Review article
The impact of dietary restrictions on the expression of FOXO3 as an anti-ageing biomarker

Muhammad Alifian Remifta Putra¹, Radiana Dhewayani Antarianto², Novi Silvia Hardiany³

¹Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
²Department of Histology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
³Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

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Corresponding author: Novi Silvia Hardiany. Email: novi.silvia@ui.ac.id

ABSTRACT
Ageing involves destruction on the molecular, cellular, and organ level, causing disease and death. Previous research has discovered that both diet and genetics can affect ageing. Forkhead helix box (FOXO) transcription factors are found to be the major transcription factors correlated with longevity. In addition, one of the best-known signals that are also capable to affect ageing is a dietary restriction (DR) which is evolutionary conserved. The objective of this paper is to expand our knowledge regarding the FOXO3 gene expression as anti-ageing biomarker, and how DR could impact its expression. This review was written through a rigorous searching process from various databases including Google Scholar, Science Direct, Scopus, and PubMed, by utilizing keywords such as the ‘anti-ageing’, ‘dietary restriction’ along with ‘forkhead box O3 gene’. FOXO3 exerts its essential role through the protein kinase B (Akt/PKB) signaling pathway, as well as post-translational modifications, further activating downstream target genes. This would result in cell apoptosis, cell cycle arrest, DNA repair, and other important physiological processes. Relating to diet, the FOXO3 gene is expressed when organisms face low nutrient availability. It can also be downregulated by reducing the amount of circulating insulin-like growth factor 1 (IGF-1) level which can be achieved through fasting. Meanwhile, DR can enhance the FOXO3 expression, anti-ageing effects and further establish longevity. However, the research regarding the effect of DR in humans is still limited. Future studies need to investigate the optimal type of DR along with uncovering the molecular mechanism behind the FOXO3 expression and ageing.

Keywords: Forkhead box O3 gene; dietary restriction; anti-ageing

INTRODUCTION
Ageing is an intricate process resulting from the accumulation of various factors, including destruction on the molecular, cellular, and organ level, which lead to a loss of function and a higher chance of suffering diseases, ultimately leading to death (1). To date, the percentage of older people (i.e., aged 60 or above) is continuing to increase globally with the number being expected to double from 12% in 2015 to 22% by 2050. Further, the oldest-old group, which refers to people aged 80 or beyond, is expected to triple in size in the same time period (2). Despite the complexity of the ageing process, it has been found that diet and genetic changes are able to significantly increase the longevity of an organism (3). First, genetically speaking, many mutations can increase longevity, affecting nutrient-sensing or stress-response genes. One of the genes that has now been extensively studied and shows a promising correlation with longevity is the forkhead transcription factor of the O class (FOXO) gene, which is activated by upstream signals when an organism is facing low nutrient availability or starvation. The FOXO gene is essential for longevity as it plays a role in regulating multiple target genes that have roles in regulating metabolic balance and energy metabolism (4). Based on findings of many studies, FOXOs have an essential role in various cellular processes. FOXO transcription factor, for example, is able to either activate or inhibit downstream target genes that are able to induce different cellular processes. A member of the FOXO subfamily, FOX3, is able to respond to stimuli—such as stress resulting from changes in metabolism, oxidation, and reductions in insulin and growth factor number—further creating a response of anti-ageing; it achieves this by facilitating pivotal cell function, for instance, stem cell homeostasis, differentiation, cell apoptosis regulation and reactive oxygen species (ROS) destruction (5). Also, both the FOXO3 and FOXO1 subfamily proteins control the pivotal process of gluconeogenesis, which provides the sole energy for the brain, erythrocytes, nervous system, and testes during fasting, which is one form of dietary restriction (DR) (6). These processes have a protective function for organisms in relation to many diseases; thus, it can be concluded that FOXO3 gene expression beneficially affects ageing by regulating various cellular processes that serves as anti-ageing mechanisms (5).

