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学位記番号	乙 第 926 号
学位授与の日付	平成 29 年 7 月 20 日
学位論文題目	Grain components of Philippine rice (<i>Oryza sativa</i> L. <i>indica</i>) on potentially modulating biomarkers associated with type 2 diabetes mellitus
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論文内容の要旨

Type 2 diabetes mellitus (T2DM) is one of the four leading public health problems in the world. The risk for the disease is governed by a complex interplay of genetic and multiple lifestyle influences, of which diet is a major modifiable risk factor. In recent years, the effects of rice consumption on T2DM has been the subject of heightened interests.

Rice (*Oryza sativa* L.) exists in different types and forms around the world, and metabolic effects vary according to varietal differences. With the general aim of contributing knowledge on the role of rice in T2DM, this dissertation was thus conducted. The research was designed to determine the effects of rice grain components on selected biomarkers associated with T2DM, and to understand how the relationships may relate to T2DM risk prevention and management. The dissertation focused on Philippine rice to represent the *indica* rice subspecies. *Indica* rice are cultivated in tropical countries which have the highest projected increase in diabetes prevalence by 2030. Also, to the best of knowledge, the metabolic effects of *indica* rice are less studied compared to *japonica* rice that are cultivated in Japan and Korea.

The Philippines is rich in traditional and modern rice varieties. Filipinos have general preference for moderately tender cooked rice, typified by the popular rice variety, IR64. However, consumer preferences varied across ecosystems (**Chapter III**). Rice consumers in the upland and saline ecosystems had preferences for slightly harder cooked rice texture (18-25% apparent amylose content (AC) with intermediate gelatinization temperature (GT)), while those from cool-elevated ecosystems preferred the softer and

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sticky rice with <18% AC and low GT.

AC- the linear fraction of starch, is the main factor affecting cooked rice texture, and consumer preference. It is also cited as the most important source of variation in post-prandial glucose response (GR) from rice. In **Chapter IV**, the effects of AC on GR from milled rice was determined. Seven milled Philippine rice varieties with different AC were cooked with adjusted water-rice ratio to have similar cooked rice hardness (to address effects of cooking method and GT). The rice samples were subjected to glycemic index (GI) determination in healthy Filipino volunteers who were given a serving of the food containing 50 g of available carbohydrate. Results showed GR to be lowest for the variety with highest apparent AC (PSB Rc10), and highest for the low-AC rice (*Sinandomeng*). There were significant differences in the incremental area under the curve (IAUC) of GR among high-, intermediate- and low-AC rice varieties. GR was in close association with GI ($r = 0.99$; $p < 0.01$), and AC was negatively correlated with GI ($r = -0.65$; $p < 0.05$).

The association of milled rice AC to GI was validated in a follow-up study (**Chapter V**). In this study, it was also found that rice protein content can affect GI. Improved *Malagkit Sunsong* which has a 9% crude protein content in the previous trial had a GI of 63 ± 2 (medium), while at the current 6% crude protein content its GI was recorded at 94 ± 5 (high). The higher protein content that was covering the starch granules may have prevented rapid hydrolysis of the endosperm starch.

In **Chapter V** as well, dietary fiber (DF) was shown to affect GI. Unpolished rice or brown rice (BR) had lower GI than its milled counterpart. Among the four AC types, the decrease in GI from milled rice to BR was greatest for waxy rice (0–2% AC), and low-AC rice (10–17% AC), both with high GI (≥ 70). Intermediate (17–22%)-AC IR64 with medium (56–69) GI showed lesser drop, but high-AC PSB Rc10 (> 22% AC) had no significant decrease. GI was again highest for waxy (0–2% AC) rice and lowest for high-AC rice for both rice forms, but the decrease in GI with increasing AC was lower for BR than for milled rice. In the previous study (**Chapter IV**), the association of apparent AC with GI was more pronounced in the combined effect of AC and DF on GI ($r = -0.71$; $p < 0.05$).

GR and GI of black and red colored unpolished rice in comparison with BR, were also determined (**Chapter VI**). Results found black and red rice to have similar GR, which were comparable with that of the BR. GI was classified as medium for all samples (GI= 63 ± 4 , 59 ± 4 , and 59 ± 5 , for the black, red and BR, respectively), despite significant

differences in total polyphenol and anthocyanin contents. The study suggests that total polyphenol and anthocyanin contents in Philippine rice do not influence GR.

