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NUTRIGENOMIC STUDY OF GERMINATED BROWN RICE IN TYPE 2 DIABETES MELLITUS

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By

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Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philisophy

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philisophy

NUTRIGENOMIC STUDY OF GERMINATED BROWN RICE IN TYPE 2 DIABETES MELLITUS

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August 2013

Chairman : Maznah Ismail, PhD

Institute : Bioscience

Lifestyle factors, especially dietary, significantly affect the course of type 2 diabetes. The disease continues to debilitate millions around the world with significant impacts in the developing world. There are continuous efforts to curb this disease and despite considerable advances in its management, morbidity and mortality due to type 2 diabetes keep rising. As more evidence suggests events triggered by lifestyle factors cause significant impact on type 2 diabetes, there is a need for search of more alternatives to already available options for the effective management of this disease. Particularly, reports of its association with diets taken are now widely acknowledged, and with an increasing awareness of the role of diet in its etiopathogenesis,

functional diets are being studied for their promising role. Consumption of white rice (WR) is one such factor especially in developing countries where it is used as a staple food. WR increases risk of diabetes and worsens it, and growing evidence now suggests germinated brown rice (GBR) to have potentials as a functional diet for managing this disease. The presence of bioactive compounds in GBR like γ-aminobutyric acid (GABA), γ-Oryzanol, dietary fibre, phenolics, vitamins, acylated steryl β-glucoside (ASG), and minerals confer functional properties including antihyperglycemia and antioxidative and hypocholesterolemic effects.

In a type 2 diabetic rat model, induced by a combination of high fat diet and low dose streptozotocin injection (35 mg/kg body weight, dissolved in 100 mmol/l sodium citrate buffer, pH 4.5, injected intraperitoneally), effects of GBR, brown rice (BR) and WR in comparison to metformin were studied on fasting plasma glucose, lipid profile (total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and triglyceride), liver enzymes (alanine transaminase, γ-glutamyltranspeptidase and aspartate transaminase), serum urea and creatinine, hydroxyl radical scavenging activities of liver and kidneys, and plasma total antioxidant status. Nutrigenomic regulation of genes related to glucose metabolism, cholesterol metabolism, antioxidants and xenobiotic metabolism were equally evaluated to determine the effects of the different diets on transcriptional regulation of genes, as underlying mechanisms for the functional effects observed. Additionally, the contribution of individual bioactives (ASG, GABA, oryzanol and phenolics) towards such nutrigenomic mechanisms was evaluated using HEPG2 cells.

BR and GBR reduced plasma glucose (9 % and 34 % reduction) more than metformin (3 %) while WR worsened glycemic control (28 % increase) over 4 weeks of intervention. Through nutrigenomic suppression, BR and GBR were shown to downregulate gluconeogenic genes (*Fbp* and *Pck*) in a similar manner to, but better than metformin, while WR upregulated the same genes. The fold changes in expression of the *Fbp* and *Pck* genes, for the WR, BR and GBR groups in comparison to diabetic untreated group, were 1.2, 0.5 and 0.4, and 1.2, 0.7 and 0.5, respectively. Also, upregulation of apolipoprotein A1 and low-density lipoprotein receptor genes were involved in GBR's hypocholesterolemic effects, with fold changes of 2.9 and 1.2, respectively, while the genes were upregulated in WR and BR groups by 1 fold. The bioactive compounds mostly produced similar patterns of effects on transcriptional regulation of the glucose- and cholesterol-metabolism genes in HEPG2 cells. This suggested that synergism due to the bioactive compounds may have contributed to the overall functional effects of GBR. Furthermore, GBR improved antioxidant status better than BR, WR and metformin. It improved total antioxidant status, and liver and kidney hydroxyl radical scavenging activities in type 2 diabetic rats, and also preserved their liver enzymes, as well as serum creatinine. Upregulation of the catalase and superoxide dismutase genes was shown to be involved in GBR's antioxidant effects. Also, synergistic effects of the different bioactive compounds likely contributed to the transcriptional regulation of the antioxidant genes. In HEPG2 cells, all bioactives upregulated SOD 2 gene in a dose dependent manner, while only GABA and oryzanol upregulated SOD 1 and catalase genes respectively. Also, upregulation of some xenobiotic metabolism genes in type 2 diabetic rats was potentiated by WR, with the likely consequence of faster drug metabolism, less drug efficacy and more toxicity. GBR did not produce as much upregulation as WR, and BR showed significantly lower expression values than both WR and GBR. Interestingly, the

bioactive compounds in GBR upregulated the peroxisome proliferator activated receptor gamma (*PPARγ*), while in combination they downregulated the gene.

