

Does Chronic Glycolysis Accelerate Aging? Could This Explain How Dietary Restriction Works?

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ABSTRACT: The mechanisms by which dietary restriction (DR) suppresses aging are not understood. Suppression of glycolysis by DR could contribute to controlling senescence. Many glycolytic intermediates can glycate proteins and other macromolecules. Methylglyoxal (MG), formed from dihydroxyacetone- and glyceraldehyde-3-phosphates, rapidly glycates proteins, damages mitochondria, and induces a prooxidant state to create a senescent-like condition. *Ad libitum*-fed and DR animals differ in mitochondrial activity and glycolytic flux rates. Persistent glycolysis in the unrestricted condition would increase the intracellular load of glycating agents (e.g., MG) and increase ROS generation by inactive mitochondria. Occasional glycolysis during DR would decrease MG and reactive oxygen species (ROS) production and could be hormetic, inducing synthesis of glyoxalase-1 and anti-glycating agents (carnosine and polyamines).

KEYWORDS: calorie; diet; methylglyoxal; glycation; hormesis

IS GLYCOLYSIS POTENTIALLY DELETERIOUS?

Mitochondria are frequently regarded as major sources of age-associated cellular disorder/dysfunction because of reactive oxygen species (ROS) generated within them. Glycolysis, however, is another source of endogenous molecular toxicity; most glycolytic intermediates possess reactive carbonyl groups (being aldehydes or ketones) that modify protein amino groups and DNA, in the cytosol and mitochondria, via mechanisms similar to those of nonenzymic glycosylation (glycation).¹⁻⁴ Glyceraldehyde-3-phosphate and dihydroxyacetone-phosphate rapidly glycate proteins, producing advanced glycosylation end-products (AGEs) that are implicated in age-related pathologies, diabetes⁵

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and its secondary complications,⁵ brain aging,⁶ and Alzheimer's disease.⁷ Methylglyoxal (MG), generated both spontaneously and enzymically from the glyceraldehyde-3- and dihydroxyacetone-phosphates, as well as from threonine, glycine, and fatty acids,⁸ is highly deleterious; it glyicates and cross-links proteins, and damages lipids and DNA (see Ref. 8 and references therein). It induces peroxides in cortical neurons,⁹ has pro-oxidant effects in smooth muscle cells,¹⁰ inhibits heart mitochondria,³ and reacts with arginine residues of mitochondrial permeability transition pore proteins⁴ to provoke organelle dysfunction and ROS production.¹¹ MG-induced protein glycation creates active centers for one-electron oxidation/reduction reactions and ROS generation.¹² MG inactivates glutathione peroxidase irreversibly, which increases cellular peroxide concentration and oxidative damage. Prolonged MG administration induces microvascular damage and other diabetes-like complications, even within a normo-glycemic context.¹³

METHYLGLYOXAL AND DIETARY RESTRICTION

Intracellular MG concentration is determined by the rate and duration of glycolytic activity.^{14,15} The MG formation rates range between 0.1% and 0.4% of the glycolytic flux; the free intracellular MG concentration ranges from 0.16 μM to 2.4 μM ; reversibly bound MG is 2–3 orders of magnitude higher.⁸ It is proposed that more MG is produced in *ad libitum*-fed animals during continued (chronic) glycolysis than under food restriction, where glycolysis is both brief and infrequent because of constrained food availability. Low cellular proliferation rates also increase cellular MG concentrations in *ad libitum*-fed animals on account of decreased use of glycolytic intermediates as precursors for DNA and protein synthesis.⁸ Deficiency or inactivation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) raises the concentrations of MG and glycated products.¹⁶

As MG can provoke many, or most, of the biochemical changes that accompany normal aging (protein carbonyl groups and cross-linking, lipid and DNA damage, mitochondrial dysfunction, ROS production, and apoptosis as described above), it is proposed that chronic glycolysis in *ad libitum*-fed animals is detrimental because of continuous generation of relatively high levels of MG. In DR animals glycolysis is transient; any MG that is generated would persist for only short periods of time. That dietary restriction's effects on aging can be induced by fasting or intermittent (e.g., every other day) feeding, without any decrease in overall caloric intake,¹⁷ is consistent with the current proposal.

