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NÍVEL MESTRADO

Avaliação do tempo de vida cronológico de *Saccharomyces cerevisiae* em diferentes fontes de carbono associadas com o metabolismo e com os mecanismos de reparação de DNA

FERNANDA BAREA

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Dissertação apresentada ao Programa de Pós-graduação em Biotecnologia da Universidade de Caxias do Sul, visando a obtenção do grau de Mestre em Biotecnologia.

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DISSERTAÇÃO APROVADA EM 05 DE DEZEMBRO DE 2008

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Dedico este trabalho á minha querida vozita, aos meus pais, irmão e namorado.

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ESTRUTURA DA DISSERTAÇÃO

Esta dissertação de mestrado está caracterizada da seguinte maneira: uma introdução geral, seguida dos objetivos e de três capítulos principais. Na parte final, encontra-se uma discussão geral, as conclusões obtidas, as perspectivas e um adendo. Os três capítulos aparecem na forma de artigos científicos e seguem as normas utilizadas pelos periódicos para os quais foram e/ou serão submetidos.

Na introdução são levantados aspectos importantes sobre a formação dos produtos finais de glicação avançada (AGEs) e como estes produtos aparecem associados não somente com a manifestação de uma série de patologias, mas também com os processos de envelhecimento e de longevidade.

A seguir, aparecem os três capítulos principais: O primeiro capítulo, intitulado **"**A BRIEF REVIEW ABOUT AGES: MECHANISM OF INDUCTION, INTERMEDIATE METABOLITES AND ITS ASSOCIATION WITH DIFFERENT PATHOLOGIES AND AGING" trata de uma revisão sobre os produtos finais de glicação avançada (AGEs), seus mecanismos de formação, sua associação com diferentes intermediários metabólicos e a sua implicação na ocorrência de diferentes patologias como a diabetes melito e doenças neurodegenerativas do envelhecimento. Além disso, descreve aspectos como a presença de receptores de AGEs (RAGEs) e a ocorrência de diferentes mecanismos inibidores. Esta revisão será submetida para um periódico especializado da área.

O segundo capítulo, intitulado "RELATIONSHIP AMONG CARBOHYDRATE INTERMEDIATE METABOLITES AND DNA DAMAGE AND REPAIR IN YEAST FROM A SYSTEMS BIOLOGY PERSPECTIVE", foi recentemente publicado no periódico *Mutation Research: Fundamental and Molecular Mechanisms of*

Mutagenesis. Este artigo tem como base um estudo de Biologia de Sistemas que aponta a presença e a participação de proteínas relacionadas com o metabolismo de carboidratos em leveduras atuando na ativação de diferentes mecanismos de reparação do DNA. Estes resultados evidenciam a presença de mecanismos protetores de DNA frente ao processo de formação dos produtos finais de glicação avançada (AGEs). Além disso, com base na literatura, o artigo mostra os aspectos genotóxicos dos principais intermediários metabólicos provenientes do metabolismo da glicose e da frutose.

O terceiro capítulo intitulado **"**THE INFLUENCE OF DIFFERENT CARBON SOURCES ON THE CHRONOLOGICAL AGE OF *Saccharomyces cerevisiae* AND ITS ASSOCIATION WITH METABOLISM AND DNA REPAIR MECHANISMS**"** analisa o tempo de vida cronológico de linhagens deficientes para o metabolismo de carboidratos e para os mecanismos de reparação de DNA frente a diferentes fontes e concentrações de carbono. Este estudo teve como suporte a utilização dos dados da Biologia de Sistemas do segundo capítulo e será submetido para um periódico especializado da área.

Seguindo a ordem estabelecida, a tese apresenta a discussão geral inter-relacionando os conhecimentos dos três capítulos e o relato das conclusões e das perspectivas pretendidas.

O artigo intitulado "*IN SILICO* ANALYSES OF A NEW GROUP OF FUNGAL AND PLANT RECQ4-HOMOLOGOUS PROTEINS" finaliza a presente tese, e refere-se a caracterização *in silico* de uma família de proteínas homólogas presentes em organismos eucariotos e procariotos denominadas de RecQ helicases. Estas RecQ helicases participam de eventos associados com a manutenção da integridade genômica durante o ciclo celular e nos eventos de reparação do DNA. Este trabalho foi recentemente publicado no periódico *Computational Biology and Chemistry*.

ÍNDICE

LISTA DE TABELAS

CAPÍTULO II

CAPÍTULO III

LISTA DE FIGURAS

CAPÍTULO I

CAPÍTULO II

mitochondrial malic enzyme (Mae1p)]. The arrows indicate the flux of metabolites in the different pathways. Numbers inside an empty circle indicate enzymes that participate only in the carbohydrate metabolic pathways (1, cytoplasmatic malic enzyme). *Abbreviations:* Glyceraldehyde 3-phosphate (GAP); di-hydroxyacetone phosphate (DHAP); mitochondria membranes (MM).

Figura 3. Mechanism of nucleotide-AGEs formation from carbohydrate-derived metabolites (A and B). In (A), the reaction of a nucleotide (represented by deoxyguanosine) with methylglyoxal (MG) results in a Schiff base taht undergoes a spontaneous rearrangement to a more stable Amadori compound (a nucleotide-AGE). In (B), the glycolytic intermediate metanolites, like dihydroxyacetona (DHAP) and glyceraldehyde 3-phosphate (GAP), react with both deoxyguanosine and deoxycytidine originating different nucleotide-AGEs (indicated by black boxes). By its turn, DHAP and GAP can spontaneously form glyoxal and MG, two potent nucleotide-glycating agents. *Abbreviations*: N2-(1, Carboxymethyl)deoxyguanosine, MdG; N2-(1-carboxyethyl)deoxyguanosine, CEdG; N2-(carboxy-3-hydroxypropyl)deoxyguanosine, ChPdG; N2-(1-carboxy-3,4,5-trihydroxypentyl)deoxiguanosine, dG-Gluc.

Figura 4. A physical protein-protein interaction network between yeast DNA repair pathways (nodes represented as black circles) and carbohydrate metabolic associated enzymes (nodes represented as white circles). Proteins that act in different biological mechanisms are indicated by a gray shadow. The number of edges linking the nodes indicate the strength of connection among proteins.

Figura 5. Schematic model representing the association of DNA repair mechanisms with specific enzymes of carbohydrate metabolic pathways. In growth conditions in which glucose and/or fructose were present, the Pgm2p enzyme was associated with base excision repair (BER), homologous recombination (HR), and a damage detection

circuitry (composed of Mec1p and Dun1p; gray box). BER and HR mechanisms can act together to repair the damage generated by glucose/fructose-derived reactive carbonyl species (RCS). The damage detection circuitry activates:(i) the nucleotide excision repair (NER) pathway that controls mismatch repair and dNTP synthesis and (ii) mismatch repair, which is linked to DNA replication and probably acts during DNA synthesis to prevent the permanent incorporation of mutagenic dNTP-advanced glycation end products (AGEs). The glycolytic enzyme Pgk1p, however, is also associated with DNA replication and HR and appears to controls the major protein of SUMOylation (Smt3p), which coordinates the post-translational modification of non-homologous end joining (NHEJ) proteins and glycolytic/gluconeogenesis enzymes. In turn, the action of yeast calmodulin (Cmd1p) represses the activity of Pfk1p while activating Pyc2p under gluconeogenic conditions seen with growth. The latter, in conjuction with the senataxin homologous protein, Sem1 (2), recruits NER enzymes. *Abbreviations*: glucose-6-phosphate (G6-P); glucose 1-phosphate (G1-P); fructose 6-phosphate (F6-P); fructose 1,6-bisphosphate (F,16-BP); pyruvate (PYR); oxaloacetato (OXA).

CAPÍTULO III

Figura 1. Survival of WT, *tor1*Δ, *pfk1*Δ, *pfk2*Δ e *rad1*Δ strains. Proficient and deficient strains for carbohydrate metabolism and DNA repair mechanisms of *S. cerevisiae*, respectively. error bars represent the individual standard deviation for each carbon source.

Figura 2. Glycogen accumulation assays in the WT, *rad1*Δ. *tor1*Δ, *pfk1*Δ e *pfk2*Δ strains. The higher the iodine accumulation, the greater the glycogen amount stored by the strain.

LISTA DE ABREVIATURAS

3-DG	*3-deoxyglucosone* (3-desoxiglucosona)
AGEs	*Advanced glycation end-products* (Produtos finais de glicação avançada)
ALEs	*Advanced lipooxidation end-products* (Produtos finais de lipooxidação avançada)
CEL	*N-(carboxyethyl)lysin* [*N*-(carboxietil)lisina]
CML	*N-(carboxymethyl)lysine* [*N*-(carboximetil)lisina]
DHA	*Dihydroxyacetone-phosphate* (Dihidroxiacetona-fosfato)
ESRD	*End-Stage Renal Disease* (Doença renal avançada)
ERK	*Extracellular signal-regulated kinase* (Cinase regulada por sinal extracelular)
F-1-P	*Fructose-1-phosphate* (Frutose-1-fosfato)
F-3-P	*Fructose-3-phosphate* (Frutose-3-fosfato)

F-1,6-P	*Fructose-1,6-bisphosphate* (Frutose-1,6-bisfosfato)
G-1-P	*Glucose-1-phosphate* (Glicose-1-fosfato)
G-6-P	*Glucose-6-phosphate* (Glicose-6-fosfato)
G-3-P	*Glyceraldehyde-3-phosphate* (Gliceraldeído-3-fosfato)
GA/GAP	*Glyceraldehyde* (Gliceraldeído)
GO	*Glyoxal* (Glioxal)
MAP	*Mitogen-Activated Protein* (Proteína ativada por mitógenos)
MGO/MG	*Methylglyoxal* (Metilglioxal)
NFκB	*Transcription nuclear factor* (Fator de transcrição nuclear)
RAGEs	*Receptors for Advanced glycation end-products* (Receptores de membrana dos produtos finais de glicação avançada)
RCS	*Reactive carbonyl species* (Espécies reativas de carbonila)

ROS	*Reactive oxygen species* (Espécies reativas de oxigênio)
BER	*Base excision repair* (Reparação por excisão de bases)
BiNGO	*Biological Network Gene Ontology* (Rede Biológica de Ontologia Gênica)
CEdG	N^2*- (1- Carboxyethyl) deoxyguanosine* [N^2-(1-Carboxietil) desoxiguanosina]
CMdG	N^2*- (1- Carboxymethyl) deoxyguanosine* [N^2-(1-Carboximetil) desoxiguanosina]
ChPdG	N^2*-(1-carboxy-3-hydroxypropyl) deoxyguanosine* [N^2-(1-Carboxi- 3-hidroxipropil) desoxiguanosina]
dG-Gluc	N^2 *–(1-carboxy-3,4,5- trihydroxypentyl) deoxyguanosine* [N^2-(1-Carboxi-3,4,5-trihydroxypentil) desoxiguanosina]
DSB	*Double Strand Breaks* (Quebra dupla do DNA)
DHAP	*Di-hydroxyacetone phosphate* (Di-hidroxiacetona fosfato)
GO	*Gene Ontology* (Ontologia Gênica)

HR	*Homologous Recombination* (Recombinação homóloga)
LS	*Leigh`s syndrome* (Síndrome de Leigh)
MM	*Mitochondrial membranes* (Membranas mitocondriais)
MMS	*Methyl-methanesulfonate* (Metil-metanossulfonato)
NER	*Nucleotide Excision Repair* (Reparo por excisão de nucleotídeos)
NHEJ	*Non-Homologous End-Joining* (Recombinação não homóloga de extremidades terminais de DNA)
OXA	*Oxaloacetate* (Oxaloacetato)
PPPI	*Physical Protein- Protein Interaction* (Interação física proteína-proteína)
PYR	*Pyruvate* (Piruvato)
RPA	*Replication Protein A* (Proteína A replicativa)

RNR complex	*Ribonucleotide Diphosphate Reductase complex* (Complexo da redutase de ribonucleotideos difosfatos)
SGD	*Saccharomyces Genome Database* (Banco de Dados Genômicos de *Saccharomyces*)
SSBs	*Single strand breaks* (Quebra simples do DNA)
SUMO	*Small Ubiquitin-related Modifier* (Modificador semelhante a pequenas ubiquitinas)
RCS	(*Replicative lifespan)* (Ciclo de vida replicativo)
CLS	*(Chronological lifespan)* (Ciclo de vida cronológico)

RESUMO

Uma dieta rica em carboidratos aparece como um dos poucos fatores ambientais capazes de interferir tanto na longevidade quanto no envelhecimento de um organismo. Neste sentido, a geração aumentada e o acúmulo dos AGEs (do inglês *Advanced glycation end-products*), formados por reações não enzimáticas entre os monossacarídeos glicose e frutose e/ou seus intermediários metabólicos com os ácidos nucleicos e grupos amina de proteínas, determinam a importância que estes produtos representam para a duração do ciclo de vida dos organismos.

Os AGEs aparecem associados a uma série de patologias relacionadas com a longevidade e com a ocorrência do envelhecimento precoce, aparecendo em número aumentado nos casos de diabetes melito e nas doenças neurodegenerativas como o Alzheimer e o Parkinson.

Nesta dissertação de mestrado buscou-se mostrar uma associação entre as proteínas do metabolismo de carboidratos e as vias de reparação do DNA. Os dados obtidos evidenciaram uma importante interação entre as principais enzimas do metabolismo de carboidratos com as proteínas de reparação de DNA e mostraram que ambos parecem ser essenciais para a manutenção da integridade genômica em leveduras.

Nesta dissertação também foi verificado o tempo de vida cronológico de diferentes linhagens da levedura *Saccharomyces cerevisiae* frente a diferentes fontes de carbono e os resultados obtidos foram relacionados com a atuação do metabolismo de carboidratos e com as vias de reparação do DNA. Soma-se ao trabalho os dados de idade cronológica associados com a ausência do complexo Tor1 (*Target of Rapamycin*),

uma via relacionada com a indução de autofagia e que está associada com os mecanismos promotores da longevidade e do envelhecimento.

Os dados gerados por esta dissertação também permitiram relacionar informações importantes sobre o metabolismo de carboidratos e sua interferência nos mecanismos genéticos e bioquímicos associados com a longevidade e com o envelhecimento de leveduras, abrindo caminhos para a realização de novos estudos que visem buscar maiores conhecimentos sobre a caracterização dos mecanismos genéticos associados com a longevidade dos organismos.

Palavras-chaves: idade cronológica, frutose, glicose, envelhecimento, biologia de sistemas.

ABSTRACT

A diet rich in carbohydrates is one of the few environmental factors capable of interfering in longevity and in an aging of organisms. In this sense, the increased generation and/or accumulation of AGES (Advanced glycation end-products) formed by reactions between monosaccharides glucose and fructose and/or their metabolic intermediates with nucleic acid and amine group of proteins determine the importance of these products for the lifespan.

The AGES appear to be associated with a series of diseases related with longevity and aging. In addition, it has been reported an increased in AGEs in diabetes mellitus and neurodegenerative diseases, e.g. Alzheimer's and Parkinson's diseases.

In this work we search for an association between carbohydrate metabolism and DNA repair mechanisms. The data showed a significant interaction between key enzymes of carbohydrates metabolism with DNA repair mechanisms and indicated that both processes seems to be essential for the maintenance of genomic integrity in yeast.