The fact that GBR downregulates gluconeogenic genes (*Fbp* and *Pck* genes) similar to metformin, but has a better glycemic control in Type 2 diabetic rats, suggests other mechanisms are involved in GBR's antihyperglycemic properties. Also, upregulation of the genes studied here cannot be said to account for all GBR's hypocholesterolemic and antioxidant effects since bioactives in GBR are proven to have other transcriptional and non-transcriptional mechanisms for lowering cholesterol and improving antioxidant status. Potentially as data from the current study shows, multiple mechanisms of antihyperglycemic, hypocholesterolemic and antixodant effects could mean stricter control of metabolic indices by GBR. It could potentially provide enhancements in metabolic outcomes in type 2 diabetes mellitus better than metformin. Although, the data from the current findings are from chemically induced diabetes mellitus, there are other studies that have reported on GBR's effects on human diabetes. It appears that the findings from human studies mirror those of the current animal experiments and other closely related ones, suggesting that the current findings may likely be reproduced in humans also. If GBR replaces WR as the staple food of choice for 3 billion people around the world, the implications would be profound on the prevention of diabetic complications. This could effectively reduce burden of the disease especially in poor countries where there is limited access to expensive medications and state-of-the-art healthcare facilities. In view of these benefits and the chronic nature of diabetes, the antidiabetic properties of GBR are worth studying further on a long term basis, especially in humans.

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KAJIAN NUTRIGENOMIK BERAS PERANG CAMBAH TERHADAP DIABETES MELLITUS JENIS 2

Oleh

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Faktor gaya hidup, terutamanya faktor pemakanan memberi kesan yang ketara kepada perkembangan diabetes jenis 2. Penyakit ini terus melemahkan berjuta-juta orang di seluruh dunia dengan kesan ketara di negara-negara membangun. Usaha untuk membendung penyakit ini berlaku secara berterusan. Sungguhpun terdapat kemajuan yang besar dalam pengurusan penyakit, morbiditi dan kematian terus meningkat. Lebih banyak bukti menunjukkan faktor gaya hidup memberi impak yang ketara mengenai diabetes jenis 2. Oleh itu, adalah menjadi satu kemestian untuk mencari lebih banyak alternatif kepada pilihan yang sedia ada dalam pengurusan penyakit ini. Dengan laporan yang mengaitkan penyakit dengan pemakanan kini

diakui secara meluas, dan dengan kesedaran yang semakin meningkat peranannya dalam etiopatogenesis, potensi peranan makanan berfungsi telah dikaji. Pengambilan nasi putih atau 'White Rice' (WR) merupakan salah satu faktor terutamanya di negara-negara membangun di mana ia digunakan sebagai makanan ruji. WR meningkatkan risiko dan memburukkan lagi keadaan penyakit diabetes. Kini, semakin banyak bukti mencadangkan beras perang cambah atau 'Germinated Brown Rice' (GBR) mempunyai potensi sebagai makanan berfungsi dalam pengurusan penyakit ini. Kandungan bioaktif seperti γ-aminobutyric acid (GABA), γ-oryzanol, serat, fenolik, vitamin, acylated steryl β-glucoside (ASG) dan mineral yang terdapat dalam GBR menyumbang kepada kesan dan aktiviti antihiperglisemia, antioksidan dan hipokolesterolemia.