NATURALLY OCCURRING PROTECTION AGAINST MG

Most cells possess glyoxalases¹⁸ as well as other aldehyde-scavenging enzymes¹⁹ that detoxify MG. Glyoxalase-1 expression in certain areas of the brain

varies during the human life span²⁰ and a large increase in plasma AGEs is associated with erythrocyte glyoxalase-1 deficiency.²¹ Upregulation of glyoxalase-1 activity can lower cell-associated MG and possibly suppress Alzheimer's disease (AD).²² DR attenuates amyloid- β deposition in an AD animal model,²³ although the mechanisms involved are uncertain.

Glutathione, pyridoxamine, thiamine, the polyamines spermine and spermidine, and carnosine can scavenge MG.²⁴ Spermine, spermidine, and carnosine are present at high concentrations in many tissues.

Carnosine, present in long-lived tissues (muscles and nerves) particularly, inhibits MG-induced generation of protein carbonyl groups and cross-linking of MG-modified lysine and MG-treated ovalbumin to normal proteins,²⁵ and forms adducts with MG-induced protein-bound carbonyl groups²⁶ (see Ref. 25 and references cited therein). Adducts of carnosine with acrolein, MG, and hydroxynonenal (HNE) adducts have been characterized²⁵ and carnosine-HNE adducts were recently detected in muscle tissue;²⁵ "carnosylated" amino-lipid has been detected in human muscle.²⁵ Carnosine suppresses senescence in cultured human fibroblasts and some antiaging effects were observed in mice and fruit flies;²⁵ it also delays onset of diabetic complications in mice.²⁷ Hence, carnosine might protect against reactive carbonyl compounds *in vivo*.

Spermine, spermidine,²⁸ and pyridoxamine²⁹ may perform similar antiglycating functions. Millimolar quantities of spermine are present in nuclei that may help protect DNA and histones against glycation.²⁸ Spermine can inhibit formation of the AGE pyrraline during long-term exposure of albumin to glucose.³⁰ Pyridoxamine scavenges aldehydes and MG-pyridoxamine adducts have been characterized.³¹ Pyridoxamine may act synergistically as it stimulates glyoxalase activity in erythrocytes.³² Deglycating³³ and trans-glycating³⁴ roles for fructosamine-3-kinase have been proposed; the enzyme either recycling spermine-carbonyl adducts³³ or the sugar-derived component of Maillard reaction products (Schiff bases) is transferred to taurine, carnosine, anserine, or glutathione. Tissue concentrations of polyamines³³ and carnosine²⁵ possibly decrease with age, increasing the potential for MG-induced dysfunction in older animals, although more research is needed to substantiate this.

Thiamine, in the form of a lipid-soluble derivative benfotiamine,³⁵ stimulates transketolase, the rate-limiting enzyme in the pentose pathway, whose substrates are fructose-6-phosphate and glyceraldehyde-3-phosphate, which would decrease MG generation.

DR AND HORMESIS: IS GLYCOLYSIS A STRESSOR?

Transient exposure to stress could induce a hormetic response that increases long-term protection against deleterious age-related changes, such as protein oxidation and glycation.³⁶ It is suggested that persistent glycolysis is deleterious, but brief glycolysis is hormetic. There is some evidence to support this idea: increased glucose uptake upregulates glyoxalase-1 synthesis in yeast,³⁷

while a nematode glyoxalase-1 gene promoter region contains an insulin-responsive element and responds to oxidative stress.³⁸ MG enhances chaperone functions of α -crystallin and Hsp-27 (stress proteins),³⁹ an action that may be hormetic; the amount of extra protection afforded by MG would be limited by the number of chaperone protein molecules available for modification; excess MG generation during persistent glycolysis in *ad libitum*-fed animals would be deleterious.

