggest that other insulin-stimulated pathways are, however, normally responsive to the hormone, becoming therefore overstimulated following carbohydrate intake. Such conditions occur, for example at renal tubular level, where day-long insulin-mediated sodium retention was enhanced in insulin-resistant, hyperinsulinemic humans, despite identical dietary intake of both sodium and carbohydrate [75]. In addition, greater insulin-mediated inhibition of protein catabolism was also demonstrated in insulin-resistant as compared to control subjects [42].

We suggest that this overall increase of other insulin signalling pathways is a pro-aging factor, probably of greater relevance than hyperglycemia. The degree of hyperglycemia, seen in glucose-tolerant individuals with selective muscle resistance to insulin-stimulated glucose

metabolism, is, in fact, only of mild (~20–50%) entity until diabetes ensues.

This hypothesis, although speculative, is supported by studies in invertebrate and mammalian models where it was determined that whether overall insulin signalling is reduced by mutations, like in *C. elegans*, or by lowered plasma glucose and insulin levels, as it occurs in the calorie-restricted rodent, a similar outcome becomes apparent: extension of lifespan and, at any given age after maturity, decreased prevalence of ARD.

REFERENCES

[1] Ames, B. N.; Shigenaga, M. K.; Hagen, T. M. Oxidants, antioxidants and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. USA* **90:**7915–7922; 1993.

[2] Harman, D. Aging: a theory based on free radical and radiation chemistry. *J. Gerontol.* **11:**298–300; 1956.

[3] Harman, D. The biologic clock: the mitochondria? *J. Am. Geriatr. Soc.* **20:**145–147; 1972.

[4] Terman, A.; Brunk, U. T. Lipofuscin: mechanism of formation and increase with age. *APMIS* **106:**265–276; 1998.

[5] Lexell, J. Evidence for nervous system degeneration with advancing age. *J. Nutr.* **127:**1011S–1013S; 1997.

[6] Beckman, K. B.; Ames, B. N. The free radical theory of aging matures. *Physiol. Rev.* **78:**547–581; 1998.

[7] Shigenaga, M.; Hagen, T. M.; Ames, B. N. Oxidative damage and mitochondrial decay in aging. *Proc. Natl. Acad. Sci. USA* **91:** 10771–10778; 1994.

[8] Stadtman, E. R. Protein oxidation and aging. *Science* **257:**1220–1224; 1992.

[9] Stadtman, E. R.; Berlett, B. S. Reactive oxygen-mediated protein oxidation in aging and disease. *Drug Metab. Rev.* **30:**225–243; 1998.

[10] Honda, S.; Matsuo, M. Lifespan shortening of the nematode caenorhabditis elegans under higher concentrations of oxygen. *Mech. Ageing Dev.* **63:**235–246; 1992.

[11] Honda, Y.; Honda, S. The daf-2 gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in Caenorhabditis elegans. *FASEB J.* **13:**1385–1393; 1999.

[12] Gross, L.; Dreyfuss, Y. Prevention of spontaneous and radiation-induced tumors in rats by reduction of food intake. *Proc. Natl. Acad. Sci. USA* **87:**6795–6797; 1990.

[13] Yu, B. P.; Masoro, E. J.; Murata, I.; Bertrand, H. A.; Lynd, F. T. Life span study of SSPF Fisher 344 male rats fed ad libitum or restricted diets: longevity, growth, lean body mass and disease. *J. Gerontol.* **37:**130–141; 1982.

[14] Sohal, R. S.; Weindruch, R. Oxidative stress, caloric restriction and aging. *Science* **273:**59–63; 1996.

[15] Lee, C. K.; Klopp, R. G.; Weindruch, R.; Prolla, T. A. Gene expression profile of aging and its retardation by caloric restriction. *Science* **285:**1390–1393; 1999.

[16] Masoro, E. J.; McCarter, R. J. M.; Katz, M. S.; McMahan, C. A. Dietary restriction alters characteristics of glucose fuel use. *J. Gerontol.* **47:**202–208; 1992.

[17] Dean, D. J.; Brozinick, J. T. Jr.; Cushman, S. W.; Cartee, G. D. Calorie restriction increases cell-surface GLUT4 in insulin-stimulated skeletal muscle. *Am. J. Physiol.* **275:**E957–E964; 1998.

[18] Lane, M. A.; Ball, S. S.; Ingram, D. K.; Cutler, R. G.; Engel, J.; Read, V.; Roth, G. S. Diet restriction in rhesus monkeys lowers fasting and glucose-stimulated glucoregulatory end points. *Am. J. Physiol.* **268:**E941–E948; 1995.