In this work it was also verificated the chronological lifespan (CLS) in several strains of yeast *Saccharomyces cerevisiae* grown on different carbon sources. The results generated were related with carbohydrate metabolism and DNA repair mechanisms. In addition, CLS was associated with the absence of Tor1 (*Target Of Rapamycin*), a genetic mechanism that has been associated with aging and longevity.

Furthermore, the data generated by the current study allowed to obtain important data about carbohydrate metabolism and its association with longevity and the aging of yeast.

Key words: chronological lifespan, fructose, glucose, aging, systems biology

INTRODUÇÃO GERAL

Os monossacarídeos glicose e frutose são capazes de reagir com proteínas, aminoácidos e DNA por meio de reações químicas denominadas de glicação não-enzimática. Esta glicação origina-se da reação entre os grupos cetona ou aldeído dos açúcares com o grupo amino livre das proteínas (Singh *et al*., 2001). No DNA, a glicação ocorre com os grupos amino livres das bases nitrogenadas de nucleotídeos e nucleosídeos (Barea & Bonatto, 2008).

Os produtos iniciais formados por estas reações são denominados de bases de Schiff, estes por sua vez, originam outros compostos chamados de adutos de Amadori e/ou Heyns. A presença destes adutos nas proteínas preconiza uma série de eventos que induzem a desidratação, a ciclização e a oxidação destas moléculas, formando assim, compostos heterogêneos altamente reativos conhecidos como produtos finais de glicação avançada (*Advanced Glycation End-Products*; AGEs) (Levi & Werman, 2001; Thorpe & Baynes, 2003). A formação dos AGEs também é uma característica presente na molécula do DNA, e nesse caso, a sua ocorrência aparece associado com a formação de diferentes dNTPs-AGEs (Barea & Bonatto, 2008).

Essas etapas que caracterizam a formação dos AGEs são também conhecidas como Reação de Maillard, esse processo foi descrito pela primeira vez na década de 1900, quando foi observado que na presença de açúcares reduzidos, diferentes aminoácidos aquecidos desenvolviam uma aparência caramelizada (Gugliucci, 2000; Singh *et al*., 2001).

A formação e o acúmulo dos AGEs aparece associado com o início e a progressão de diferentes patologias. Sua presença nas proteínas leva a ocorrência de ligações irreversíveis entre estas moléculas, alterando as suas propriedades bioquímicas

e iniciando uma grande variedade de respostas celulares anormais. No DNA, a presença dos AGEs aparece relacionado com a ocorrência de mutações e danos ao genoma, eventos estes que aparecem associados com a ativação de mecanismos protetores, como as vias de reparação do DNA (Barea & Bonatto, 2008). Desta forma, os AGEs agem como mediadores nas complicações de diabetes melito, nas disfunções relacionadas com o envelhecimento e nas doenças neurodegenerativas, como o Alzheimer, o Parkinson e a aterosclerose (Dutta *et al*., 2005).

Tendo em vista a implicação destes produtos em diferentes patologias e no processo de envelhecimento é de fundamental importância a realização de estudos que busquem avaliar de forma efetiva, o verdadeiro papel dos AGEs no ciclo de vida dos organismos, na regulação das vias metabólicas e na manutenção dos sistemas biológicos como um todo (Baynes, 2000).

Sendo assim, este trabalho de mestrado procurou avaliar o tempo cronológico de leveduras submetidas a diferentes concentrações e fontes de carbono. Os resultados obtidos permitiram associações interessantes sobre o metabolismo dos carboidratos, permitindo também, inferências relacionadas com a sobrevivência e a formação dos AGEs. Além disso, a utilização de ferramentas de Biologia de Sistemas mostrou a presença de um circuito de interações até então inédito entre as proteínas do metabolismo de carboidratos com os mecanismos de proteção e de manutenção da integridade genômica, sendo parte desta associação corroborada nos ensaios práticos realizados por este trabalho.

OBJETIVOS

OBJETIVO GERAL

Analisar por meio da Biologia de Sistemas, as interações proteína-proteína para o metabolismo de carboidratos e para as vias de reparação de DNA em leveduras, avaliando o tempo de vida cronológico de *Saccharomyces cerevisiae* em linhagens proficientes e deficientes para o metabolismo de carboidratos e para as vias de reparação do DNA em diferentes fontes de carbono (glicose, frutose, glicerol) sob condição ou não de restrição calórica.

OBJETIVOS ESPECÍFICOS

- Analisar, por meio de programas específicos de Biologia de Sistemas, as interações proteína-proteína para o metabolismo de carboidratos e as vias de reparação de DNA em leveduras.

- Avaliar o tempo de vida cronológico de *S. cerevisiae* em linhagens deficientes e proficientes para o metabolismo de carboidratos e para as vias de reparação de DNA, usando para isso, meios de cultura contendo diferentes fontes e concentrações de carbono (glicose, frutose e glicerol).

- Verificar a acumulação de glicogênio nas linhagens de *S. cerevisiae* proficientes e deficientes para o metabolismo de carboidratos e para os mecanismos de reparação de DNA utilizadas neste trabalho.

Capítulo I

A BRIEF REVIEW ABOUT AGES: MECHANISM OF INDUCTION, INTERMEDIATE METABOLITES AND ITS ASSOCIATION WITH DIFFERENT PATHOLOGIES AND AGING

Manuscrito a ser submetido.

A brief review about AGEs: mechanism of induction, intermediate metabolites and its association with different pathologies and aging

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Abstract

Advanced glycation end–products (AGEs) are heterogeneous compounds formed by a nonenzymatic reaction between proteins and reducing carbohydrates. The structure and mechanism of several AGEs have been characterized. The AGEs formation is a natural phenomenon in biological systems, but its accumulation can be accelerate in many cells and tissues during aging and also in the presence of physiopathological condition, e.g. diabetes mellitus. AGEs also are associated in a diverse group of diseases related to the neurodegeneration disorders, such Alzheimer and Parkinson. In the DNA, the presence of genotoxics lesions of AGEs is associated with significant structural and functional alterations. The interaction of AGEs with membrane receptors in mammalian cells induces an intracellular signaling that leads to enhanced oxidative stress and formation of inflammatory process. In this sense, there are different cellular protective mechanisms that prevents DNA glycation, which are classified as non-enzymatic and enzymatic mechanisms Thus, the purpose of this review is to give a broad vision about the importance of reducing carbohydrates to the induction of AGEs, its implication for different pathologies, and the cellular mechanisms associated to with AGEs metabolism.

Keywords: advanced glycation end-products (AGEs); glucose; fructose; DNA glycation; AGEs receptors.

1. Introduction

Reducing carbohydrates such as glucose and fructose are able to react with proteins, lipids, amino acids, and DNA by means of chemical reactions known as non-enzymatic glycation (Singh et al. 2001). This non-enzymatic glycation occurs by the reaction between the aldehyde or ketone groups of sugars with the free amino group of amino acids lysine and arginine of proteins (Levi and Werman 2003; Thorpe and Baynes 2003; Jakus and Rietbrock 2004). This process also known as the Maillard reaction was described in the early 1900's, when it was noted that amino acids heated in the presence of reducing sugars developed a characteristic yellow-brown colour (Gugliucci 2000; Singh et al. 2001).

The non-enzymatic glycation is initiated by the reaction of a reducing sugar with a protein amino group to generate Schiff, also called early glycation products. Subsequently more stable rearranges form the Amadori products and/or Heyns base adducts. Some of the early glycation products on long-lived proteins continue to undergo complex series of chemical rearrangements *in vivo* to form complex compounds and crosslinks known as advanced glycation end-products (AGEs) (Aronson 2004; Dutta et al. 2005).

Both intracellular and extracellular proteins are subject to non-enzymatic glycation process, where the formation of covalent crosslinks can affect the functions of proteins (Laurean et al. 1996; Singh et al. 2001). The formation of AGEs has been suggested to occur both *in vitro* and *in vivo* and has been demonstrated to play a key role in the long-term complications of diabetes (Dutta et al. 2005). In this sense, glycation is a type of chemical modification that takes place slowly and continuously throughout the life span (Baynes 2000; Levi and Werman 2003; Thorpe and Baynes 2003).

Short-lived molecules, such as proteins in plasma and blood, are expected to be influenced primarily by the early glycation products, while long-lasting molecules like collagen, lens crystalline, myelin and DNA are expected to be altered as they irreversibly accumulate substancial amounts of AGEs (Baynes 2000)

The accumulation of AGEs in long-lived molecules has been implicated in the pathophysiology of aging and neurodegenerative amyloid diseases such as Alzheimer and diabetes (Thorpe and Baynes 2003; Booth et al. 1997). In diabetes, the hyperglycemia eventually causes serious and irreversible microvascular and macrovascular complications, including retinopathy, neuropathy, nephropathy, atherosclerosis and cerebrovascular disease (Khalifah et al. 1999).

In this sense, AGEs have pathophysiological roles that are related in the development and progression of different pathologies and aging. Therefore, in this review, we describe the process of formation and accumulation of AGEs, the structure of major AGES, the intermediate metabolites involved in the formation of AGEs, compounds inhibitors, and receptors

2. How AGEs are formed?

The first stage of AGEs formation occurs through sugars forms chemically reversible (early glycation products) with reactive amino groups of proteins to form the Schiff bases (Fig. 1) (Jakus and Rietbrock 2004). This reaction is relatively fast and highly reversible, and represents an equilibrium reaction in which the amount of Schiff base formed is dictated by monossacharids concentration (Aronson 2004). Therefore, the reactivity is proportional to the percentage of sugar present in the open chain form, and inversely proportional to the length of the carbon chain of the sugar (Tessier et al. 2003; Aronson 2004).

Over a period of days, begins the second stage, where Schiff base subsequently rearranges to form Amadori product and/or Heyns aadducts (Fig. 1). Formation these products is slower, but of difficult reverse reaction, and therefore accumulate on proteins (Aronson 2004). As observed for Schiff base, the amount of Amadori product and/or Heyns adducts formed is related to the monossacharids concentration (Ulrich and Cerami 2001; Talasz et al. 2002).

The class of heterogeneous compounds collectively referred to as AGEs, occur in the final stage, when Amadori products and /or Heyns continue to undergo complex series of chemical rearrangements *in vivo* in long live proteins to form complex compounds can undergo dehydration, cyclization, oxidation, and rearrangement to form secondary products, as various dicarbonyls compounds, reactive carbonyl species (RCS) glyoxal (GO), methylglyoxal (MGO), deoxyglucosones**,** protein bound and crosslinks (Singh et al. 2001; Thorpe and Baynes 2003). An important characteristic of AGEs compared with the Amadori products is that, once formed, AGE-protein adducts are stable and irreversible (Aronson 2004).

The formation of AGEs is catalysed by transition metals such as iron and copper and is inhibited by reducing compounds such as ascorbate (Singh et al. 2001; Thorpe and Baynes 2003; Levi and Werman 2003).

In recent years, was demonstrated that AGEs are formed not only from hexoses and pentoses (Barea and Bonatto 2008), but also from threose-derivated structures, oxoaldehydes, dicarbonyl compounds, short chain sugars, such as glycolaldehyde, and reactive intermediates, such as GO and MGO which are characterized by their high ability to cause protein-protein crosslinks (Laurean et al. 1996; Tessier et al. 2003).

Additionally, the formation of AGEs also involves neutrophils, monocytes, and macrophages, which upon inflammatory stimulation produce myeloperoxidases and

NADPH oxidase enzymes that oxidizes amino acids. Other pathways are receptor for AGEs (RAGEs) associate with the production of reactive oxygen species (ROS) that promotes more AGEs via NADPH oxidase pathway (Tezel 2006). The fructose entering the polyol pathway also may directly form AGEs via 3-deoxyglucosone (3-DG), a potent AGE intermediate (Fig. 2) (Huebschmann et al. 2006).

a) Structures of AGEs

There are a multiplicity of AGEs structures in tissues as a result of reducing sugars (Singh et al. 2001; Thorpe and Baynes 2003). In this sense, the major AGEs structures have been identified by different techniques (Singh et al. 2001).

A pathway for the occurrence the AGEs is the formation of reactive intermediate products known as 3-DG and MGO (Singh et al. 2001). Both are formed by non-oxidative rearragement and hidrolysis of Amadori products (Singh et al. 2001; Thorpe and Baynes 2003) and also formed in the degradation of glucose and fructose (Usui et al. 2007).

The MGO is also formed from non-oxidative mechanisms in anaerobic glycolysis and can be derived from fructose by fragmentation of triose phosphate or catabolism of ketone bodies and threonine (Singh et al. 2001). The MGO is highly active glycating agent which is thought to be responsible for the increased protein glycation detected during hyperglycemic conditions and for much of the protein glycation associated with diabetic complications (Hipkiss 2007).

The MGO, GO and 3-DG have recently been proposed to be formed from all stages of the glycation process, by degradation of glucose or Schiff base, or from Amadori products. Thus, the presence these intermediates could be considered an important point in the process of formation of AGEs by the classical metabolic pathway (Singh et al. 2001).

The AGEs crosslines (Fig. 3A) and vesperlysines (Fig. 3B) are derivated directly from glucose and retain the intact carbon structure. The formation of the others AGEs such as pyrraline (fig. 3D) from glucose is non-oxidative process (Singh et al. 2001; Thorpe and Baynes 2003).

The formation of pentosidine (fig. 3C) requires oxidation, may also be formed by hexoses, pentoses, or by glycololytic intermediaries glyceraldehyde-3-phosphate (G-3P) and dihydroxyacetone-phosphate (DHAP), which can spontaneously decompose into MGO (Hamada et al. 1996; Hipkiss 2007). The pentosidine is a casual factor in aging, where the kinetic of pentosidines accumulation, rather than absolute levels of this biomarker, must reflect on some process that rate-determining in longevity (Baynes 2000).

The AGEs *N*-(carboxymethyl)lysine (CML) (Fig. 3E) and *N*-(carboxyethyl)lysine (CEL) (Fig. 3F) are characterised as glycoxidation products, formed by sequential glycation and oxidation reactions (Singh et al. 2001; Thorpe and Baynes 2003). In addition, both compounds can also be formed by other precursor as hexoses, pentoses, glycolytic intermediates, or ascorbic acid. (Thorpe and Baynes 2003). These compounds may also be formed from a variety of non-carbohydrate sources, including lipids and amino acids, thus, cannot be readly applied to specific products of AGEs, because when both is formed from lipids it is described as an advanced lipooxidation end-products (ALEs) (Hamada et al. 1996; Khalifah et al. 1999).

b) *Intermediates metabolites in the formation of AGEs*

All reducing sugars whethever aldoses, ketoses or even molecules related to sugars can initiate glycation and crosslinks *in vivo*. Several studies indicate that a

number of sugars in addition to glucose can form AGEs (Levi and Werman 2003; Tessier et al. 2003; Barea and Bonatto 2008). Some intermediate metabolites of glycolytic pathway and of the polyol pathway have been shown to be potent glycating agents, whereas alterations in the glycolytic and the polyol pathway metabolism and changes in the levels of intermediate metabolites seem to be potentially involved in enhanced AGEs formation (Hamada et al. 1996; Barea and Bonatto 2008).