Aside from its direct effect on GR, rice can also be linked to T2DM via its effects on satiety and food intake. Very few studies however, investigate rice satiety. Hence, the satiating capacity of milled and brown rice of different AC types, and of colored rice were evaluated. In all trials, the estimation of satiety was done in conjunction with GI measurement. Satiety quotient (SQ) and/or satiety index (SI) were computed based on VAS, and was used as index of short-term satiety, together with 2-h post-meal cooked rice intake. There was no difference in the SQ between black and red colored rice, and between colored rice and BR (**Chapter VI**). Short-term satiety was also similar across the seven milled rice samples, and satiety was found to be independent of both AC and GI (**Chapter VII**). On the other hand, a tendency for BR to be more satiating than milled rice was observed in **Chapter VIII**. Both SQ and SI tended to be higher in BR than milled rice, regardless of AC type. SI was also found to have strong positive correlation with DF content. Contrary to hypothesis, however, the higher SQ and SI for brown rice was not translated into lower subsequent food intake after 2-h, and common cooked rice intake was not affected by DF content. The satiety of BR using a longer time span (4-h post meal or at lunch time) was then pursued using a different set of human volunteers (**Chapter IX**). BR remained to be perceived as more filling and suppresses hunger better than milled rice. However, the difference in adjusted satiety scores at ~4-h post meal between milled rice and BR, was only significant for the high-AC variety. Mean post meal cooked rice intake also remained similar across rice AC types and rice forms, but intake based on dry weight decreased with increasing AC. Further estimation of energy intakes at post meal and within 24-h indicated that energy intake was independent of rice DF content, but was negatively associated with rice AC.

Hyperlipidemia, which is also prevalent worldwide, is associated with risk of T2DM. Non-alcoholic fatty liver disease (NAFLD) is being linked with the development and progression of T2DM, including related complications. Polyphenols notably anthocyanin were suggested to prevent cellular damage in hepatocytes associated with NAFLD through different mechanisms of action. In **Chapter X** of this dissertation, the effect of the rice powder extract (RPE) from an unpolished Philippine black rice variety and BR on hepatic lipid accumulation was compared. The extracts were prepared out of cooked, freeze-dried samples, and were tested using obese and diabetic mice model (KKAy). The study found that RPE from black rice (BL) and BR improves hepatic lipid accumulation

via peroxisomal fatty acid oxidation. A histochemical analysis also revealed that BL and BR RPE feeding ameliorated triacylglycerol (TG) accumulation in the liver. To elucidate the mechanism underlying the improvement in hepatic TG accumulation, gene expression was evaluated using real-time PCR. The mRNA expression levels of *acyl-CoA oxidase (AOX)*, a rate-limiting enzyme of peroxisomal fatty acid oxidation, were significantly higher in the BL and BR groups than in the control-K group. RPE showed peroxisome proliferator activated receptor α (PPAR α) agonistic activity which did not differ between BL and BR RPE despite a higher anthocyanin content in black RPE. It is likely that another compound has PPAR α agonistic activity in RPE or that RPE contains compounds that modify the activity, and further study was recommended.

This dissertation revealed that grain components of Philippine rice affect biomarkers associated with T2DM. AC was the most important property that modulate T2DM metabolic response. AC was inversely associated with GR and post-meal cooked rice intake, and rice varieties with at least intermediate-AC is suggested for the prevention and management of T2DM in populations where *indica* rice is the staple food, such as the Philippines. Revisiting the breeding objectives in the Philippine rice varietal development program, is recommended, in reference to the increasing preference for softer cooked rice among Filipinos. Switching from milled rice to BR can also contribute to T2DM risk reduction, through the combined effect of dietary fiber with AC in lowering GR and GI. Effects of DF from rice to satiety and food intake are yet to be established, with full account of other factors such as cognitive cues and eating behaviors. Nonetheless, the perceived better satiating potential of BR than milled rice may provide opportunities to maximize promotion of BR as a functional food, if taken as entry point for nutrition education and as part of a holistic dietary intervention under the national diabetes prevention and control plan.

The dissertation also showed that protein content can lower GR and GI of *indica* rice. More importantly, *in vitro* and *in vivo* experiments demonstrated that RPE from black unpolished rice and BR, have PPAR α agonistic activities, and can improve hepatic TG accumulation. These are new findings and are worthy of further investigations

審査報告概要

本研究では、フィリピン米中のアミロース(AC)含量が、グルコース応答(GR)および炊飯

米摂取量とそれぞれ負の相関を示し(ヒト介入試験), インディカ米が主要食糧であるフィリピンなどでは, 2型糖尿病(T2DM)の予防管理のために中~高 AC を有するイネ品種が望まれることが示唆された。また, 食物繊維と AC との併用効果によって GR および血糖指数(GI)が低下することから, 精白米から玄米(BR)への切り替えは T2DM のリスク低減に寄与する可能性が示された。インディカ米のタンパク質が GR および GI を低下させることも示された。本研究の成果は, 栄養教育のエントリーポイントとして, 或いは全国的な糖尿病予防および管理計画の下での全体的な食事介入の一環として BR の摂取促進を考慮する際の有用な根拠情報となると考えられた。さらに, *in vitro* および *in vivo*(動物)試験では, 黒米および BR 由来の抽出物が PPAR α アゴニスト活性を有し, 肝臓の中性脂肪蓄積を改善し得ることも確認された。よって, 審査委員一同は, Angelina DR. Felix 氏に博士(食品栄養学)の学位を授与する価値があると判断した。