Dalam model tikus diabetik jenis 2 yang dicetus daripada gabungan diet tinggi lemak dan suntikan streptozotocin (35 mg/kg berat, dilarutkan dalam 100 mmol/l larutan penimbal natrium sitrat, pH 4.5, disuntik secara intraperitoneal), kesan GBR, beras perang atau 'Brown Rice' (BR) dan WR berbanding metformin telah dikaji ke atas paras glukosa plasma semasa puasa, profil lipid, enzim hati, urea serum dan kreatinin, aktiviti radikal hidroksil hati dan buah pinggang, serta status jumlah antioksidan dalam plasma. Kawal atur nutrigenomik oleh gen-gen yang berkaitan dengan metabolisma glukosa, metabolisma kolesterol, antioksidan, antikoagulasi, dan metabolisma xenobiotic telah dinilai untuk menentukan kesan kepelbagaian diet ke atas kawal atur transcriptional gen, sebagai mekanisma asas bagi kesan berfungsi yang didokumentasikan. Di samping itu, sumbangan bioaktif (ASG, GABA, oryzanol dan fenolik) secara individu terhadap mekanisma nutrigenomik tersebut dikaji dengan menggunakan sel HEPG2.

BR dan GBR mengurangkan glukosa plasma (penurunan sebanyak 9% dan 34%) lebih daripada metformin (3%) manakala WR memburukkan kawalan glisemik (peningkatan sebanyak 28%) bagi empat minggu intervensi. Menerusi penyekatan nutrigenomik, BR dan GBR telah menunjukkan penurunan kawal atur terhadap gen-gen glukoneogenik (*Fbp* dan *Pck*) menerusi kaedah yang sama, malahan lebih baik daripada metformin. Ekspresi gen *Fbp* dan *Pck* untuk WR, BR dan GBR berbanding kumpulan yang tidak dirawat masing-masing adalah sebanyak 1.2, 0.5 dan 0.4, dan 1.2, 0.7 dan 0.5 kali ganda. Peningkatan kawalaturan gen apoliporotein A1 dan reseptor lipoprotein berketumpatan rendah juga terlibat dalam kesan hipokolesterolemik GBR, masing-masing dengan peningkatan sebanyak 2.9 dan 1.2 kali ganda, manakala pengawalaturan gen-gen tersebut meningkat sebanyak 1 kali ganda bagi kumpulan WR dan BR. Dalam sel HEPG2 bioaktif tersebut kebanyakannya menunjukkan corak kawalatur transkriptional metabolisma gluokosa dan kolesterol yang serupa. Hal ini mencadangkan bahawa kesan sinergistik sebatian bioaktif mungkin menyumbang kepada kesan berfungsi GBR secara menyeluruh. Tambahan pula, GBR meningkatkan status antioksidan lebih baik berbanding BR, WR dan metformin. GBR meningkatkan status jumlah antioksidan dan aktiviti memerangkap radikal hidroksil hati dan buah pinggang dalam tikus diabetes jenis 2 serta memelihara enzim hati, urea serum dan kreatinin. Peningkatan kawal atur oleh catalase dan gen superoxide dismutase didapati mempunyai penglibatan dalam menyumbang kepada kesan antioksidan. Di dalam kes ini juga, sinergisma memainkan peranan dalam peningkatan kawal atur nutrigenomik gen-gen antioksidan oleh bioaktif GBR. Semua bioaktif yang dikaji meningkatkan kawal atur gen SOD 2 dan catalase secara bergantung kepada dos. Kawalatur gen metabolisma xenobiotik dalam tikus diabetes jenis 2 ditingkatkan oleh WR,berkemungkinan disebabkan oleh metabolisma ubat yang lebih cepat, efikasi ubat yang berkurang dan lebih toksik. GBR tidak

menunjukkan peningkatan sebagaimana ditunjukkan WR, dan BR menunjukkan penurunan ekspresi yang signifikan berbanding WR and GBR. Menariknya, bioaktif dalam GBR meningkatkan *PPARγ* manakala gabungan bioaktif menurunkan kawalatur gen tersebut.