There are many glycolytic intermediate metabolites involved directly or indirectly in metabolism of AGEs as glucose-1-phosphate (G-1-P), glucose-6-phosphate (G-6-P), glyceraldehyde-3-phosphate (G-3P) and dihydroxyacetone-phosphate (DHAP) (Barea and Bonatto 2008).

Glucose is the most abundant monosaccharide in blood and tissues being associated with hyperglycemia in diabetes mellitus (Levi and Werman 2003; Jakus and Rietbrock 2004). However, the glycation induced by glucose is slow when compared with many other monosaccharides, whereas intracellular sugars, such as G-6-P and fructose form AGE at a faster rate (Singh et al. 2001; Levi and Werman 2001). These compounds, like other reducing sugars, can react with proteins trough the glycation, which may account for several complications of diabetes mellitus and accelerating aging (Levi and Werman 2001).

Hamada *et al.* (1996) showed that the intermediate metabolites like trioses phosphates, fructose-1-6-bisphosphate (F-1,6-P), fructose-3-phosphate (F-3-P) rather than glucose, play tangible roles in the glycation of intracellular proteins, despite the fact their concentrations are much lower than the glucose. A possible explanation for these evidences is that the bulk of both sugars are present as the cyclic (pyranose and furanose) structures, where the open ring cetone form represents 0.7% of the total fructose, but only 0.002% of open aldehyde of the total glucose. Because of this

difference in the level of the free aldehyde form, fructose can initiate glycation in a higher rate than glucose (Levi and Werman 2003). *In vitro* glycation of proteins by fructose is 7.5 to 10-fold faster then glucose (Talasz et al. 2002; Levi and Werman 2003).

The fructose metabolism include different biochemical pathways (Hagopian et al. 2005), such as the synthesis of sorbitol from fructose through the polyol pathway in some organs (ocular lens, kidney and peripheral nerves). Fructose can also be produced by glucose conversion via glycolysis and the pentose phosphate (Barea and Bonatto 2008). This distinct metabolism estimulates the formation of several metabolites as fructose-1-phosphate (F-1-P), dihydroxyacetone phosphate (DHA), glyceraldehyde (GA) and glyceraldehyde-3-phosphate (G-3-P), these two last glycolytic intermediate metabolites (Levi and Werman 1998; Levi and Werman 2001; Tessier et al. 2003).

The G-3-P is a potent protein crosslink agent and a precursor for argpyrimidine, which appears to be the major modification of amino acids. Its phosphorylated form is also a source of CML and CEL. DHA and its dephosphorylated forms are also potent glycation and cross-linking agents (Tessier et al. 2003).

2. DNA damage and AGEs

The G-6-P, glucose and possibily other reducing sugars can react with DNA *in vitro* to produce marked biological and structural alterations. Accumulation of these products might be a mechanism for the decreased genetic viability and increased mutagenesis observed in the aged organism (Bucala et al. 1984; Bucala et al. 1985; Barea and Bonatto 2008). A Systems Biology investigation have shown that DNA structure and functions can be affected in the presence of intracellular reducing sugars,

as glucose, G-6-P and the reactive glycated products formed from G-3-P and lysine (Barea and Bonatto 2008).

It has been related that *in vitro* G-3-P and lysine produce base modification and apurinic/apyrimidic sites (Thornalley 2003) that are converted to strand-breaks and double strands in plasmid DNA in *Escherichia coli* (Levi and Werman 2001). Additionaly, GO and MGO induce multi-base deletions, base repair substitutions and transversions. The introduction of the specific nucleotide AGE into DNA was also associated with increased mutation frequency and increased DNA strands breaks in *E. coli* (Thornalley 2003).

The fructose, fructose-6-phosphate and their phosphorylated metabolites inflicted cell damage, causing DNA modifications, apoptosis and mutation in mouse lymphoma cells. Therefore, have been suggested that glycation *in vivo* in mammalian tissues is a possible event that may negatively affect cell viability and DNA integrity (Levi and Werman 2003). In this sense, the observations that intracellular metabolites can modify DNA suggests a mechanism for accumulation of glycated DNA (Barea and Bonato, 2008) that may lead genetic lesions that may lead to cellular senescence, (Bucala et al.1984; Levi and Werman 2001).

In addition to DNA, the histones are another example of target for glycation. Being extremely abundant molecules, which have a long –half-life in the cell (Gugliucci 1994), it has been demonstrate that the histones are susceptible nonenzymatic glycation, with the formation *in vitro* of pentosidine, protein crosslinks and fluorescent AGEs. Moreover, these studies clearly showed that the concentration on histones-AGEs is increased in cells from diabetic subjects (Gugliucci 1994; Gugliucci and Bendayan 1995; Talasz et al. 2002).

Histones modifications by AGEs during aging or diabetes may alther chromatin structures and function in turn leading to changes in gene expression (Talasz et al. 2002). The decline in DNA repair mechanisms and persistence of lesions in DNA with increased age may also exacerbate the effects of nucleotide glycation, relacionated with the increased mutation and that appear associated with presence of mutagenesis and carcinogenesis in mammals (Thornalley 2003; Barea and Bonatto, 2008).

3. Diseases and AGEs association

The AGEs are generated normally in the body at homeostatic concentrations of glucose, resulting in AGEs accumulation with age, and in accelerated rate in diabetes. However, proteins with long half-lives such collagen will accumulate substantial amounts of AGEs *in vivo*. This ability of AGE-modified proteins to form proteins-proteins crosslinks on collagen *in vivo* is a key determinant observed with aging and in diabetes (Baynes 2000; Aronson 2004).

Therefore, the accumulation of AGEs and reactive precursors *in vivo* that have been implicated in many pathologies such diabetes, atherosclerosis, end-stage renal disease (ESRD), rheumatoid arthritis, and neurodegenerative diseases of aging as an Alzheimer's and Parkinson (Booth et al. 1997; Jakus and Rietbrock 2004). Therefore, the formation and accumulation of AGEs in high concentration is common in the vascular wall in atherosclerosis, in the protein deposits in neurodegenerative diseases and amyloid plaques (Thorpe and Baynes 2003).

Diabetes is characterized by high glucose concentrations in plasma, this disease can be caused by a quantitative deficiency in insulin secretion or a resistance to insulin action. Actually it is estimated that diabetes mellitus afflict 5% to 7% of worldwide population, being a major risk factor for cardiovascular disease and atherosclerosis (Gugliucci 2000; Jakus and Rietbrock, 2004). In diabetes the ability to maintain

homeostasis and to control glucose concentration in plasma is decreased, consistent with a role for excess glucose that appears related in age-related chronic diseases (Thorpe and Baynes 2003). Moreover, hyperglycemia is considered a key factor in the development of diabetic vascular complications (retinopathy, neuropathy, nephropathy) and macrovascular disease such as atherosclerosis, potentially resulting in heart disease (Singh et al. 2001; Jakus and Rietbrock 2004).

Patients with diabetes have higher levels of AGEs that non-diabetic subjects as a consequence of hyperglycemia. In erythrocytes, Giardino et al. (1996) shown that AGE-hemoglobin accounts for 0.42% of circulating hemoglobin in normal subjects and 0.75% in diabetics. Other studies have shown 20-30% higher AGEs levels in people with uncomplicated diabetes and 40-100% higher levels in subjects with type 2 diabetes complicated by coronary artery disease or micro albuminuria (Huebschmann et al. 2006). The level serum AGEs and serum CML have been found to be elevated in both types of diabetes (Jakus and Rietbrock 2004).

In subjects with diabetics the nephropathy has been a leading cause of ESRD, patients with ESRD have shown significant elevations in circulating AGEs compared with healthy control subjects (Huebschmann et al. 2006), this is evidence suggest that AGEs play a critical role in the development of nephropathy (Yan et al. 2007). In retinopahy the AGEs are directly linked to an increased retinal vascular endothelial growth factor expression, resulting in vascular leakage (Jakus and Rietbrock 2004).

In collagen AGEs form crosslinks, making collagen less susceptibility to proteolytic and chemical degradation. The decreased proteolytic results in an accumulation and continued AGE-derived crosslink of myocardial and vessel wall collagen, leading to the lost of elasticity and flexibility that are necessary for normal activity of the arteries (Aronson 2004; Huebschmann 2006; Goh et al 2008). In addition,

it has been reported that glycation can disturb calcium homeostasis by inhibiting calcium pumps. Thus, the detrimental effects of glycation reaction on calcium metabolism are further supported by the findings that intracellular calcium concentrations are increased in most of the tissues of animal models with diabetes (Ying 1997).

As mentioned before, there are evidences that links hyperglycemia to complications of health, and apparently one key biochemical mechanisms is the direct effect of glucose and other sugars on proteins (Gugliucci 2000; Huebschmann et al. 2006). These evidences converge to glycation as the major molecular basis of diabetes vascular complications and support the fact that AGEs clearly contribute to the progression of micro and macrovascular complications (Huebschmann et al. 2006).

a) Ages and aging

Aging can be considered as a fundamental and highly complicated characteristic of life and of majority of senescence pathologies, including Alzheimer's, atherosclerosis and Parkinson's diseases (Ying 1997). Aging is a complex process that involves multiple mechanisms (Slijepcevic 2008). Moreover, the relationships among these multiple mechanisms are complex processes and appear to be paradoxical (Ying 1997).

The aging can be defined as the loss of physiologic aptitude of an organism that begins after the same reach its reproductive competence (Kirchman et al. 1999). True biomarkers do not exist for the aging and its phenotype is extremely complex, being many of its biological consequences can be part of diseases that afflict the organism (Martin et al. 1996; Vijg and Suh 2005).

Researches on biochemical mechanism of aging in recent years give more attention to the nonenzymatic glycation as such an important point in the aging theory

(Ying 1997; Yin et al. 2005). At the biochemical level, aging is characterized by the accumulation of altered protein molecules. Therefore, the aging and cumulative damage to protein is the result of a balance between the rates of synthesis, exposure and turnover of proteins, and its half-life is the determinant point to be considered for the extension of lifespan, being used as in integrator of aging (Ying 1997; Baynes 2000).

Moreover, the crosslink and denaturation of proteins caused by glycation are the key factors for early aging- related alterations of blood vessel, kidney, lung and joint. This may results in the hardening of structural proteins and damages of functional proteins, like of anti-oxidative enzymes and DNA repair. Additionally, aging changes such as decreasing of energy supply, decline of metabolizing functions and disruption of physiological homeostasis also are observed and can occur increased in cases of glycation (Ying 1997).

The collagen is a long half-life molecule that allows the accumulation of nutritional and age-related lesions, these proteins provide a historical perspective on the aging process (Baynes 2000). The glycation affect both biochemical, mechanical and physiologic functioning of collagen, and their deleterious effects play a significant role in the pathogenesis of the aging process (Levi and Werman 1998). Other example of presence of AGES in normal aging of humans is the gradual decrease in the elasticity of the cardiovascular system, which leads to increased arterial and myocardial stiffness (Aronson 2004). Therefore, the formation of AGEs can is an important consequence for the aging (Hipkiss, 2008), accelerating multiple aging processes and is an important age-related biological reaction responsible for various diseases initiated also by diabetes (Ying 1997).

In addition, is important to consider that lifespan is genetically programmed, being aging a stochastic process affecting the integrity of the genome. In this sense, the

formation of AGEs can be viewed as a significant source of genomic damage (Baynes 2000). Thus, the accumulation of chemical damage by Maillard and other nonenzymatic reactions can be also a major determinant of the natural lifespan of species (Thorpe and Baynes 2003) and should provide an indirect index of genomic damage, being a of the mechanisms associated with the longevity and aging in organisms (Baynes 2000).

4. AGEs receptors (RAGEs)

AGEs receptors have been identified in monocytes, macrophages, endothelial, mesangial cells and microglia (Gugliucci 2000; Singh et al. 2001; Jakus and Rietbrock 2004). AGEs interaction with RAGEs results in receptor activation and receptor-mediated induction of oxidative stress, inflammatory responses and cellular dysfunction (Aronson 2004; Huebschmann et al. 2006).

The major studied pro-inflammatory RAGE is a member of the immunoglobulin superfamily (Singh et al. 2001; Fan et al. 2004; Huebschmann et al. 2006). This RAGE acts as a scavenger, mediates intracellular signaling and promotes multiple signaling pathways that generate ROS. These pathways include p21, the extracellular signal-regulated kinase (ERK) 1-and-2, mitogen-activated protein (MAP) kinases. In addition, AGE-RAGE interaction results in cellular activation and inflammation, followed by lipoproteins accumulation in atherosclerosis, leading to chronic inflammation and further accelerating this pathology (Singh et al. 2001; Basta et al. 2005; Huebschmann et al. 2006; Mikulíková et al. 2007). It should be noted that there are receptors that normally aid in clearing AGEs from the blood circulation and may help to mitigate the prooxidant effects of AGEs (Huebschmann et al. 2006) these types of AGE receptor include AGE-R1 and lysozyme (Goh et al. 2008).

The lysozyme is one soluble receptor important in the detoxification of AGEs, this member of the human immune defense system and exhibits high AGE-binding

affinity, recognizing at least two structurally distinct AGEs, CML and MGO derivatives (Huebschmann et al. 2006). The overexpression of AGE-R1 confirmed enhanced endocytosis and degradation of AGEs; however, AGE-R1 also revealed an inhibitory action on AGEs and RAGE induced mitogen-activated and active the free radical sensitive transcription nuclear factor NFκB, a multifaceted coordinator of numerous response to injury genes that suggested that AGE-R1 may mitigate AGE-induced oxidative species and cellular toxicity (Fan et al. 2004). Accordingly, the colocalization of receptors and AGEs at the microvascular sites of injury suggests that their interaction may play a significant role in the pathogenesis of diabetic vascular lesions (Gugliucci 2000; Huebschmann et al. 2006).

5. Inhibitors of AGES

The discovery of compounds that can inhibit deleterious glycation reaction shown a great therapeutic benefit for all pathologies and represents a promising target for research (Booth et al. 1997). The action of these compounds occur through diverse pathways, including decreasing AGEs by inhibiting the production of Amadori products, binding and detoxifying dycarbonil compounds and interrupting biochemical pathways that impact the AGEs levels (Huebschmann et al. 2006).

Cellular protective processes that prevent glycation of DNA can occur via non-enzymatic and enzymatic mechanisms. The major non-enzymatic anti-glycating mechanisms are mediated by small molecules like amino acids (l-cysteine and l-arginine), polyamines (spermine and spermidine), carnosine and anserine, thiols and thiolamines (taurine and glutathione) (Barea and Bonatto 2008). These protections non-enzymatic mechanism are the use to eliminate or to reduce the presence of AGEs in DNA and in other macromolecules (Monnier 2003).

Both the spermine as the spermidine they are present in high concentrations in different tissues, and the carnosin is associated with tissues with a long time of life (muscles and the nervous system) (Abe 2000), they are associated with the protection front the glycolytics intermediates and aldehydes reagents (Hipkiss 2005). Additionally, carnosin, piridoxin, spermine, and spermidine suppresses the crossed connections between proteins and MG, delaying the senescence of fibroblasts cultivated human *in vitro* (McFarland and Holliday 1994) and the aging in mice and *Drosophila melanogaster* (Yuneva et al. 2002).