Carnosine synthesis is metabolically controlled in astroglia-rich primary cultures, where cAMP downregulates carnosine synthetase by up to 80%.⁴⁰ Because the dipeptide suppresses MG reactivity by its glyoxalase-1 mimetic activity,⁴¹ its ability to form adducts with MG (see above), its disaggregation effects on MG-glycated protein,⁴² and, when complexed with zinc ions, its ability in rat mucosal tissue to induce synthesis of the stress protein hsp72,⁴³ short-term glycolysis could increase carnosine synthesis and increase protection against MG. Persistent glycolysis could increase MG production to an extent that overwhelms all the protective activities to increase intracellular glycation potential. Increased carnosine synthesis could help explain the improved cancer resistance observed when DR is imposed⁴⁴ because the dipeptide can selectively kill transformed cultured cells.²⁵

Spermine synthesis may be stimulated by stress; ornithine decarboxylase (ODC), the first enzyme of polyamine synthesis pathway, is upregulated under oxidative stress and UVB irradiation,³³ conditions that provoke glycoxidation. The increased ODC levels in rat kidney in early diabetes may be a glycation-inhibiting response in this tissue,³³ consistent with the proposal that antiglycating mechanisms are hormetically activated during brief periods of glycolysis.

Macrophages undergo adaptive responses when exposed to glycated serum: exposure to subtoxic amounts of AGE (5%) increases antioxidant activity and protects against subsequent treatment with 10% AGE, while 10% AGE is lethal to nonadapted cells,⁴⁵ observations consistent with the suggestion that responses to glycation are hormetic.

In a study of the effects of DR on diurnal rat metabolism, McCarter and Palmer⁴⁶ showed that DR and *ad libitum*-fed animals differed in terms of fuel utilization. The restricted animals metabolized more carbohydrate immediately after feeding and then switched to a predominantly lipid/protein-based metabolism. In contrast the *ad libitum*-fed animals' metabolism was substantially glycolytic for the whole 24 hours of each day. These observations are consistent with the assumption in the present report that glycolysis is transient in DR animals but persistent in those fed *ad libitum*.⁴⁷

GLYCOLYSIS AND MECHANISMS OF AGING

It is suggested that under certain circumstances age-related cellular dysfunction may not derive entirely from mitochondrially generated ROS.

Extra-mitochondrial ROS, induced by MG and glycated polypeptides, can damage the mitochondrial membranes, including the permeability transition pore, to produce features characteristic of senescence. Excessive and persistent glycolysis could provide a source of mitochondrial damage and ROS generation in *ad libitum*-fed animals, whereas DR would suppress production of glycating agents such as MG and decrease the occurrence of macromolecular damage, including that to mitochondria.

The present proposals do not exclude the operation of any other mechanism(s) by which DR mediates aging suppression, it being unreasonable to assume that senescence in different tissues and cells is controlled by a single, universal, mechanism. The possible effects of glycolysis, occasional or persistent, outlined here may overlap or supplement other possible mechanisms of DR's effects on aging, such as increased plasma membrane NAD(P)H oxidase activity, decreased electron supply to mitochondria, protein acetylation/deacetylation, protein turnover, stress proteins synthesis, and increased ROS production by underemployed mitochondria.

CONCLUSION

The mechanisms by which DR mediates its protective effects toward aging and related pathologies may be due, at least in part, to infrequent glycolysis, rather than any direct action affecting mitochondrial function. It is suggested that the glycolytic by-product MG, which induces many of the biochemical and subcellular changes associated with ageing and related pathologies, including mitochondrial dysfunction, may play a causal role in the aging of *ad libitum*-fed animals. In DR animals occasional glycolysis could be hormetic, and protective activities (e.g., synthesis of glyoxalase, carnosine, spermine, and other agents as yet unidentified) may be induced. It is interesting that *ad libitum* feeding increases glycolysis and decreases mitochondrial usage, both conditions being deleterious on account of increased MG and ROS generation, while DR suppresses glycolysis and increases mitochondria-mediated transfer of electrons to oxygen to decrease stressor production.

[NOTE ADDED IN PROOF: After submission of this manuscript, Ramasamy *et al.*⁴⁸ and Yao *et al.*⁴⁹ have provided evidence demonstrating the role of glycolytically derived MG in both protein modification and gene expression, consistent with the ideas outlined here.]

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