Second, in terms of dietary changes, it has been found that nutrient and stress sensors are able to mediate lifespan extensions by allowing organisms to adapt to various environmental and physiological signals. This leads to the selective investment of energy into various protective systems, minimising the damage that can...
Forkhead helix box (FOX) gene: definition, structure, and classification of the FOX gene

The FOX gene is categorized into the forkhead family of transcription factors, and it mainly relates to the function of apoptosis by triggering the expression of genes necessary for cell death. FOX proteins produced by the FOX gene are a group of transcription factors that are conserved from evolutionary processes, and they play the important role of being a major integration hub for various important cellular stimuli (6). The main characteristic of the FOX gene is the forkhead/winged helix-turn-helix (HTH) DNA-binding domain, which is composed of three α-helices along with two loops, known as ‘wing domains’. Even though the forkhead DNA-binding domain contains approximately a hundred amino acid residues; as these are highly conserved, the other domains of the FOX are very divergent in FOX proteins, meaning that they can have very different cellular effects and binding specificities (7). FOX proteins regulate diverse biological processes, including normal homeostasis and development; through phosphoinositide 3-kinase (PI3K)/Akt, FOXO3 is able to perform biological processes that are essential to increasing lifespan, such as protein turnover, substrate metabolism, and cell death and survival (8).

The FOXO3 protein (i.e., FOXO3, FOXO3a, or FKHRL1) is 1 of about 40 FOX transcription factors expressed within the mammalian genome (9). As a member of the FOXO subfamily, chromosome 6q21 becomes the location of the FOXO3 gene, and it plays a major role in regulating diverse cellular processes by targeting the activity and expression of effector genes (10). FOXO3a subcellular localization is important in determining its functions and activities. FOXO3a phosphorylation induces translocation process from the nucleus into the cytoplasm, and it is further associated with 14-3-3 proteins, ultimately blocking re-entry into the nucleus (11).

Function and regulation of the FOXO3 gene

The FOXO family was discovered to play a role in developmental processes, energy metabolism, and tumorigenesis in different tissues. All these functions could occur with specific activation from a coordinated transcriptional program. Deregulation occurring through FOXO functions could cause a build-up of DNA damage and uncontrolled cell proliferation, leading to carcinogenesis as one of the consequences (12).

FOXO3a is the main transcription factor that facilitates multiple pathological and physiological processes by triggering target genes’ transcription involved in proliferation, apoptosis, survival, cell-cycle progression, and DNA damage (8). The FOXO3a gene family also has an essential role in responding to various cellular stresses, such as oxidative stress and ultraviolet (UV) irradiation (13). FOXO3a is also tightly associated with the longevity of humans and the regulation of the autophagy process in cancer and muscle cells. Disruption in normal regulation of FOXO3a expression can cause various diseases, especially cancer. Meanwhile, the FOXO3a overexpression mainly inhibits tumorigenesis, such as in breast cancer (14).

This review was conducted by gathering and further analysing articles from several scientific journals. The data sources were filtered from several online databases, namely: Science Direct, Scopus, PubMed, and Google Scholar. The initial search from four databases yielded 167 studies and from these, some papers were excluded due to not passing the inclusion criteria by conducting abstract readings of the authors. Only 20 papers were further analysed to identify the effect of DR on FOXO3 gene expression. In the end, 11 papers were retrieved and satisfyingly meet the objective and eligibility criteria. Further details for the 11 papers were 5 papers discussing the effect of DR within the lower-level organism, 2 papers on the high-level organism, and 4 papers discussing the expression FOXO3 gene on humans and how DR affect its function. The keywords used in this review include the following: ‘forkhead box O3 gene’ AND ‘dietary restriction’ AND ‘anti-aging’. The beginning of this review discussed the forkhead helix box (FOX) gene in detail, starting with its definition, structure, and classification. Next, it discussed the function and regulation of the FOX gene, along with its role as an ageing biomarker. The second part mainly discussed DR, including its definition, function, and effect on FOXO3 gene expression, along with the impact of fasting on the ageing process.
Post-translational modifications (PTMs) are fundamental for proteins’ function regulation, as these lead to changes in subcellular location, DNA-binding affinity, molecular half-life, and/or interactions with different cellular proteins. Major PTMs that are commonly found include the process of phosphorylation, methylation, acetylation, sumoylation, neddylation, ubiquitination, sulphation, glycosylation, and prenylation. FOXO3a activity itself is mainly modulated by various types of PTMs, such as the processes of acetylation, methylation, phosphorylation, and ubiquitination. These processes of reversible PTMs can alter FOXO3a translocation, which subsequently causes changes in DNA-binding affinity and pattern changes in the transcriptional activity located at specific sites of target genes. These FOXO3a modifications occur with the help of various enzymes combined with many signaling molecules (15,16).