Spermine and spermidine are both poliamines found in high concentrations in the nucleus, possibly protecting DNA and histones from AGEs (Gugliucci and Menini 2003). In this sense, it was demonstrated that the concentrations of poliamines in tissues (Gugliucci 2005) and of carnosin (Stuerenburg and Kunze, 1999) decreased with age, which could be related with the physiologic dysfunctions caused for the AGEs in senescent animals.

In the case of the enzymatic mechanisms, the main active proteins are the glyoxalase and the frutosamin 3-cinase (Thornalley 2003; Gugliucci 2005). The superexpression of enzyme glyoxalase has been demonstrated in certain areas of the brain, and it could suppress the incidence of Alzheimer. Frutosamin 3-cinase act in the recycling of carbonils adducts with espermin (Gugliucci 2005) and/or in the transfer of bases Schiff for taurine and other thiol-containing compounds (Szwergold 2005; Barea and Bonatto 2008).

Moreover, *in vitro* and animal models have shown the effectiveness of the chemical agents to reduce AGE accumulation. These agents act by inhibiting oxidant activity such as vitamin C and nicarnitine, others by cleaving crosslink formation such as phenacyl thiazolium bromide. Agents such aminoguanidine (Booth et al. 1997;

Khalifah et al. 1999) 2,3 di-amino phenazine, pyruvate and the monoclonal antibody A717 also have effects on AGEs accumulation, but their mechanisms of action has not been fully determined (Singh et al. 2001).

The reduced ingestion of calories (also known also by caloric restriction) affect the chronological lifespan drastically in most of the studied organisms (Hipkiss 2006; Hipkiss 2007; Smith et al. 2007) and appears as other factor that can inhibit in an indirect way the formation of AGEs. This hypothesis suggests that in conditions of the caloric restriction, the ingestion of few calories would act as an agent inductor of hormesis (Parsons 2007) and, consequently, activate a cellular response that it would promote an increase in the efficiency of performance of the reducing carboydrates metabolism for the formation/production of AGEs for classical pathway. However, it is little still known regarding the performance of molecular mechanisms of the hormese in the longevity and in the aging (Sinclair 2005).

6. Oxidative stress and AGEs: two sides of the same coin?

One of the main discoveries in the study of the process of macromolecules glycation occurred during the decade of 1980, when was shown the importance of the reactive oxygen species (ROS) in the generation of AGEs, through formation of species reactive of carbonila (RCS) from the carboydrates oxidation, lipids and amino acids (Thorpe and Baynes 2003; Tezel 2006).

The glyoxal (GO) and methylglyoxal (MGO) are two examples of reactive intermediates responsible for formation of RCS (Giardino et al. 1996), and in different culture cells Schmitt et al. (2006) related the formation of ROS as the primary effect cellular of AGEs.

The increase in intracellular ROS also was associated with concomitant increase in intracellular AGE formation in bovine endothelial cell, these data suggested that there is causal relation-ship between ROS generation and intracellular AGE formation (Giardino et al. 1996). In addition, DNA glycation also gives to some characteristic nucleotide adducts that have been found to increase oxidative stress by the generation of hydrogen peroxide, superoxide, and hydroxyl radicals, resulting in oxidative damage in nucleotides and DNA (Barea and Bonatto 2008).

Moreover, both non-enzymatic and enzymatic anti-glycating mechanisms have many similarities with protection against oxidative stress. For example, cells resists to oxidative stress through a variety of complementary mechanisms including enzymatic (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic (glutathione and vitamin E) detoxification and synthesis of DNA repair enzymes. These similarities between anti- oxidant and anti-glycating mechanisms could be more indicative of the association and interaction among both in the formation of the AGEs (Barea and Bonatto 2008)

In the aging oxidative stress increases with age in the brain and the ability of neurons to respond to oxidative stress declines with age mostly due to an imbalance between increasing oxidant production and decreasing antioxidant capacity (Tezel 2006). The AGEs also accumulate in various tissues in the course of physiological aging, being related with the presence of neurodegenerative diseases (Baynes 2000). In the other hands, two important consequences of hyperglycemia in diabetes are increase formation of AGEs and development of oxidative stress (Tan et al. 2007; Gupta et al. 2007).

These associations between AGEs and ROS show a synergism of both in the occurrence of different pathologies and in presence of anti-oxidant and anti-glycating

mechanisms. However, little data exist in the literature related the association between ROS and RCS in the formation of AGEs and larger studies are needed so that these associations in common can be better explained scientifically.

7. Concluding remarks

All studies referred to AGEs nowadays have a great importance for aging and associated pathologies. Data from the literature shows an increased presence of AGEs in the different diseases such as diabetes mellitus (macro and microvascular complications) and neurodegenerative disorders of aging such as Alzheimer's and Parkinson's (Singh et al. 2001; Thorpe and Baynes 2003).

The carbohydrates glucose and fructose in addition to intermediate metabolites formed by classical monosaccharide pathways are associate with formation of AGEs (Barea and Bonatto 2008). In this sense, diet may be one of the few environmental aspects associated with the interference in the longevity and aging of organisms. Therefore, is very important studies showing the presence of data relationated with the formation of AGEs on condition of caloric restriction, because are the minimum data of literature that exist report this type of dietary intervention associate with reduced and/or increased formation of AGEs may interfered in the longevity.

8. References

Abe H (2000) Role of histidine-related compounds as intracellular proton buffering constituents in vertebrate muscle. Biochemistry 65: 757-765

Aronson D (2004) Pharmacological prevention of cardiovascular aging-targeting the Maillard reaction. Br J Pharmacol 7:1055-1058

Barea F, Bonatto D (2008) Relationships among carbohydrate intermediate metabolites and DNA damage and repair in yeast from a systems biology perspective. Mutat. Res. 642: 43-56

Basta G, Lazzerini G, Del Turco S, Ratto GM, Schmidt AM, De Caterina R (2005). At least 2 distinct pathways generating reactive oxygen species mediate vascular cell adhesion molecule-1 induction by advanced glycation end products.Arterioscler Thromb Vasc Biol. 25:1401-1407

Baynes JW (2000). From life to death--the struggle between chemistry and biology during aging: the Maillard reaction as an amplifier of genomic damage. Biogerontology 1: 235-246

Booth AA, Khalifah RG, Todd P et al (1997) In vitro kinetic studies of formation of antigenic advanced glycation end products (AGEs). Novel inhibition of post-Amadori glycation pathways. J Biol Chem 272: 5430-5437

Bucala R, Model P, Cerami A (1984) Modification of DNA by reducing sugars: a possible mechanism for nucleic acid aging and age-related dysfunction in gene expression. Proc Natl Acad Sci USA 81: 105-109

Bucala R, Model P, Russel M et al. (1985) Modification of DNA by glucose 6-phosphate induces DNA rearrangements in an Escherichia coli plasmid. Proc Natl Acad Sci USA 82: 8439-8442

Dutta UU, Cohenford MA, Dain JA (2005) Nonenzymatic glycation of DNA nucleosides with reducing sugars. Anal Biochem 345: 171-180

Fan Q, Liao J, Kobayashi M et al. (2004) Candesartan reduced advanced glycation end-products accumulation and diminished nitro-oxidative stress in type 2 diabetic KK/Ta mice. Nephrol Dial Transplant 19**:** 3012-3020

Giardino I, Edelstein D, Brownlee M (1996) BCL-2 expression or antioxidants prevent hyperglycemia-induced formation of intracellular advanced glycation endproducts in bovine endothelial cells. J Clin Invest 97: 1422-1428

Goh KL, Holmes DF, Lu HY, et al. (2008) Ageing changes in the tensile properties of tendons: influence of collagen fibril volume fraction. J Biomech Eng 130: 021011.

Gugliucci A (1994) Advanced glycation of rat liver histone octamers: an in vitro study. Biochem Biophys Res Commun 203: 588-593

Gugliucci A and Bendayan M (1995) Histones from diabetic rats contain increased levels of advanced glycation end products. Biochem Biophys Res Commun 212: 56-62

Gugliucci A (2000) Glycation as the glucose link to diabetic complications. J Am Osteopath Assoc 100: 621-634

Gugliucci A and Menini T (2003) The polyamines spermine and spermidine protect proteins from structural and functional damage by AGE precursors: a new role for old molecules? Life Sci 72:2603-2616

Gugliucci A (2005) Alternative antiglycation mechanisms: are spermine and fructosamine-3-kinase part of a carbonyl damage control pathway? Med Hypotheses 64:770-777

Gupta A, Tripathi AK, Tripathi RL, et al. (2007) Advanced glycosylated end products-mediated activation of polymorphonuclear neutrophils in diabetes mellitus and associated oxidative stress. Indian J Biochem Biophys 44: 373-388

Hagopian K, Ramsey JJ, Weindruch R (2005) Fructose metabolizing enzymes from mouse liver: influence of age and caloric restriction. Biochim Biophys Acta 1721: 37-43

Hamada Y, Araki N, Koh N, et al. (1996) Rapid formation of advanced glycation end products by intermediate metabolites of glycolytic pathway and polyol pathway. Biochem Biophys Res Commun 228: 539-543

Hipkiss AR (2005) Glycation, ageing and carnosine: are carnivorous diets beneficial? Mech Ageing Dev 126: 1034-1039

Hipkiss AR (2006) Does chronic glycolysis accelerate aging? Could this explain how dietary restriction works? Ann N Y Acad Sci 1067: 361-368

Hipkiss AR (2007) Could carnosine or related structures suppress Alzheimer's disease? J Alzheimers Dis 11: 229-240

Hipkiss AR (2008) Energy metabolism, altered proteins, sirtuins and ageing: converging mechanisms? Biogerontology 9: 49-55

Huebschmann AG, Regensteiner JG, Vlassara H, et al. (2006) Diabetes and advanced glycoxidation end products. Diabetes Care 29: 1420-1432

Jakus V and Rietbrock N (2004) Advanced glycation end-products and the progress of diabetic vascular complications. Physiol. Res 53: 131-142

Khalifah RG, Baynes JW, Hudson BG (1999) Amadorins: novel post-Amadori inhibitors of advanced glycation reactions. Biochem Biophys Res Commun 257: 251-258

Kirchman, PA, Kim S, Lai CY, et al. (1999) Interorganelle signaling is a determinant of longevity in Saccharomyces cerevisiae. Genetics 152: 179-190

Laurean-Cervantes D, Jacobson EL, Jacobson MK (1996) Glycation and Glycoxidation of Histones by ADP-ribose. J Biol Chem 271: 10461-10469

Levi B and Werman MJ (1998) Long-term fructose consumption accelerates glycation and several age-related variables in male rats. J Nutr 128: 1442-1449

Levi B and Werman MJ (2001) Fructose triggers DNA modification and damage in an Escherichia coli plasmid**.** J Nutr Biochem12: 235-241

Levi B and Werman MJ (2003) Fructose and related phosphate derivatives impose DNA damage and apoptosis in L5178Y mouse lymphoma cells. J Nutr Biochem14: 49-60

Martin GM, Austad SN, Johnson TE (1996) Genetic analysis of ageing: role of oxidative damage and environmental stresses. Nat Genet 13: 25-34

McFarland GA, Holliday R (1994) Retardation of the senescence of cultured human diploid fibroblasts by carnosine. Exp Cell Res 212: 167-175

Mikulíková K, Eckhardt A, Kuneš J, et al. (2007) Advanced glycation end-product pentosidine accumulates in various tissues of rats with high fructose intake. Physiol Res 57: 89-94

Monnier VM (2003) Intervention against the Maillard reaction *in vivo.* Arch Biochem Biophys 419: 1-15

Parsons PA (2007) The ecological stress theory of aging and hormesis: an energetic evolutionary model Biogerontology 8: 233-242

Schmitt A, Bigl K, Meiners I, et al. (2006) Induction of reactive oxygen species and cell survival in the presence of advanced glycation end products and similar structures. Biochim Biophys Acta 1763: 927-936

Sinclair DA (2005) Toward a unified theory of caloric restriction and longevity regulation. Mech Ageing Dev 126: 987-1002

Singh R, Barden A, Mori T et al. (2001) Advanced glycation end-products: a review. Diabetologia 44: 129-146

Slijepcevic P (2008) DNA damage response, telomere maintenance and ageing in light of the integrative model. Mech Ageing Dev 129: 11-16

Smith DL Jr, Mc Clure JM, Materic M et al. (2007) Calorie restriction extends chronological lifespan of Saccharomyces cerevisiae independently of the Sirtuins. Aging cell 6: 649-662

Stuerenburg H, J Kunze, K. (1999) Concentrations of free carnosine (a putative membrane-protective antioxidant) in human muscle and rat muscles. Arch Geront Geriat 29: 107-113

Szwergold BS (2005) Intrinsic toxicity of glucose, due to non-enzymatic glycation, is controlled *in vivo* by deglycation systems including: FN3K-mediated deglycation of fructosamines and transglycation of aldosamines. Med Hypotheses 65: 337-348

Talasz, H, Wasserer S, Puschendorf B (2002) Nonenzymatic glycation of histones in vitro and in vivo. J Cell Biochem 85: 24-34

Tan AL, Forbes JM, Cooper ME (2007) AGE, RAGE, and ROS in diabetic nephropathy. Semin Nephrol 27:130-143

Tessier FJ, Monnier VM, Sayre LM, et al. (2003) Triosidines: novel Maillard reaction products and cross-links from the reaction of triose sugars with lysine and arginine residues. Biochem J 369: 705-719

Tezel G (2006) Oxidative stress in glaucomatous neurodegeneration: mechanisms and consequences. Prog Retin Eye Res 25: 490-513

Thornalley PJ (2003) Protecting the genome: defence against nucleotide glycation and emerging role of glyoxalase I overexpression in multidrug resistance in cancer chemotherapy. Biochem Soc Trans 6: 1372-1377

Thorpe SR and Baynes JW (2003) Maillard reaction products in tissue proteins: new products and new perspectives. Amino Acids 25: 275-281

Ulrich P, Cerami A (2001) Protein glycation, diabetes and aging. Prog Horm Res 56: 1-21

Usui T, Yanagisawa S, Ohguchi M, et al. (2007) Identification and determination of alpha-dicarbonyl compounds formed in the degradation of sugars. Biosci Biotechnol Biochem 71: 2465-2472

Vijg J and Suh Y (2005) Genetics of longevity and aging. Annu Rev Med 56: 193-212

Yan HD, Li XZ, Xie JM et al. (2007) Effects of advanced glycation end products on renal fibrosis and oxidative stress in cultured NRK-49F cells. Chin Med J Engl 120: 787-793

Yin WH, Chen JW, Tsai C et al. (2005) L- arginine improves endothelial function and reduces LDL oxidation in patients with stable coronary artery disease. Clin Nutr 24: 988-997

Ying W (1997) Deleterious network hypothesis of aging. Med Hypotheses 48: 143-148

Yuneva AO, Kramarenko GG, Vetreshchak TV, et al. (2002) Effect of carnosine on Drosophila lifespan. Bull Exp Biol Med 133: 559-561

Figure legends

Figure 1. The classical pathway of the AGEs. The open chain of glucose reacts with lysine to form Schiff base (imine) that undergoes an Amadori product to form aduct to protein. Both the Schiff base and Amadori products are stabilized in cyclic furanose and pyranose configurations.