[19] Bogardus, C.; Lillioja, S.; Foley, J.; Christin, L.; Freymond, D.; Nyomba, B.; Bennett, P. H.; Reaven, G. M.; Salans, L. Insulin resistance predicts the development of non-insulin dependent diabetes mellitus in Pima Indians. *Diabetes* **36**(S1):47A; 1987.

[20] Warram, J. H.; Martin, B. C.; Krolewski, A. S.; Soeldner, J. S.; Kahn, C. R. Slow glucose removal rate and hyperinsulinemia precede the development of type II diabetes in the offspring of diabetic parents. *Ann. Intern. Med.* **113:**909–915; 1990.

[21] Lillioja, S.; Mott, D. M.; Spraul, M.; Ferraro, R.; Foley, J. E.; Ravussin, E.; Knowler, W. C.; Bennett, P. H.; Bogardus, C. Insulin resistance and insulin secretory dysfunction as precursors of non-insulin dependent diabetes mellitus. *N. Engl. J. Med.* **329:**1988–1992; 1993.

[22] Pyorala, K. Relationship of glucose tolerance and plasma insulin in the incidence of coronary heart disease: results from two population studies in Finland. *Diabetes Care* **2:**131–141; 1979.

[23] Despres, J. P.; Lamarche, B.; Mauriege, P.; Cantin, B.; Dagenais, G. R.; Moorjani, S.; Lupien, P. J. Hyperinsulinemia as an independent risk factor for ischemic heart disease. *N. Engl. J. Med.* **334:**952–957; 1996.

[24] Yip, J.; Facchini, F. S.; Reaven, G. M. Resistance to insulin-mediated glucose disposal as a predictor of cardiovascular disease. *J. Clin. Endocrinol. Metab.* **83:**2773–2776; 1998.

[25] DeFronzo, R. A.; Alvestrand, A.; Smith, D.; Hendler, R.; Hendler, E.; Wahren, J. Insulin resistance in uremia. *J. Clin. Invest.* **67:** 563–568; 1981.

[26] Mak, R. H.; DeFronzo, R. A. Glucose and insulin metabolism in uremia. *Nephron* **61:**377–382; 1992.

[27] Reaven, G. M.; Lithell, H.; Landsberg, L. Hypertension and associated metabolic abnormalities—the role of insulin resistance and the sympathoadrenal system. *N. Engl. J. Med.* **334:**374–381; 1996.

[28] Stoll, B. A.; Secreto, S. New hormone-related markers of high risk in breast cancer. *Ann. Oncol.* **3:**435–438; 1992.

[29] McKeown-Eyssen, G. Epidemiology of colorectal cancer revisited: are serum triglycerides and plasma glucose associated with risk? *Cancer Epidemiol. Biomarkers Prev.* **3:**687–695; 1994.

[30] Del Giudice, M. E.; Fantus, I. G.; Ezzat, S.; McKeown-Eyssen, G.; Page, D.; Goodwin, P. J. Insulin and related factors in premenopausal breast cancer risk. *Breast Cancer Res. Treat.* **47:**111–120; 1998.

[31] Reaven, G. M.; Miller, R. Study of the relationship between glucose and insulin responses to an oral glucose load in man. *Diabetes* **17:**560–569; 1968.

[32] Hollenbeck, C.; Reaven, G. M. Variations in insulin stimulated glucose disposal in healthy individuals with normal glucose tolerance. *J. Clin. Endocrinol. Metab.* **64:**1169–1173; 1987.

[33] Monnier, V. M.; Cerami, A. Nonenzymatic browning in vivo: possible process for aging of long-lived proteins. *Science* **211:** 491–493; 1981.

[34] Rivett, A. J. Purification of a liver alkaline protease which degrades oxidatively modified glutamine synthase. Characterization as a high molecular weight cysteine proteinase. *J. Biol. Chem.* **260:**12600–12606; 1985.

[35] Starke-Reed, P. E.; Oliver, C. N. Protein oxidation and proteolysis during aging and oxidative stress. *Arch. Biochem. Biophys.* **275:** 559–567; 1989.

[36] Carney, J. M.; Starke-Reed, P. E.; Oliver, C. N.; Landum, R. W.; Cheng, M. S.; Wu, J. F.; Floyd, R. A. Reversal of age-related increase in brain protein oxidation, decrease in enzyme activity and loss in temporal and spatial memory by chronic administration of the spin-trapping compound N-tert-butyl-alfa-phenyl nitrone. *Proc. Natl. Acad. Sci. USA* **88:**3633–3636; 1991.