The main mechanism of FOXO3a activity regulation and its genes that are targeted depends on the translocation control of FOXO3a between the cytoplasm and nucleus, which can be performed using the phosphorylation activity of a series of kinases. The balance of nuclear export and import is very important in order to maintain proper FOXO3a function. The initiation and further progression of various diseases, including cancer, are caused mainly by the disruption of this balance (17).

**Fig. 1:** Cellular processes that are maintained by FOXOs play an important role as homeostatic regulators, especially in response to stress, further affecting lifespan and ageing (8).
Role of the FOXO3-gene as a biomarker in anti-ageing processes

FOXO3 is one of the few genes that is associated with human longevity, proven by the genetic variants of FOXO3 (shown in Figure 1) being correlated with exceptional longevity in many levels of organisms, such as worms, flies, and mammals (8). Stimuli—such as stress that comes from changes in metabolism, oxidation, and reduction in insulin and growth factor numbers—are able to induce FOXO3 to create the response of anti-ageing, by facilitating pivotal processes including DNA repair, stopping the cell cycle, stem cell homeostasis, immunity system modulation, differentiation, cell apoptosis regulation, redox balance, and ROS destruction. All these processes have one similarity: they perform a protective function in relation to many diseases, such as type 2 diabetes, cardiovascular disease, cancer, and neurological problems. Therefore, FOXO3 has a beneficial effect by regulating various cellular processes, serving as an anti-ageing mechanism (8).

The main role of FOXO3 in ageing mainly occurs via maintaining stem cell homeostasis of crucial cells, such as brain, blood, and skeletal muscle cells. For example, in skeletal muscle stem cells, known as ‘satellite cells’, FOXO3 induces an increase in stem cell self-renewal by activating Notch signaling and maintaining satellite cell pools that have divided but remain in an undifferentiated state. In addition, FOXO proteins also induce senescence and cell cycle arrest during cellular damage, while independently causing stemness signaling to be repressed. Therefore, FOXO3 genetic variants can be concluded to contribute to ageing, but more research is needed to uncover the impact of FOXO3 on healthy ageing (18).

Dietary restriction (DR): definition and function of DR

DR can be defined as food intake reduction that increases a healthy lifespan. DR alone has been proven to be able to prolong the lifespan of many already healthy organisms, for instance, yeast, worms, and even mammals and primates (as shown in Table 1) (3). This implies that this mechanism is evolution-conserved, which allows researchers to create studies in short-lived model organisms in an effort to identify suitable interventions to ameliorate the negative aspects of human ageing. DR, along with reduced growth factor signaling, can elevate resistance to oxidative stress, decrease macromolecular damage and increase lifespan in organisms that become the model of the studies (3,19).

<table>
<thead>
<tr>
<th>Type of organism</th>
<th>Lifespan Increase</th>
<th>Beneficial Health Effects</th>
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<tbody>
<tr>
<td></td>
<td>Dietary Restriction</td>
<td>Mutations/drugs</td>
</tr>
<tr>
<td>Yeast</td>
<td>3 fold</td>
<td>10 fold</td>
</tr>
<tr>
<td>Worms</td>
<td>2-3 fold</td>
<td>10 fold</td>
</tr>
<tr>
<td>Flies</td>
<td>2 fold</td>
<td>60-70%</td>
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<tr>
<td>Mice</td>
<td>30-50%</td>
<td>30-50% (~100% in combination with DR)</td>
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<tr>
<td>Monkeys</td>
<td>Trend noted</td>
<td>Not tested</td>
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<tr>
<td>Humans</td>
<td>Not determined (GHR deficient subjects reach old age)</td>
<td>Not determined</td>
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Meanwhile, in humans, studies show the beneficial effects of DR against insulin resistance, inflammation, oxidative stress, obesity, and disruption in left ventricular diastolic function, in line with the functional and metabolic changes that have also been observed in DR in rodents. In humans, DR causes several hormonal adaptations process correlated with longevity as those observed in DR rodents, such as reductions in insulin and triiodothyronine, C-reactive protein, cholesterol, blood pressure, and the thickness of the carotid arteries specifically the intima-media part, which becomes main risk factors in cardiovascular disease. A high-protein diet can also alter the effects of DR in humans and mice, which raises the possibility that restrictions on protein consumption provide some benefits (3,19). Extreme DR able to cause several negative health effects, so it is important to determine whether the chronic, and intense DR increases susceptibility to wound-related pathologies, infections, and mortality in humans. Further studies are much needed to evaluate calorie intake, along with the relative micro-and macronutrient compositions needed for optimal, healthy ageing (3,19).