Figure 2: Association of mechanisms of formation of AGEs: classical pathway, *via* polyol pathway, activation receptor in process inflammatory and *via* dycarbonil compounds.

Figure 3: Structures of AGEs crosslines (A), vesperlysines (B), pentosidine (C), pyrraline (D), *N*-(carboxymethyl)lysine (CML; E) and *N*-(carboxyethyl)lysine (CEL; F).

Figure 1

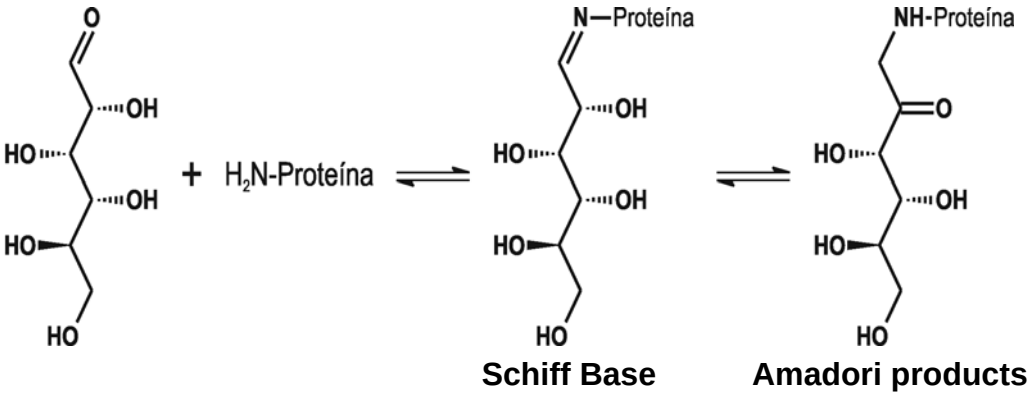


Figure 2

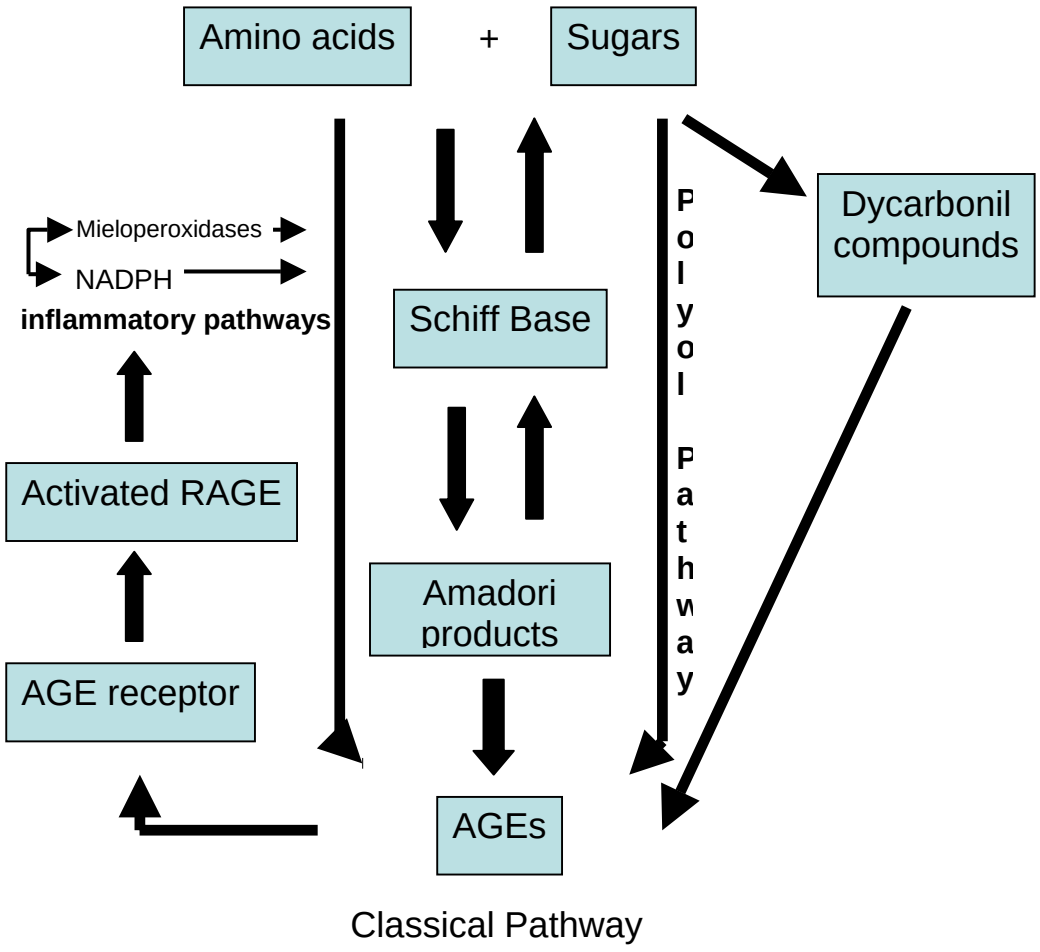
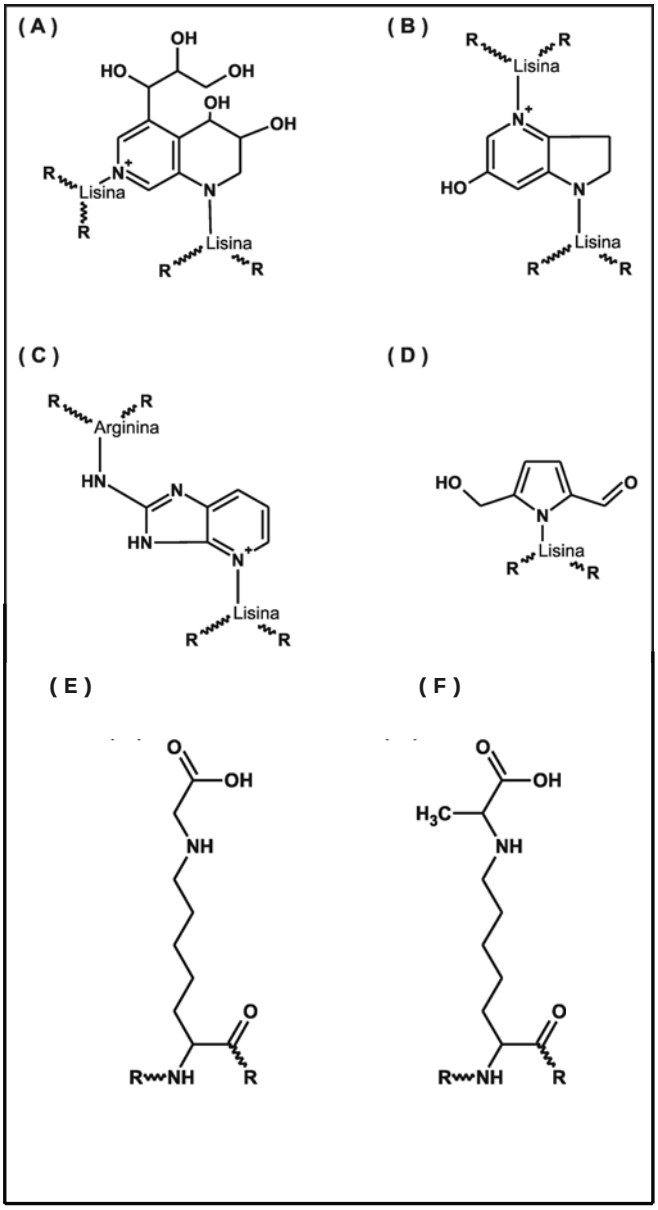


Figure 3.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Capítulo II

RELATIONSHIPS AMONG CARBOHYDRATE INTERMEDIATE METABOLITES AND DNA DAMAGE END REPAIR IN YEAST FROM A SYSTEMS BIOLOGY PERSPECTIVE

Artigo publicado no periódico ***Mutation Research: Fundamental and Molecular Mechanisms of Mutagenesis.***

Capítulo III

THE INFLUENCE OF DIFFERENT CARBON SOURCES ON THE CHRONOLOGICAL AGE OF *Saccharomyces cerevisiae* AND ITS ASSOCIATION WITH METABOLISM AND DNA REPAIR MECHANISMS

Manuscrito a ser submetido.

THE INFLUENCE OF DIFFERENT CARBON SOURCES ON THE CHRONOLOGICAL AGE OF *Saccharomyces cerevisiae* AND ITS ASSOCIATION WITH METABOLISM AND DNA REPAIR MECHANISMS

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ABSTRACT

Diet is one of the few environmental factors that may influence the chronological age of an organism. A diet rich in the monosaccharides glucose and fructose is associated with AGEs formation, which seem to be involved in aging. On the other hand, a condition known as caloric restriction appears to be involved in the increase of the lifespan of many organisms, including yeasts. Therefore, the purpose of this study was to evaluate how two different fementative carbon sources, glucose and fructose in high 5% (w/v) and low 0.5% (w/v) concentrations, and the non-fermentative carbon source, glycerol [3% (v/v)], may interfere with the metabolism and chronologic aging of different strains of *Saccharomyces cerevisiae*. While our results show a correlation between high monosaccharides concentration and a decrease in lifespan, caloric restriction had a positive impact on increasing longevity. In strains deficient for various enzymes controlling the glycolytic flux, we obtained novel data on chronological age, which have allowed us to draw associations and interesting explanations for the metabolic interactions seen in these strains.

KEYWORDS: AGEs, glucose, fructose, aging and caloric restriction

1. INTRODUCTION

The number of reports using the yeast *Saccharomyces cerevisiae* as a model organism for the understanding of biochemical and molecular mechanisms of aging has increased in the last years (Jazwinski, 2000; Chen et al., 2005). In this sense, four processes appear to be crucial to lifespan in yeast, i.e., metabolism, stress resistance, genic regulation, and genomic instability. Any alteration in these processes may results in cellular dysfunctions, mutagenesis, or cellular death. In order to prevent these events, a series of mechanisms may be required for the maintenance of survival, e.g., alternative metabolic pathways, DNA repair pathways, and cell cycle adjustments (Jazwinski, 2005; Kitanovic and Wolfl, 2006).

An unclear aspect from literature about aging is its association with metabolic intermediates products formed by very well studied pathways (glycolysis, glyconeogenesis, polyol pathway, and pentose phosphates). In this sense, a recent data by Barea and Bonatto (2008) using Systems Biology shows a circuitry of interactions between proteins of carbohydrate metabolism with DNA repair mechanisms in yeast. These results shown that both mechanisms prevent the AGES-generated damages in DNA and are responsible for maintaining the genomic integrity. Moreover, this study identified that the major carbohydrates intermediates could be associated with DNA and dNTP AGEs.

Therefore, the present work has the purpose of assessing the chronological lifespan of the *Saccharomyces cerevisiae* yeast by using different carbon sources under conditions of caloric restriction or not. Moreover, we associate the chronological life span by qualitative measuring the levels of glycogen stored in the cells. The results showed an association between glycogen accumulation and an increase in the lifespan of different yeast strains deficient for the carbohydrate metabolism.

2. MATERIALS AND METHODS

2.1 Culture media, solutions, and yeast strains

The complete liquid YEPD medium with 2% (w/v) yeast extract, 2% (w/v) peptone and 2% (w/v) glucose was used for growing the *S. cerevisiae* strains (Table 1). The solid YEPD medium was used for cultivation and maintenance of the yeast strains. For solid YEPD medium, 2% (w/v) bacto agar was added in their formulation. The Synco medium was used for culture the yeast strains during the chronological age assays. Synco formula contains 0.67% (w/v) of nitrogenous yeast base without amino acids or ammonium sulfate; 0.5% (w/v) ammonium sulfate and the following amino acids and nitrogen bases: 2 mg adenine, 2 mg arginine, 5 mg lysine, 1 mg histidine, 2 mg leucine, 2 mg metionine, 2 mg uracil, 2 mg tryptophan, and 24 mg threonine. These amounts were used for every 100 mL of medium. Synco used herein was supplemented with different carbon sources, e.g. 3% (v/v) glycerol, 5% (w/v) glucose, 5% (w/v) fructose, 0.5% (w/v) glucose, and 0.5% (w/v) fructose. Saline solution [NaCl 0.9% (w/v)] was used for washing cells and diluting the cell suspensions.

2.2 Chronological lifespan assay

Chronological lifespan assay of proficient and deficient *S. cerevisiae* strains for carbohydrate metabolism and DNA repair mechanisms was performed by inoculating cells in liquid YEPD medium. Cells were allowed to grow for 48 h at 28^{o}C until reaching the stationary phase. Thus, the cells number was determined by Neubauer counting chamber following by the adjustment of the cells number to 2×10^{8} cells/mL^{-1}. This inoculum was added to Synco medium supplemented with different carbon sources. The yeast cultures were incubated for 12 d at 28^{o}C. Aliquots of 1 mL were

retrieved from the cultures every 48 h, and a further counting of cell number was performed in the Neubauer counting chamber. The cells were diluted to a concentration of 2×10^3cells/mL^{-1} and 100 μL were taken from the cell suspension and seeded on solid YEPD. The plates were incubated for 2-3 d at 28°C. Survival of the different strains was determined by counting the number of colony-forming units (CFUs). All assays were performed in triplicate with three repetitions for each strain.

2.3 Glycogen accumulation assay in *S. cerevisiae*

The qualitative verification of glycogen accumulated in different strains subject to the chronological age assay was performed according to Chester (1968). Cells were counted in the Neubauer counting chamber and diluted to a concentration of 2×10^8 cells/mL^{-1}. Cell suspension of each strain (8 μl) was dripped onto plates containing solid Synco supplemented with different carbon sources. The cells were incubated for 48 h at 28 C followed by the exposure of plates by iodine vapor for 30 s. The qualitative assay was performed three times for each strain.

3. RESULTS AND DISCUSSION

3.1 Chronological lifespan assays with different strains of *Saccharomyces cerevisiae*

Being an eukaryotic organism, having a short lifespan, a small well-defined genome and many orthologous genes with human, *Saccharomyces cerevisiae* is a very useful model for studies involving an association of metabolic pathways and DNA repair mechanisms (Bitterman et al., 2003; Jazwinski, 2005). Characteristically, yeast cells show two distinct mechanisms related to lifespan, i.e. the replicative lifespan (RLS) and the chronological lifespan (CLS) (Bitterman et al., 2003; Reverter-Branchat et al., 2004; Chen et al., 2005; Qin and Lu, 2006).

In the CLS assay, yeasts are routinely grown in Synco medium until all nutrients are consumed, which occurs around the second day, when cells stop dividing and enter a stationary phase for a period of time during which they are able to stay viable (Chen et al., 2005). Interestingly, it has been reported that the metabolic activity of yeast cells in stationary phase is similar to the metabolism of post-mitotic cells found in a variety of adult mammalian tissues (Chen et al., 2005; Smith et al., 2007).

In this sense, during the CLS assay in Synco medium, yeasts accumulate intracellular damages and show cell alterations similar to those observed in aging human tissues, such as decrease in metabolism and protein synthesis and morphological cell alterations (Chen et al., 2005; Smith et al., 2007). The utilization of Synco medium is important for CLS in yeasts because the cells rapidly deplete the nutrients found in this medium and cease to divide despite retaining a relatively accelerated metabolism. Such cells have a short chronological life span relative to cells grown in rich medium (Bitterman et al., 2003).