Hakikat bahawa GBR menurunkan kawal atur gen-gen glukoneogenik (*Fbp* dan *Pck*) pada paras yang sama dengan metformin, tetapi mampu mengawal keadaan glisemik dalam tikus diabetes jenis 2 secara lebih baik, membayangkan bahawa terdapat mekanisme lain yang berperanan dalam kesan antihyperglisemik GBR. Peningkatan kawal atur gen-gen yang dikaji tidak dapat menjelaskan semua kesan hypokolesterolemik dan antioksidan kerana bioaktif dalam GBR telah dibuktikan mempunyai mekanisme transkripsional dan bukan transkripsional yang lain dalam menurunkan kolesterol dan memperbaiki status antioksidan. Seperti dibuktikan oleh data dari kajian semasa, pelbagai mekanisme antihiperglisemik, hipokolesterolemik dan kesan antioksidan oleh GBR berpotensi dalam pengawalan indeks ini. GBR berpotensi untuk memberi penambahbaikan kesan metabolik yang lebih baik berbanding metformin dalam pengurusan diabetes jenis 2. Walaupun data daripada kajian sekarang adalah berdasarkan diabetes mellitus yang dicetus oleh bahan kimia, terdapat kajian lain yang menunjukkan kesan GBR ke atas manusia. Penemuan daripada kajian ke atas haiwan dan kajian seumpamanya telah didapati mencerminkan hasil kajian ke atas manusia, justeru menyarankan bahawa kajian sekarang dapat diteruskan ke atas manusia. Sekiranya GBR menggantikan WR sebagai makanan asasi pilihan bagi tiga billion penduduk di seluruh dunia, implikasi yang lebih berkesan terhadap pencegahan komplikasi diabetes dapat dilaksanakan. Hal ini akan dapat mengurangkan bebanan penyakit terutamanya di negara-negara miskin dimana akses kepada ubat-ubatan mahal dan kemudahan penjagaan kesihatan adalah terhad. Berdasarkan manfaat yang ditunjukkan dan kesan kronik

diabetes, sifat antidiabetik GBR adalah bernilai untuk dikaji dengan lebih mendalam bagi masa yang panjang, terutamanya ke atas manusia.

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Approval Sheet 1

I certify that a Thesis Examination Committee has met on 2nd August, 2013 to conduct the final examination of Mustapha Umar Imam on his thesis entitled "Nutrigenomic Study of Germinated Brown Rice and its Bioactives on Type 2 Diabetes Mellitus" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philisophy in Molocular Biotechnology.

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philisophy. The members of the Supervisory Committee were as follows:

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CHAPTER 1

INTRODUCTION

Morbidity and mortality associated with type 2 diabetes is severe and significant, respectively. An estimated 371 million people were reported to be suffering from this disease in 2012 [1]. About 5% of all deaths globally each year are due to this disease, almost 80% of which occur in low and middle-income countries [1]. These figures are expected to grow especially in developing nations in near future. Rising incidence and prevalence of the disease points to factors that are prevalent among vulnerable populations around the world.

The primary underlying problem in type 2 diabetes is insulin deficinecy and/or insensitivity, which results in hyperglycemia [2]. Eventually, metabolic perturbations lead to other secondary metabolic abnormalities including oxidative stress and hypercholesterolemia that together complicate the disease through cardiovascular diseases, nephropathy, neuropathy, and other medical problems [3]. Often times, diabetes necessitates the consumption of multiple medications because of the many medical problems resulting from it. The medications may have side effects on organ sysmtems especially considering that the metabolic abnormalities caused by type 2 diabetes affect the functioning of every system. Therefore, any xenobiotic that may further worsen the functioning of the already compromised organ systems due to type 2 diabetes may significantly affect the mangement of the disease. Complications arising from type 2 diabetes are debilitating and result in a huge expenditure in health care [1, 4]. The total cost of management

of the disease, coupled with the accompanying economic loss due to lack of productivity makes the search for remedies even more necessary. Genetic factors have largely been implicated in the past, and of recent it has come to light that lifestyle factors significantly contribute in its pathogenesis [2]. Also, the importance of dietary factors is acknowledged as a fundamental variable in the course of this disease [5, 6].