[37] Shinohara, K.; Tomioka, M.; Nakano, H.; Tone, S.; Ito, H.; Kawashima, S. Apoptosis induction resulting from proteasome inhibition. *Biochem. J.* **317:**385–388; 1996.

[38] Louard, R. J.; Fryburg, D. A.; Gelfand, R. A.; Barrett, E. J. Insulin sensitivity of protein and glucose metabolism in human forearm muscle. *J. Clin. Invest.* **90:**2348–2354; 1992.

[39] Hamel, F. G.; Bennett, R. G.; Harmon, K. S.; Duckworth, W. C. Insulin inhibition of proteasome activity in intact cells. *Biochem. Biophys. Res. Commun.* **234:**671–674; 1997.

[40] Hamel, F. G.; Bennett, R. G.; Duckworth, W. C. Regulation of multicatalytic enzyme activity by insulin and the insulin-degrading enzyme. *Endocrinology* **139:**4061–4066; 1998.

[41] Fukagawa, N. K.; Minaker, K. L.; Rowe, J. W.; Goodman, M. N.;

Matthews, D. E.; Bier, D. M.; Young, V. R. Insulin mediated reduction of whole body protein breakdown. *J. Clin. Invest.* **76:**2306–2311; 1985.
[42] Luzi, L.; Petrides, A.; DeFronzo, R. A. Different sensitivity of glucose and amino acid metabolism to insulin in NIDDM. *Diabetes* **42:**1868–1877; 1993.
[43] Xu, L.; Badr, M. Z. Enhanced potential for oxidative stress in hyperinsulinemic rats: imbalance between hepatic peroxisomal hydrogen peroxide production and decomposition due to hyperinsulinemia. *Horm. Metab. Res.* **31:**278–282; 1999.
[44] Krieger-Brauer, H. I.; Kather, H. Human fat cells possess a plasma membrane-bound H2O2 generating system that is activated by insulin via a mechanism bypassing the receptor kinase. *J. Clin. Invest.* **89:**1006–1013; 1992.
[45] Kashiwagi, A.; Shinozaki, K.; Nishio, Y.; Maegawa, H.; Maeno, Y.; Kanazawa, A.; Kojima, H.; Haneda, M.; Hidaka, H.; Yasuda, H.; Kikkawa, R. Endothelium-specific activation of NADPH oxidase in aortas of exogenously hyperinsulinemic rats. *Am. J. Physiol.* **277:**E976–E983; 1999.
[46] Haber, F.; Weiss, J. J. The catalytic decomposition of hydrogen peroxide by iron salts. *Proc. R. Soc. London* **A147:**332–351; 1934.
[47] Halliwell, B.; Gutteridge, J. M. C. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem. J.* **219:**1–14; 1984.
[48] Facchini, F. S. Effect of phlebotomy on plasma glucose and insulin concentrations. *Diabetes Care* **21:**2190; 1998.
[49] Steinberg, H. O.; Brechtel, G.; Johnson, A.; Fineberg, N.; Baron, A. D. Insulin mediated skeletal muscle vasodilation is nitric oxide dependent. A novel action of insulin to increase nitric oxide release. *J. Clin. Invest.* **94:**1172–1179; 1994.
[50] Sherrer, U.; Randin, D.; Vollenweider, P.; Vollenweider, L.; Nicod, P. Nitric oxide release accounts for insulin's vascular effects in humans. *J. Clin. Invest.* **94:**2511–2515; 1994.
[51] Brown, G. C. Nitric oxide and mitochondrial respiration. *Biochim. Biophys. Acta* **1411:**351–369; 1999.
[52] Boveris, A.; Costa, L. E.; Cadenas, E.; Poderoso, J. J. Regulation of mitochondrial respiration by adenosine diphosphate, oxygen and nitric oxide. *Methods Enzymol.* **301:**188–198; 1999.
[53] Beckman, J. S. Ischemic injury mediator. *Nature (Lond.)* **345:**27–28; 1990.
[54] MacMillan-Crow, L. A.; Crow, J. P.; Kerby, J. D.; Beckman, J. S.; Thompson, J. A. Nitration and inactivation of manganese superoxide dismutase in chronic rejection of human renal allografts. *Proc. Natl. Acad. Sci. USA* **93:**11853–11858; 1996.
[55] Brown, G. C. Reversible binding and inhibition of catalase by nitric oxide. *Eur. J. Biochem.* **232:**188–191; 1995.
[56] Turrens, J. F.; Boveris, A. Generation of superoxide anions by the NADH dehydrogenase of bovine heart mitochondria. *Biochem. J.* **191:**421–427; 1980.