Considering the analyses of a PPPI (physical protein-protein interaction) network evidenced by Barea and Bonatto (2008) it was showed a series of interpolations between proteins acting on the metabolism of carbohydrates and in DNA repair mechanisms. Among the many interestingly associations observed, the presence of an interaction circuitry used by yeasts for preventing and repairing the potential damages induced about by advanced glycation end-products (AGEs) or carbon reactive oxygen species (RCS) in DNA appears to be the most important (Barea and Bonatto, 2008). In this sense, we observed that the WT strain, proficient for carbohydrate metabolism and DNA repair mechanisms, has shown the highest survival rates for the non-fermentative carbon source glycerol (87%; Fig. 1A). It was also observed that the survival of WT strain under caloric restriction was increased, i.e., 69% and 63% for the

monosaccharides glucose and fructose, respectively (Fig. 1A). For higher monosaccharide concentrations, survival rates were smaller when compared to caloric restriction conditions, i.e. 48% for both glucose and fructose (Fig. 1A).

These CLS results obtained for in WT strain (Figure 1-A) show a negative correlation, between the increase in monosaccharide concentration and the decrease in survival rates. These results, together with data found in the literature, suggest that a decrease in survival rates might be associated with an increase in concentration of metabolic intermediate products formed by glycolytic pathway (Hamada et al., 1996; Lin et al., 2002; Yin and Chen, 2005; Barea and Bonatto, 2008). Thus, it might be suggested that the increase in monosaccharide concentration in this study might be related to AGEs formation, corroborating the data of the literature that associate the presence of AGEs with a decrease in lifespan and a higher susceptibility to aging events (Ying, 1997; Hagopian et al., 2003; Bitterman et al., 2003). In conditions of caloric restriction, an increase in survival was observed in BY4741 strain. These data evidence a correlation between the decrease in monosaccharide quantity and an increase in lifespan, corroborating data already reported in literature (Jazwinski, 2000; Hagopian et al., 2003; Bitterman et al., 2003; Smith et al., 2007). In this case, is suggests that caloric restriction could optimize the action of the glycolytic pathway. This condition would function as a marker of metabolic adjustment, optimizing the use of nutrients by this pathway, events that seem to be associated with the extension of yeasts lifespan (Lin et al., 2002; Smith et al., 2007). In the presence of a non-fermentative source, the respiratory condition was related to the increase of lifespan. This condition may be compared to the human cardiac muscle, where the respiratory condition is the only one performed and which provides a long lifespan to the muscle.

The *tor1*Δ strain is deficient for the Tor1 complex that in yeasts seems to be involved in the regulation of many cellular processes, including cell growth in face of nutrients availability and stress mechanisms (Powers et al., 2006). A series of factors seem to be involved in signaling the TOR pathway, amongst which are the transcription of enzymes involved in several metabolic pathways and the control of autophagy (Kamada et al., 2000; Raught et al., 2001; Cherra and Chu, 2008). This complex is inhibited by rapamycin, an immunosuppressive drug (Carracedo et al., 2008; Medvedik et al., 2007). When exposed to various carbon sources, the *tor1*Δ strain showed an increased survival rates for the respiratory condition (79%; Fig. 1B) and in caloric restriction condition for glucose (84%; Fig. 1B) and fructose (92%; Fig. 1B), respectively. It should be noted that the survival values observed for the condition of caloric restriction were higher in *tor1*Δ strain than those evidenced by the WT strain under the same conditions. In the fermentation condition tested, were a decrease in survival rates (56% and 68% for glucose and fructose, respectively; Fig. 1B). Nevertheless, it should be noted that these values were higher than the values observed for the WT strain in the fermentation condition. The results of CLS in *tor1*Δ strain (Figure 1B) show an increase in survival in all conditions. It has been reported that the inhibition of complex Tor1 seems to be involved in the increase of CLS in yeasts (Powers et al., 2006). An increase in survival rates for *tor1*Δ strain in condition of caloric restriction has been also noted suggesting that autophagy under these conditions might be maximized in relation to the fermentation condition. In this sense, data from literature show a link between the caloric restriction condition and an increase in autophagy associated with a decrease in aging rates (Shintani and Klionsky, 2004; Rubinsztein, 2007; Cherra and Chu, 2008).

The *pfk1*Δ strain is deficient for the α subunit of phosphofructokinase (Pfk), a key enzymatic complex of the glycolytic pathway in yeast cells. The phosphofructokinase complex is characteristically an oligomeric enzyme with four α subunits and four ß subunits (Kirchberger et al., 1999; Rodicio et al., 2000). The Pfk1p is a major enzyme related with the regulation of the glycolytic flux and catalyzes the third reaction of glycolysis, with the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate. (Kirchberger et al., 1999; Rodicio et al., 2000). In this sense, ours results showed that *pfk1*Δ strain have a better survival rates at higher glucose (65%; Fig. 1C) and fructose (43%; Fig. 1C) concentrations when compared to the WT strain. Under caloric restriction conditions, survival rates were lower (46% for glucose and 42% fructose, respectively; Fig. 1C) In addition, it was observed that *pfk1*Δ strain showed a significant decrease in survival rate (36%; Fig. 1C) in the presence of non-fermentative carbon source when compared to the WT strain (Fig. 1A).

A hypothesis that may explain the data gathered with *pfk1*Δ strain is the channeling of glycolytic intermediates to accumulate glycogen through the glycogenic pathway, which can be induced by the high concentrations of glucose-6-phosphate. The increase in glucose 6-phosphate for *pfk1*Δ and *pfk2*Δ mutant strains has already been evidenced in other studies (Pederson et al., 2004). Thus, instead of the monosaccharide being oxidized by the glycolytic pathway it is directed to the glycogenic pathway to form and accumulate glycogen. This hypothesis was confirmed by the positive results of the glycogen accumulation assay for the *pfk1*Δ strain under fermentation condition (Fig. 2). In conditions of caloric restriction, a possible explanation for the survival rates decreasing in might be due to the incidence of futile cycles. In starvation situations, the gluconeogenic pathway is activated and the carbon flow is focuses on forming glucose from other precursors such as pyruvate. However, at the same time as this metabolic

event takes place, the *pfk1*Δ strain presents a beta subunit of the enzyme phosphofructokinase (Pfk2p), which in yeasts has a catalytic site independent from Pfk1p and, therefore, imparts a capacity for oxidizing monosaccharides by the glycolytic pathway. Thus, the occurrence of glycolysis together with gluconeogenesis should explain the decrease in survival for *pfk1*Δ strain observed under these conditions. For the non-fermentative source, the survival rate observed was even smaller, and a possible explanation for this could also be related to futile cycles

The *pfk2*Δ strain is deficient for the β subunits of PFK complex (Heinisch et al., 1996; Kirchberger et al., 1999). This subunit catalyzes the conversion of fructose-6-phosphate to fructose-2,6-bisphosphate, the last an activator of the glycolytic pathway, also playing a regulatory role in the gluconeogenic pathway (Heinisch et al., 1996). The CLS assay indicated that the *pfk2*Δ strain shown survival rates considered high for all conditions studied. For higher monosaccharide concentrations, the rates were 60% and 79% for glucose and fructose, respectively (Fig. 1D). The survival rates for the condition of caloric restriction in glucose and fructose were 50% and 66%, respectively (Fig. 1D). For the non-fermentative source, the survival rate of 62% obtained is also considered high (Fig. 1D).

In general, the results obtained for *pfk2*Δ strain indicate a high survival rates for all conditions studied (over 50%). These results allow us to point out two aspects: (i) the differing survival response of the *pfk1*Δ and *pfk2*Δ strains, which show opposing results under the same conditions of study; (ii) a rather high survival rate for the *pfk2*Δ strain.

At first moment, these results might suggest an inhibition of the glycolytic pathway in the *pfk2*Δ strain and that this could be associated with a smaller production of AGEs and there would be an increase in survival rates (Kassi and Papavassiliou,

2008). However, more studies are needed to better explain this increase of survival in CLS of *pfk2*Δ strain.

By its turn, the *rad1*Δ strain is deficient for nucleotide excision repair (NER) system (Mitchell et al., 2003; Costa et al., 2003; Boiteux and Guillet, 2004; Park and Gerson, 2005), a pathway that appears to be associated with DNA repair induced by damages caused by carbohydrate metabolic intermediate products (Barea and Bonatto, 2008). The lack of such repair mechanism in face of the various concentrations and carbon sources resulted in a significant decrease in survival rates for the higher concentrations of the monosaccharides glucose (35%; Fig. 1E) and fructose (36%; Fig. 1E). Interestingly, the survival rates of *rad1*Δ in fermentative conditions were the lowest amongst all results obtained for the other strains. Under caloric restriction conditions, the survival rates are high for glucose (81%; Fig. 1E) and fructose (69%; Fig. 1E). For the non-fermentative source, the survival rate (85%; Fig. 1E) was highest when compared to other carbon sources. Thus, the results for *rad1*Δ strain allow us to infer the importance of the NER pathway in repairing DNA damages caused by AGEs.

3.2 Assays on glycogen accumulation in the different *Saccharomyces cerevisiae* strains used herein

Similarly as most organisms, the yeast *S. cerevisiae* uses glucose and fructose as its major energy and carbon source. The glucose inside in the cell is phosphorylated to glucose-6-phosphate, and then oxidized by glycolytic pathway. In this sense, glucose-6-phosphate may also be used in the pentose phosphate pathway and in the glucogenic pathway. Concerning the latter, glucose-6-phosphate may be converted to glycogen via UDP-glucose, where glycogen acts as energy reserve under famine conditions

(Pederson et al., 2004). The metabolism of fructose in yeast is also associated with the a presence of glucose-6-phosphate

In *S. cerevisiae* some studies have shown the importance of allosteric activation by glucose-6-phosphate on glycogen accumulation in cells, where the activation of the enzyme glycogen synthase by glucose-6-phosphate *in vivo* shows to be necessary for glycogen accumulation (Pederson et al., 2004).

We observed that the *pfk1*Δ and *pfk2*Δ strains has shown a higher incorporation of iodine in under fermentative condition. These results indicate a higher accumulation of the glycogen in both strains. For caloric restriction condition, all strains have shown similar results with low glycogen accumulation.

In this sense, the present study of CLS showed significant associations on the mechanism of aging and longevity in yeasts: (i) the diet appears to be a factor that factor that interferes in lifespan in yeast. (ii) the condition of caloric restriction (WT) and the induction of autophagy in *tor1*Δ, which seem to be related to an increase in lifespan. (iii) from the original results for the *pfk1*Δ strain, it was observed an incidence of interesting metabolic interactions, whereby glycogen accumulation seems to be the response for survival increase in the higher concentrations of monosaccharides. (iv) survival rates in the *rad1*Δ strain associate the important action of (NER) in protecting the products from carbohydrate metabolism.

6. REFERENCES

Barea, F., Bonatto, D., 2008. Relationships among carbohydrate intermediate metabolites and DNA damage and repair in yeast from a systems biology perspective. Mutat. Res. 642, 43-56.

Bitterman, K.J., Medvedik, O., Sinclair, D.A., 2003. Longevity regulation in Saccharomyces cerevisiae: linking metabolism, genome stability, and heterochromatin. Microbiol. Mol. Biol. 67, 376-399.

Boiteux, S., Guillet, M., 2004. Abasic sites in DNA: repair and biological consequences in Saccharomyces cerevisiae. DNA Repair (Amst).3:1-12. Review.

Carracedo, A.M.L., Teruya-Feldstein, J., Rojo. F., Salmena, L., Alimonti, A., Egia, A., Sasaki, A.T., Thomas, G., Kozma, S.C., Papa, A., Nardella. C., Cantley, L.C., Balsega. J., Pandolfi, P.P., 2008. Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. J. Clin. Invest. 118, 3065-3074.

Chen, Q., Ding, Q., Keller, J. N., 2005. The stationary phase model of aging in yeast for the study of oxidative stress and age-related neurodegeneration. Biogerontology. 6, 1-13.

Cherra, S.J., Chu, C.T., 2008. Autophagy in neuroprotection and neurodegeneration: A question of balance. Future Neurol. 3, 309-323.

Chester, V. E., 1968. Heritable glycogen-storage deficiency in yeast and its induction by ultra-violet light. J. Gen. Microbiol. 51, 49-56.

Costa, R.M., Chinganças, V., Galhardo, Rda. S., Carvalho, H., Menck, C.F., 2003. The eukaryotic nucleotide excision repair pathway.Biochimie. 85, 1083-1099. Review.

Hagopian, K., Ramsey, J.J., Weindruch, R., 2003. Influence of age and caloric restriccion on liver glycolytic enzyme activies and metabolite concentrations in mice. Exp. Gerontology 38, 253-266.

Hamada, Y., Araki, N., Koh, N., Nakamura, J., Horiuchi, S., Hotta, N., 1996. Rapid formation of advanced glycation end products by intermediate metabolites of glycolytic pathway and polyol pathway. Biochem. Biophys. Res. Commun. 228, 539-543.

Heinisch, J.J., Boles, E., Timpel, C., 1996. A yeast phosphofructokinase insensitive to the allosteric activator fructose 2,6-bisphosphate. Glycolysis/metabolic regulation/allosteric control. J. Biol. Chem. 271, 15928-15933.

Jazwinski, S.M., 2000. Metabolic mechanisms of yeast ageing. Exp. Gerontol. 35, 671-676.

Jazwinski, S.M., 2005. Yeast longevity and aging-the mitochondrial connection. Mech. Ageing Dev. 126, 243-248.

Kamada, Y., Funakoshi, T., Shintani, T., Nagano, K., Ohsumi, M., Ohsumi, Y., 2000. Tor-mediated induction of autophagy via an Apg1 protein kinase complex. J. Cell Biol. 150, 1507-1513.

Kassi, E., Papavassiliou, A.G., 2008. Could glucose be a proaging factor? J. Cell. Mol. Med. 12, 1194-1198.

Kirchberger, J., Edelmann, A., Kopperschläger, G., Heinisch, J.J., 1999. A single point mutation leads to an instability of the hetero-octameric structure of yeast phosphofructokinase. Biochem J. 341, 15-23.

Kitanovic, A., Wolfl, S., 2006. Fructose-1,6-bisphosphatase mediates cellular responses to DNA damage and aging in Saccharomyces cerevisiae. Mutat. Res. 594, 135-147.

Lin S.J., Kaeberlein M., Andalis A.A., Sturtz L.A., Defossez P.A., Culotta V.C., Fink G.R., Guarente L. 2002. Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration**.** Nature. 418, 344-348.

Medvedik, O., Lamming, D.W., Kim, K.D., Sinclair, D.A., 2007. MSN2 and MSN4 link calorie restriction and TOR to sirtuin-mediated lifespan extension in Saccharomyces cerevisiae. PLoS Biol. 5, 2330-2341.

Mitchell, J.R**.,** Hoeijmakers, J.H**.,** Niedernhofer, L.J**.,** 2003. .Divide and conquer: nucleotide excision repair battles cancer and ageing. Curr Opin Cell Biol. 15, 232-240.

Park, Y., Gerson, S.L., 2005. DNA repair defects in stem cell function and aging. Annu. Rev. Med. 56, 495-508.