**Effect of DR on FOXO3 gene expression**

Fig. 2 shows the correlation between calorie restriction intervention and the increase in longevity, along with the role of calorie restriction in maintaining a cell redox state by means of cycling calories using mitochondria, in order to restore nicotinamide adenine dinucleotide (NAD+) (8). Calorie restriction activates sirtuins, which eventually cause FOXO activation, leading to improved autophagy and amino acid recycling by inhibiting mammalian target of rapamycin (mTOR) activity; these, along with other mechanisms, in turn, lead to a healthy ageing phenotype. In contrast to calorie restriction, excess calories-especially carbohydrates which lead to an increase in the NAD + hydrogen (NADH)/NAD+ ratio, causing lipogenesis, ROS overproduction by the mitochondria, mTOR activation when protein intake becomes excessive and poor autophagy (8).

**Effect of fasting on the ageing process**

Recent studies show that fasting plays role in adaptive cellular responses, further reducing inflammation and oxidative damage, bolstering cellular protection, and optimizing energy metabolism. In lower-level eukaryotes, the implementation of chronic fasting can extend longevity by means of reprogramming stress resistance and metabolic pathways. Experiments in rodents have shown that periodic or intermittent fasting gives protection against many diseases, such as cancers, diabetes, heart disease, and neurodegeneration processes. In humans, it can reduce the rates of obesity, asthma, hypertension, and rheumatoid arthritis. Thus, it can be concluded that fasting has significant potential to delay the ageing process and to aid in the prevention—or even in the management of certain diseases, while minimizing the adverse effects caused by chronic dietary interventions (20).

Both fasting and DR were found to increase longevity and promote stress resistance in model organisms, starting from the experiment in unicellular yeast until mammals (as shown in Figure 1) (8). This can be conducted through down-regulating conserved nutrient-signaling proteins, such as IGF-1 and other growth factors. Fasting is able to significantly reduce IGF-1 level, which circulates throughout the body; it has an essential role in that it negatively regulates FOXO transcription factors through Akt. Other signaling molecules, such as Tor and Ras, play important roles and functions are downstream of IGF-1. Both Tor and Ras are important in regulating ageing and stress resistance, but the extensive molecular
mechanisms behind this are still under investigation. It has also been found that adenylyl cyclase (AC) is just as important as Tor and Ras in protein kinase A (PKA), however, its exact mechanism is still unclear. These pathways have various regulatory effects, including those related to metabolism, cellular growth, and cellular protection against toxins and oxidants, all of which serve to delay the process of ageing (21).

CONCLUSION

DR induces an increase in FOXO3 gene expression, thus amplifying its effects in anti-ageing and further expanding longevity. Future research needs to investigate the optimum type of DR, and also uncover the detailed molecular mechanism of FOXO3 gene expression modulation and its impact on longevity. In clinical settings, DR has the potential to be used in the treatment of many diseases, such as diabetes, cancer, neurodegeneration, and heart disease. DR can also reduce the rates of asthma, obesity, hypertension, and rheumatoid arthritis, and to some extent, it can be utilized in the management of diseases. Lastly, chronic dietary interventions in the form of DR can help to delay the ageing process and minimize the side effects of ageing.

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CONFLICT OF INTEREST

None

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