Consumption of white rice (WR) has been reported to increase risk of type 2 diabetes and because of its high glycemic index, it could worsen glycemic control [5, 7]. Sun et al. [8] have shown, in a study involving over 200,000 subjects, that WR increased the risk of type 2 diabetes, while replacing one-third of daily serving with brown rice reduced the risk of developing type 2 diabetes. Following up almost 60,000 men and women for five years, Nanri et al. [9] also reported an increased risk of type 2 diabetes in women who consumed WR, but the association in men was less clear. Interestingly, Zhang et al. [10] did not report similar findings after substituting WR with BR for 16 weeks in middle aged Chinese men and women, but low population size could have resulted in lack of significant difference, prompting them to recommend more studies with larger sample size. In a meta-analysis and systematic review [11], however, involving more than 350,000 subjects followed up for 4-22 years, the risk of developing type 2 diabetes was clearly shown to be higher especially in Asians who consumed WR instead of brown rice (BR). Interestingly, WR is the main staple for majority of people living in developing countries, where the burden of type 2 diabetes is expected to be highest in the near future. On the other hand, the presence of bioactive compounds in BR and especially germinated brown rice (GBR) underlies their usefulness in the management of type 2 diabetes [12].

In the light of recent evidence therefore, lifestyle factors especially diet need to be given more attention in the management of type 2 diabetes. In low and middle income countries, there is no diet more befitting of closer scrutiny than WR to try and find alternatives devoid of the risks associated with WR. Also, there is evidence that wide spread dysregulation of metabolism, at the transcriptional and non-transcriptional levels, in type 2 diabetes contributes immensely to the problems in the disease. Transcriptional changes most often preceed other metabolic abnormalities, and hence studying transcriptional regulation of pathways that are deranged in type 2 diabetes has provided insights into the significant events leading up to the development of type 2 diabetes and its complications there after. Transcriptional changes are the earliest to happen in cells in response to stimuli, and their regulation could prevent derangements in metabolism, when an appropriate stimulus is used. Therefore, targeting such molecular events in the therapy of type 2 diabetes would therefore be immensely valuable in prevention of the disease and/or its complications. This hypothesis has generated several leads, and the use of dietary components for their potential benefits in managing type 2 diabetes, based on their effects on molecular targets, is expected to form the basis for the future use of diets in its management. The evidence-based nature of this approach to dietary management of type 2 diabetes will likely prove better than current approaches, and more extensive studies are needed in this regard. Specifically, reports on the regulation of molecular events related to type 2 diabetes by WR, BR and GBR are lacking. Such data could provide insights into potential mechanisms modulated by these rice types and their implications in type 2 diabetes.

General objective:

To study the nutrigenomic effects GBR and its bioactive compounds in comparison to WR and BR on type 2 diabetes mellitus, *in vitro* and *in vivo*.

Specific objectives:

1. To isolate and characterize bioactives (acylated steryl glucoside [ASG], gamma amino butyric acid [GABA], oryzanol, ferrulates) from WR, BR and GBR
2. To determine the effects of WR, BR and GBR and its bioactives on weight, glycemia and glucose metabolism genes in type 2 diabetic rats and HEPG2 cells.
3. To determine the effects of WR, BR and GBR and its bioactives on lipid profile and cholesterol metabolism genes in type 2 diabetic rats and HEPG2 cells.
4. To determine the effects of WR, BR and GBR and its bioactives on antioxidant status and genes in type 2 diabetic rats and HEPG2 cells.
5. To determine the effects of WR, BR and GBR on of xenobiotic metabolism genes in type 2 diabetic rats.

Hypotheses of the study were:

1. GBR and its bioactives could improve metabolic indices like glycemia, lipid profile and antioxidant status better than WR or BR.
2. GBR and its bioactives could delay and/or prevent oxidative stress related complications in type 2 diabetes mellitus better than WR or BR.
3. GBR and its bioactives could regulate several metabolic pathways including those of glucose metabolism, cholesterol metabolism and antioxidants as mechanisms of improving metabolic indices in diabetic rats and HEPG2 cells better than WR or BR.
4. GBR could prevent upregulation of xenobiotic metabolism genes in type 2 diabetic rats better than WR or BR.

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