Pederson, B.A., Wilson, W.A., Roach, P.J., 2004. Glycogen synthase sensitivity to glucose-6-P is important for controlling glycogen accumulation in Saccharomyces cerevisiae. J. Biol. Chem. 279, 13764-13768.

Powers, R.W., Kaeberlein, M., Caldwell, S.D., Kennedy, B.K., Fields, S., 2006. Extension of chronological life span in yeast by decreased TOR pathway signaling. Genes Dev. 20, 174-184.

Qin, H., Lu, M., 2006. Natural variation in replicative and chronological life spans of Saccharomyces cerevisiae. Exp. Gerontol. 41, 448-456.

Shintani, T., Klionsky, D.J., 2004. Autophagy in health and disease: a double-edged sword. Science 306, 990-995.

Smith, D.L.Jr., Mc Clure, J.M., Materic, M., Smith, J.S., 2007. Calorie restricion extends chronological lifespan of Saccharomyces cerevisiae independently of the Sirtuins. Aging Cell 6, 649-662.

Raught, B., Gingras, A.C., Sonenberg, N., 2001. The target of rapamycin (TOR) proteins. Proc. Natl. Acad. Sci. U. S. A. 98, 7037-7044.

Reverter-Branchat, G., Cabiscol, E., Tamarit, J., Ros. J., 2004. Oxidative damage to specific proteins in replicative and chronological-aged Saccharomyces cerevisiae: common targets and prevention by calorie restriction. J. Biol. Chem. 279, 31983-31989.

Rodicio, R., Strauss, A., Heinisch, J.J., 2000. Single point mutations in either gene encoding the subunits of the heterooctameric yeast phosphofructokinase abolish allosteric inhibition by ATP. J. Biol. Chem. 275, 40952-40960.

Rubinsztein, D.C., 2007. Autophagy induction rescues toxicity mediated by proteasome inhibition. Neuron. 54, 854-856.

Ying, W., 1997 Deleterious network hypothesis of aging. Med. Hypotheses 48, 143-148.

Yin, D., Chen, K., 2005. The essential mechanisms of aging: Irreparable damage accumulation of biochemical side-reactions. Exp. Gerontol. 40, 455-465.

Table 1. *S. cerevisiae* strains used in this study.

Strains	Genotype	Reference
BY4741	BY4741; *Mat* a; *his3*Δ1; *leu2*Δ0; *met15*Δ0; *ura3*Δ0	Euroscarf
*pfk1*Δ	Isogenic da BY4741, except *pfk1::kanMX4*	Euroscarf
*pfk2*Δ	Isogenic da BY4741, except *pfk2::kanMX4*	Euroscarf
*rad1*Δ	Isogenic da BY4741 except *rad1::kanMX4*	Euroscarf
*tor1*Δ	Isogenic da BY4741 except *tor1::kanMX4*	Euroscarf

Figure legends

Figure 1. Survival of WT (A), *tor1*Δ (B), *pfk1*Δ (C), *pfk2*Δ (D) and *rad1*Δ (E) strains in different carbon sources after 12 days. Errors bars represent the individual standard error for each carbon source.

Figure 2. Glycogen accumulation assays for the WT, *rad1*Δ. *tor1*Δ, *pfk1*Δ e *pfk2*Δ strains for different carbon sources (indicated at right below) after two days of culture at 28°C. Dark colonies indicate a high amount of stored glycogen in the cell.

Fig. 1.

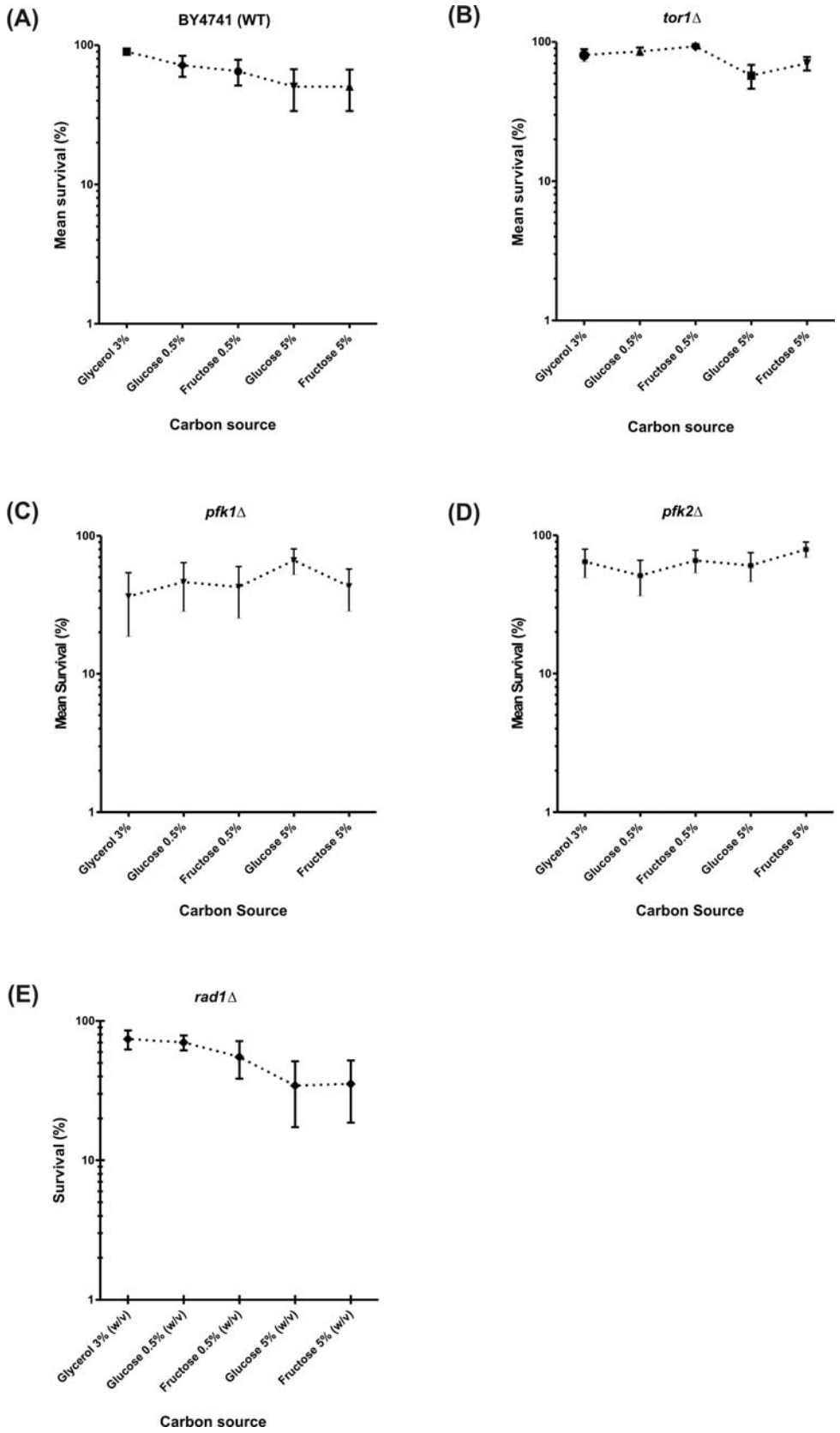
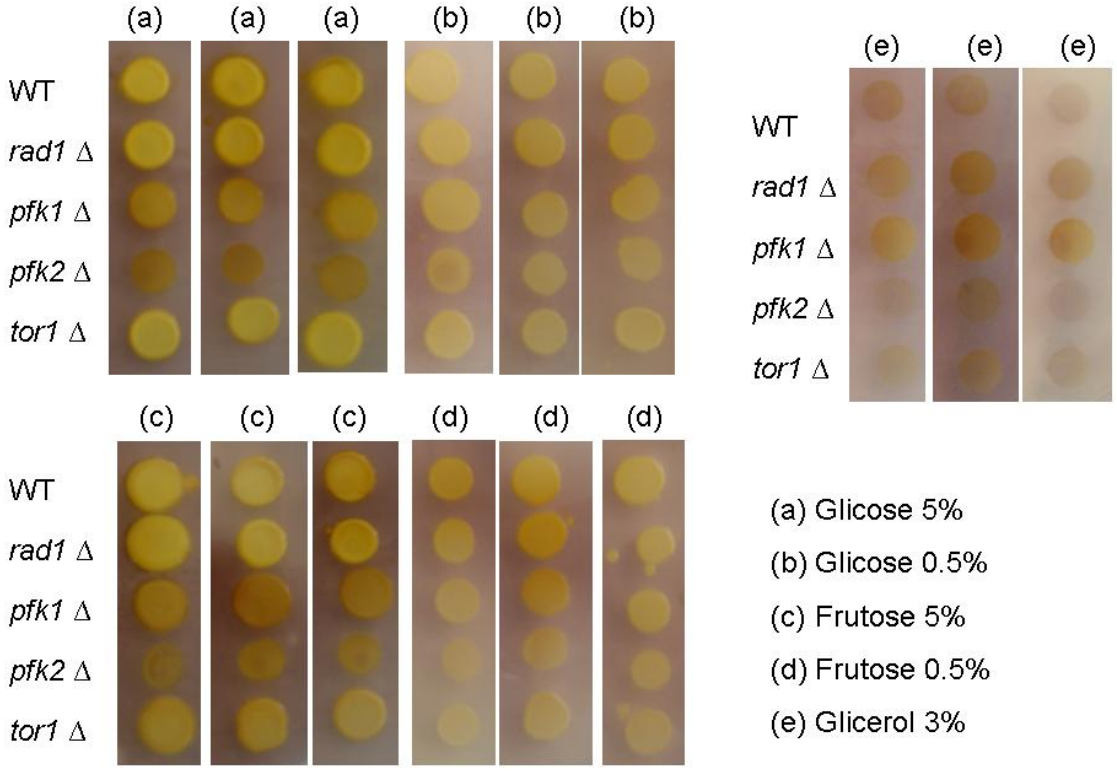

(A)
BY4741 (WT)
Mean survival (%)
100
10
1
Glycerol 3%
Glucose 0.5%
Fructose 0.5%
Glucose 5%
Fructose 5%
Carbon source
(B)
tor1Δ
Mean survival (%)
Carbon source
(C)
pfk1Δ
Mean Survival (%)
Carbon Source
(D)
pfk2Δ
Mean Survival (%)
Carbon Source
(E)
rad1Δ
Survival (%)
Glycerol 3% (w/v)
Glucose 0.5% (w/v)
Fructose 0.5% (w/v)
Glucose 5% (w/v)
Fructose 5% (w/v)
Carbon source

Fig. 2.


(a) (a) (a) (b) (b) (b)
WT
rad1 Δ
pfk1 Δ
pfk2 Δ
tor1 Δ
(e) (e) (e)
WT
rad1 Δ
pfk1 Δ
pfk2 Δ
tor1 Δ
(c) (c) (c) (d) (d) (d)
WT
rad1 Δ
pfk1 Δ
pfk2 Δ
tor1 Δ
(a) Glicose 5%
(b) Glicose 0.5%
(c) Frutose 5%
(d) Frutose 0.5%
(e) Glicerol 3%

DISCUSSÃO GERAL

A glicose e a frutose são monossacarídeos bastante comuns na dieta ocidental e são considerados a principal fonte de energia utilizada pelos organismos (Hagopian *et al*., 2005; Barea & Bonatto, 2008). Nos últimos anos, tem-se observado um aumento bastante significativo na ingestão destes compostos, principalmente pela sua ampla utilização na fabricação de alimentos processados (Gaby, 2005; Hagopian *et al.*, 2005; Levi & Werman, 1998; Barea & Bonatto, 2008). Esse aumento de consumo tem sido associado com uma maior ocorrência de patologias com caráter multimetabólico (Miller & Adeli, 2008) juntamente com as doenças neurodegenerativas que caracterizam o envelhecimento, como o Parkinson e o Alzheimer (Thorpe & Baynes, 2003; Nass *et al.*, 2007; Xanthis *et al*., 2007, Huebschmann *et al.*, 2006; Hartog *et al.*, 2007; Grillo & Colombatto, 2007).

Tendo em vista estas associações, as mudanças na concentração dos monossacarídeos glicose e frutose na dieta poderiam interferir na longevidade de um organismo unicelular? Nossos resultados evidenciaram que sim, e a condição de restrição calórica mostrou estar associada com o aumento de sobrevivência para as condições testadas. É importante salientar que os dados obtidos nesta dissertação também foram encontrados de uma forma similar por outros autores (Barros *et al.*, 2004; Reverter-Branchat *et al.*, 2004; Smith *et al.*, 2007).

Em leveduras, os ensaios de idade cronológica conduzidos por Barros *et al.* (2004) e por Reverter-Branchat *et al.* (2004) resultaram no aumento do ciclo de vida para a condição de restrição calórica [0,5% de glicose (p/v)] quando comparada com as condições usuais de 2% (p/v) de glicose. Nos dois trabalhos citados, estes resultados foram relacionados com a atividade respiratória mitocondrial, e as mudanças encontradas nas taxas respiratórias foram consideradas responsáveis pela extensão do

ciclo de vida. Estas mudanças foram relacionadas com a menor formação das espécies reativas de oxigênio (ERO) e/ou com o aumento da atividade das defesas antioxidantes. Seguindo nesta linha de raciocínio, Powers *et al.* (2006) relatam uma quantidade mínima de genes envolvidos com o envelhecimento na idade cronológica de leveduras. Dentro destes, estaria a superóxido dismutase e/ou catalase, onde o aumento da expressão de ambos os genes estariam envolvidos com o aumento de idade cronológica. A deleção, por usa vez, estaria associada a uma diminuição bastante aumentada. Estas evidências mostram a importância que o estresse oxidativo parece representar para a idade cronológica.

Nos ensaios de idade cronológica realizados por Smith *et al.* (2007), a linhagem selvagem BY4741 foi submetida a diferentes fontes de monossacarídeos sob condição ou não de restrição calórica. Os resultados encontrados por este trabalho apontaram uma relação positiva entre a condição de restrição calórica e a extensão do ciclo de vida em leveduras, muito similar aos que foram encontrados por este trabalho (Capítulo III). Para estes casos, os dados da literatura mostram que as mutações e/ou a diminuição dos níveis de proteínas cinases como a Sch9 e PKA, reguladas pela presença de glicose, aparecem associadas com a extensão da idade cronológica em leveduras (Lin *et al.*, 2002; Bitterman *et al.*, 2003; Powers *et al.*, 2006; Madia *et al.*, 2008; Wei *et al.*, 2008). Na linhagem BY4741 crescida em fonte não fermentável [glicerol 3% (p/v)] Smith *et al*., (2007) observou uma extensão da idade cronológica superior aos valores do controle [2% (p/v) de glicose]. Da mesma forma, estes resultados foram similares aos de nosso estudo (Capítulo III), onde a condição de respiração mostrou-se a melhor para a manutenção da longevidade na linhagem WT. Estes dados suportam as inferências de que uma das possíveis causas responsáveis pelo aumento de longevidade na condição de respiração (restrição calórica e fonte não fermentável; Capítulo III) seja causada por

trocas no balanço de respiração que alteram, assim, a produção energética e o estado metabólico das células (Lin *et al.,* 2002).