[57] Boveris, A.; Chance, B. The mitochondrial generation of hydrogen peroxide. *Biochem. J.* **134:**707–716; 1973.
[58] Roden, M.; Shulman, G. I. Applications of NMR spectroscopy to study muscle glycogen metabolism in muscle. *Annu. Rev. Med.* **50:**277–290; 1999.
[59] Felber, J. P.; Meyer, H. U.; Curchod, B.; Iselin, H. U.; Rousselle, J.; Maeder, E.; Pahu, P.; Jequier, E. Glucose storage and oxidation in different degrees of human obesity measured by continuous indirect calorimetry. *Diabetologia* **20:**3944; 1981.
[60] Brenner, R. R. Endocrine control of fatty acid desaturation. *Biochem. Soc. Trans.* **18:**773–775; 1990.
[61] Wagner, B. A.; Buettner, G. R.; Burns, C. P. Free-radical mediated lipid peroxidation in cells: oxidizability is a function of cell lipid bis-allylic hydrogen content. *Biochemistry* **33:**4449–4453; 1994.
[62] Pamplona, R.; Prat, J.; Cadenas, S.; Rojas, C.; Perez-Campo, R.; Lopez Torres, M.; Barja, G. Low fatty acid unsaturation protects against lipid peroxidation in liver mitochondria from long-lived species: the pigeon and human case. *Mech. Ageing Dev.* **86:**53–66; 1996.
[63] Esterbauer, H.; Wag, G.; Puhl, H. Lipid peroxidation and its role in atherosclerosis. *Br. Med. Bull.* **49:**566–576; 1993.
[64] Esterbauer, H. Cytotoxicity and genotoxicity of lipid oxidation products. *Am. J. Clin. Nutr.* **57:**779S–786S; 1993.
[65] Eckl, P. M.; Ortner, A.; Esterbauer, H. Genotoxic properties of 4-hydroxyalkenals and analogous aldehydes. *Mutat. Res.* **290:**183–192; 1993.
[66] Oberley, L. W. Free radicals and diabetes. *Free Radic. Biol. Med.* **5:**113–124; 1988.
[67] Baynes, J. W. Role of oxidative stress in development of complications of diabetes. *Diabetes* **40:**405–412; 1991.
[68] Griesmacher, A.; Kindhauser, M.; Andert, S. E.; Schreiner, W.; Toma, C.; Knoebl, P.; Prager, R.; Schnack, C.; Schernthaner, G. Enhanced serum levels of thiobarbituric acid reactive substances in diabetes mellitus. *Am. J. Med.* **98:**469–475; 1995.
[69] Nourooz-Zadeh, J.; Tajaddini-Sarmadi, J.; McCarthy, S.; Betteridge, J.; Wolff, S. P. Elevated levels of authentic plasma hydroperoxides in NIDDM. *Diabetes* **44:**1054–1058; 1995.
[70] Nourooz-Zadeh, J.; Rahimi, A.; Tajaddini-Sarmadi, J.; Tritshler, H.; Rosen, P.; Halliwell, B.; Betteridge, J. Relationships between plasma measures of oxidative stress and metabolic control in NIDDM. *Diabetologia* **40:**647–653; 1997.
[71] Facchini, F. S.; Humphreys, M. H.; Abbasi, F.; DoNascimento, C. A.; Reaven, G. M. Relation between insulin resistance and plasma concentrations of lipid hydroperoxides, carotenoids, and tocopherols. *Am. J. Clin. Nutr.* **72:**776–779; 2000.
[72] Kimura, K. D.; Tissenbaum, H. A.; Liu, Y.; Ruvkun, G. daf-2, an insulin receptor-like gene that regulates longevity and diapause in caenorhabditis elegans. *Science* **277:**942–946; 1997.
[73] Lin, K.; Dorman, J. B.; Rodan, A.; Kenyon, C. daf-16: an HNF-3/forkhead family member that can function to double the life span of Caenorhabditis elegans. *Science* **278:**1319–1322; 1997.
[74] Mihaylova, V. T.; Borland, C. Z.; Manjarrez, L.; Stern, M. J.; Sun, H. The PTEN tumor suppressor homolog in Caenorhabditis elegans regulates longevity and dauer formation in an insulin receptor-like signalling pathway. *Proc. Natl. Acad. Sci. USA* **96:**7427–7432; 1999.
[75] Facchini, F. S.; DoNascimento, C. A.; Reaven, G. M.; Yip, J. W.; Ni, X. P.; Humphreys, M. H. Blood pressure, sodium intake, insulin resistance and urinary nitrate excretion. *Hypertension* **33:**1008–1012; 1999.