Por outro lado, nosso estudo evidenciou uma diminuição do tempo cronológico em leveduras frente a uma dieta rica em açúcares. Uma das relações que explica tais resultados é a ocorrência dos produtos finais de glicação avançada (AGEs) formados pelo metabolismo de carboidratos (Capítulos I e II). Tendo em vista este aspecto, procuramos obter resultados de idade cronológica para a ausência de enzimas regulatórias da via glicolítica (Pfk1p e Pfk2p). Neste caso, nosso objetivo era verificar como a diminuição e/ou bloqueio da via glicolítica, associada com a formação dos AGEs, poderia interferir no ciclo de vida cronológico de leveduras. Sendo assim, nossos dados (Capítulo III) permitiram inferir associações e interações muito interessantes a respeito da ocorrência de diferentes vias metabólicas para as altas concentrações de monossacarídeos nas linhagens *pfk1*Δ e *pfk*2Δ.

Em humanos a ausência da isoenzima regulatória PFK1-M, encontrada na musculatura, caracteriza a síndrome conhecida como glicogenose do tipo VII ou doença de Tarui (Nichols *et al*., 1996; Ristow *et al*., 1997). Esta patologia aparece associada com o metabolismo de glicose, onde uma série de manifestações são descritas nos portadores desta síndrome, tais como um aumento na concentração intracelular de glicogênio e a diminuição da secreção de insulina (Ristow *et al*., 1997; Vora *et al*., 1987). Em leveduras, nosso estudo evidenciou um aumento do ciclo de vida para as concentrações de 5% (p/v) de monossacarídeos, quando comparadas com as condições de respiração nas linhagens *pfk1*Δ e *pfk*2Δ (Capítulo III). Estes resultados foram explicados pela acumulação de glicogênio observada nestas linhagens para as altas concentrações de monossacarídeos. Estas evidências possibilitaram inferir sobre a ocorrência de um mecanismo similar ao que ocorre nos portadores da doença de Tarui,

onde há o redirecionamento da via glicolítica para a glicogênese. A diminuição da idade cronológica observada para as condições de respiração nas linhagens *pfk1*Δ e *pfk*2Δ pode ser associada com a fisiologia observada nos portadores da síndrome de Tarui, que apresentam um significativo comprometimento do metabolismo aeróbico para condições de esforço físico (Nichols *et al*., 1996; Smith *et al*., 1996).

A presença dos AGEs, formados pelo metabolismo de carboidratos aparece envolvido também com a ocorrência de inúmeros danos no DNA e parece afetar a integridade do genoma de leveduras (Capítulo II). O número mínimo de estudos associando os AGEs com os danos genômicos, considerando a falta de dados quanto aos aspectos genotóxicos e/ou mutagênicos provocados por estes produtos, despertou o nosso interesse. Nesse sentido, os resultados práticos de idade cronológica para a linhagem *rad1*Δ (Capítulo III) indicaram uma direta associação entre a utilização da via de excisão de nucleotídeos (NER) e o metabolismo de carboidratos. Portanto, a via NER foi caracterizada em nosso estudo como um importante mecanismo que pode ser utilizado para a reparação de danos induzidos por AGEs no genoma de leveduras. Estes dados suportam os resultados obtidos pelo estudo de Biologia de Sistemas (Capítulo II).

O aumento da longevidade, nos estudos de idade cronológica em leveduras, também aparece associado com a inibição do complexo TOR1 (Bonawitz *et al.,* 2007; Medvedik *et al.,* 2007). Neste sentido, estudos mostram que a inibição do complexo TOR1 interfere na regulação da autofagia, e a ausência deste complexo aparece como um indutor deste mecanismo (Schmelzle *et al.,* 2004; Floto *et al.,* 2007). Sendo assim, nossos resultados para a linhagem *tor1*Δ mostraram médias de sobrevivência bastante aumentados para todas as condições. Estes índices foram associados com o aumento do tempo cronológico e nesse caso, parecem indicar que o processo de autofagia maximizado nesta linhagem mutante de leveduras aparece relacionado com o aumento

do ciclo de vida (Yorimitsu *et al*., 2007). Estes resultados vão de acordo com os encontrados por Powers *et al*. (2006), que mostraram o aumento da idade cronológica em linhagens de leveduras que apresentavam mutações associadas com a diminuição da atividade do complexo TOR1. Sendo assim, os dados encontrados por este trabalho (Capítulo III) ajudam a evidenciar a via TOR1 como um importante regulador do envelhecimento em leveduras, podendo ser considerada como um dos mecanismos responsáveis pela determinação das taxas de envelhecimento dos organismos.

CONCLUSÕES

CONCLUSÃO GERAL

Pelos resultados de idade cronológica e de Biologia de Sistemas realizados na levedura *Saccharomyces cerevisiae,* pode-se inferir que a dieta composta pelos monossacarídeos glicose e frutose exercem uma significativa influência nas médias de sobrevivência deste organismo. Neste sentido, a concentração aumentada destes monossacarídeos parece estar associada com a diminuição das médias de sobrevivência e a condição de restrição calórica, por sua vez, aparece relacionada com o aumento destas médias.

CONCLUSÕES ESPECÍFICAS

- Uma das possíveis explicações para a diminuição do tempo cronológico observado na dieta composta por 5% (p/v) de glicose e frutose na linhagem WT parece ser a formação aumentada de AGEs pelo metabolismo de carboidratos.
- Para as condições de respiração [restrição calórica 0,5% (p/v) e glicerol 3% (v/v)] houve aumento das médias de sobrevivência, sendo estas associadas com a extensão do ciclo de vida.
- Na linhagem *pfk1*Δ a via metabólica direcionada para o acúmulo de glicogênio parece ser a responsável pela condição de longevidade encontrada para as maiores concentrações de monossacarídeos 5% (p/v).
- A ausência do complexo Tor1, no ensaio de idade cronológica de *S. cerevisiae,* exerce influência positiva para a longevidade em todas as condições estudadas.

- A via de excisão de nucleotídeos (NER), representando neste estudo pela Rad1p, parece exercer um importante papel na reparação de danos causados ao DNA pelo metabolismo de carboidratos em leveduras.

PERSPECTIVAS

Antes de ser submetido, ainda será realizado no terceiro capítulo:

- Construção de linhagens duplo e triplo mutantes para diferentes proteínas relacionadas com o metabolismo de carboidratos e para as vias de reparação de DNA.
- Medição de proteínas carboniladas por métodos espectrofotométricos e imunoensaios.

PERSPECTIVAS

Além da *Saccharomyces cerevisiae*, o nematódeo *Caenorhabditis elegans* vem sendo amplamente utilizado nos estudos que envolvem a compreensão dos mecanismos genéticos da longevidade e do envelhecimento, sendo assim este organismo vem sendo considerado um modelo ideal tanto bioquímico quanto molecular de estudo.

Desta maneira, seguem como perspectivas futuras:

- Utilizar o nematódeo *C. elegans* para dar continuidade a este trabalho, buscando avaliar por meio de ferramentas de Biologia de Sistemas e dos dados de análise proteômica como algumas das principais proteínas relacionadas à longevidade, ao metabolismo de carboidratos e à manutenção da integridade genômica trabalham em conjunto para a indução de tolerância e reparação de danos originados a partir da formação de ERCs e dos produtos finais de glicação avançada.
- Utilizar a técnica de RNA interferente combinatório para a construção de linhagens mutantes de *C. elegans* relacionadas aos mecanismos genéticos da longevidade, à manutenção da cromatina, ao metabolismo de carboidratos e à proteção contra os produtos finais de glicação avançada.
- Avaliar o tempo de vida cronológico por meio da alteração de padrões fisiológicos nas linhagens selvagens e mutantes de *C. elegans* a partir do seu cultivo em diferentes tipos de dietas.
- Verificar os níveis de proteínas glicadas e a indução de quebras simples e duplas de DNA nas diferentes linhagens de *C. elegans* cultivadas em diferentes dietas.

REFERÊNCIAS COMPLEMENTARES

Barea F. & Bonatto D. (2008). Relationships among carbohydrate intermediate metabolites and DNA damage and repair in yeast from a systems biology perspective. Mutat. Res. 642: 43-56.

Barros, M.H.; Bandy, B.; Tahara E.B.; Kowaltowski, A.J. (2004). Reactive Oxygen Relesase Increased Life Span in *Saccharomyces cerevisiae*. J. Biol.Chem. 279: 49883-49888.

Baynes J.W. (2000). From life to death-the struggle between chemistry and biology during aging: the Maillard reaction as an amplifier of genomic damage. Biogerontology. 1:235-246.

Bitterman, K.J.; Medvedik, O.; Sinclair, D.A. (2003). Longevity regulation in Saccharomyces cerevisiae: linking metabolism, genome stability, and heterochromatin. Microbiol. Mol. Biol. 67: 376-399.

Bonawitz N.D.; Chatenay-Lapointe M.; Pan Y.; Shadel G.S. (2007). Reduced TOR signaling extends chronological life span via increased respiration and upregulation of mitochondrial gene expression. Cell Metab. 5:265-277.

Dutta U.; Cohenford M.A.; Dain J.A**.** (2005). Nonenzymatic glycation of DNA nucleosides with reducing sugars. Anal Biochem. 345:171-180.

Floto R.A.; Sarkar S.; Perlstein E.O.; Kampmann B.; Schreiber S.L.; Rubinsztein D.C. (2007) Small molecule enhancers of rapamycin-induced TOR inhibition promote autophagy, reduce toxicity in Huntington's disease models and enhance killing of mycobacteria by macrophages. Autophagy. 3:620-622.

Gaby A.R. (2005). Adverse effects of dietary fructose. Altern Med Rev. 10:294-306.

Grillo M.A. & Colombatto S. (2007) Advanced glycation end-products (AGEs): involvement in aging and in neurodegenerative diseases. Amino Acids.35:29-36.

Gugliucci A. (2000) Glycation as the glucose link to diabetic complications. J. Am. Osteopath. Assoc.100:621-634.

Hagopian, K.; Ramsey J.J.; Weindruch R. (2005). Fructose metabolizing enzymes from mouse liver: influence of age and caloric restriction. Biochim Biophys Acta. 1721: 37-43.

Hartog J.W.; Voors A.A.; Bakker S.J.; Smit A.J.; van Veldhuisen D.J. (2007) Advanced glycation end-products (AGEs) and heart failure: pathophysiology and clinical implications. Eur J Heart Fail. 9:1146-1155.

Huebschmann A.G.; Regensteiner J.G.; Vlassara H.; Reusch J.E. (2006) Diabetes and advanced glycoxidation end products. Diabetes Care. 29: 1420-1432.

Levi B. & Werman M.J. (1998). Long-term fructose consumption accelerates glycation and several age-related variables in male rats. J Nutr. 128:1442-1449.

Levi B. & Werman M.J. (2001). Fructose triggers DNA modification and damage in an Escherichia coli plasmid**.** J. Nutr. Biochem. 12:235-241.

Lin S.J.; Kaeberlein M.; Andalis A.A.; Sturtz L.A.; Defossez P.A.; Culotta V.C.; Fink G.R.; Guarente L.(2002). Calorie restriction extends *Saccharomyces cerevisiae* lifespan by increasing respiration**.** Nature. 418:344-348.

Madia F.; Gattazzo C.; Wei M.; Fabrizio P.; Burhans W.C.; Weinberger M.; Galbani A.; Smith J.R.; Nguyen C.; Huey S.; Comai L.; Longo V.D. (2008). Longevity mutation in SCH9 prevents recombination errors and premature genomic instability in a Werner/Bloom model system. J Cell Biol. 180:67-81.

Medvedik O.; Lamming D.W.; Kim K.D.; Sinclair D.A. (2007) MSN2 and MSN4 link calorie restriction and TOR to sirtuin-mediated lifespan extension in *Saccharomyces cerevisiae.* PLoS Biol.5:e261.

Miller A.; Adeli K. (2008). Dietary fructose and the metabolic syndrome. Curr Opin Gastroenterol. 24:204-209.

Nass N.; Bartling B.; Navarrete Santos A.; Scheubel R.J.; Börgermann J.; Silber R.E.; Simm A. (2007). Advanced glycation end products, diabetes and ageing. Z Gerontol Geriatr. 40:349-356.

Nichols, R.C.; Rudolphi, O.; Ek, B.; Exelbert, R.; Plotz, P.H.; Raben, N. (1996) Glycogenosis type VII (Tarui disease) in a Swedish family: two novel mutations in muscle phosphofructokinase gene (PFK-M) resulting in intron retentions. Am J Hum Genet. 59:59-65.

Powers, R.W.; Kaeberlein, M.; Caldwell, S.D.; Kennedy, B.K.; Fields, S. (2006). Extension of chronological life span in yeast by decreased TOR pathway signaling. Genes Dev. 20: 174-184.

Ristow, M.; Vorgerd, M.; Möhlig, M.; Schatz, H.; Pfeiffer, A. (1997) Deficiency of phosphofructo-1-kinase/muscle subtype in humans impairs insulin secretion and causes insulin resistance. J Clin Invest. 100:2833-2841.

Schmelzle, T.; Beck, T.; Martin, D.M.; Hall, M.N. (2004). Activation of the RAS/Cyclic AMP Pathway Supresses a TOR Deficiency in Yeast. Mol Cell Biol. 24: 338-351.

Singh R.; Barden A.; Mori T.; Beilin L**.** (2001). Advanced glycation end-products: a review. Diabetologia. 2:129-146.

Smith, B.F.; Stedman, H. Rajpurohit, Y.; Henthorn, P.S.; Wolfe J.H.; Patterson, D.F.; Giger, U. (1996). Molecular basis of canine muscle type phosphofructokinase deficiency. J Biol Chem. 271:20070-20074.

Smith, D.L.Jr.; Mc Clure J.M.; Materic M.; Smith, J.S. (2007). Calorie restricion extends chronological lifespan of *Saccharomyces cerevisiae* independently of the Sirtuins. Aging cell. 6: 649-662.

Reverter-Branchat, G.; Cabiscol, E.; Tamarit, J.; Ros J. (2004). Oxidattive damage to specific proteins in replicative and chronological-aged *Saccharomyces cerevisiae*. J. Biol. Chem. 279: 31983-31989.

Thorpe, S.R. & Baynes J.W**.** (2003). Maillard reaction products in tissue proteins: new products and new perspectives. Amino Acids. 3:275-281.

Vora, S.; DiMauro, S.; Spear, D.; Harker, D.; Danon, M.J. (1987). Characterization of the enzymatic defect in late-onset muscle phosphofructokinase deficiency. New subtype of glycogen storage disease type VII. J Clin Invest. 80:1479-1485.

Xanthis A.; Hatzitolios A.; Koliakos G.; Tatola V. (2007).Advanced glycosylation end products and nutrition-a possible relation with diabetic atherosclerosis and how to prevent it. J Food Sci. 72:125-129.

Yorimitsu T.; Zaman S.; Broach J.R.; Klionsky D.J. (2007). Protein kinase A and Sch9 cooperatively regulate induction of autophagy in Saccharomyces cerevisiae. Mol Biol Cell. 10:4180-4189.

Wei M.; Fabrizio P.; Hu J.; Ge H.; Cheng C.; Li L.; Longo V.D. (2008). Life span extension by calorie restriction depends on Rim15 and transcription factors downstream of Ras/PKA, Tor, and Sch9. PLoS Genet. 4:e13.