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Molecular science and Kampo medicine

**Nutrigenomics of Wakan-yaku (traditional crude drugs)**

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Although traditional crude vegetable drugs (Wakan-yaku) have been empirically discovered, experimental and clinical studies including multi-center, placebo-controlled, double-blind studies have demonstrated the various pharmacological effects of these drugs. In this article, advantage of scientific analysis by nutrigenomics over the present placebo-controlled evidence based medicine (EBM) is presented in the study of traditional herbs.¹ Nutrigenomics has been studied by analyzing the genomics, transcriptomics, proteomics, metabolomics and phenomics of food components including traditional crude vegetable drugs in organism (Fig. 1).¹ The recent introduction of genome-wide association study (GWAS) resulted in the development of nutrigenomics. In traditional medicine there is no clear distinction between food components and vegetable drugs. In fact, prevention and treatment of life-style-related diseases combine both low salt- high potassium diet and antihypertensive drug etc. In addition to sharing many therapeutic activities, the active components of traditional herbal medi-

![Fig. 1 Traditional medicine and Nutrigenomics](image-url)
Cine are also used in nutrient supplement for several chronic diseases. Although traditional Chinese medicine was dominated by the laws of “yang” and “yin”, and many worthless, magical remedies, modern scientific research of the crude drugs has been dominated by EBM. EBA is based on the randomized control study of large number of subjects on some drug or treatment and the average values of both placebo group and test group taking a traditional drug are evaluated by statistics to obtain average effective dose. Likewise, recommended the dietary allowance (RDA) of a nutrient is based on the estimated average requirement (EAR: the intake simply sufficient to satisfy the needs of 50% of the subjects) and the standard deviation (SD) obtained by balancing studies on large numbers of subjects (Fig. 2, upper panel). Therefore, RDA (defined as EAR+2SD) is intended to cover the nutritional requirements of 97.5% of subjects.

However, nutrigenomics is wider in scope than both EBM and RDA (Fig. 2).\(^1\) The statistics of the EBM and RDAs assumes nearly normal distribution of the data, which is not always true in populations composed of many genetic polymorphisms (Fig. 2). Single nucleotide polymorphisms (SNPs) are found every 1,000 nucleotides, and some SNPs result in the different activity of enzymes, receptors, transporters etc. in every subject. If enzyme activities of wild-type homozygote, heterozygote and mutant homozygote are very different, there will be three overlapping separate peaks instead of a single average bell-shaped distribution curve (Fig. 2, lower panel). For example, the guideline recommends ethanol intake <20g/day, but some people become intoxicated by only 1g/day, while others can drink 200g/day without significant impaired performance depending mainly on the activity of the SNP of aldehyde dehydrogenase 2.\(^1\) The daily dose of anticoagulant warfarin under the restriction of vitamin K is ranging from 1 mg to 17 mg depending on the SNP of warfarin metabolizing enzyme. In this case, the distribution curve of doses vs frequency of subjects has three peaks instead of normal single distribution curve. Thus, the use of average dose based on EBM is quite dangerous for both poor metabolizer with bleeding and high metabolizer with infarction.\(^3\) The interindividual variation corresponds to “Sho” of traditional medicine, and should be measured in nutrigenomics discussed here. The optimal nutrition guided by nutrigenomics covers wider scope than RDA and EBM due to the following 4 reasons.

1. **Polymorphism**

Since Mongoloids are genetically more susceptible to metabolic syndrome and diabetes mellitus than are Caucasians,\(^2\) the globalization of high-fat diets\(^3\) has resulted in a rapid increase in metabolic syndrome among Mongoloid populations. DNA polymorphisms in Asia-Pacific region have been identified in the genes encoding a number of the enzymes, receptors and signal transducers related to metabolic syndrome/diabetes, and drug metabolizing enzymes. Nutritional, genetic and clinical data were collected from 974 individuals in 5

![Fig. 2 Optimal Nutrition and polymorphisms](image)  
**Fig. 2** Optimal Nutrition and polymorphisms  
EAR: estimated average requirement.  
RDA: recommended dietary allowance.  
UL: tolerable upper intake level. RDA is defined as EAR + 2SD.
countries, and 105 metabolic syndrome-associated SNPs, including those of 18 nuclear receptors were genotyped. Analysis was carried out with the WEKA machine-learning system as the decision-tree software. PPARγ in adipocytes, together with RXR and LRH, express more adiponectin, which prevents metabolic syndrome. Incidentally, a famous component of traditional herb, glycyrhrizin is a ligand of PPARγ. Metabolic syndrome-sensitive Pro-homozygote of PPARγ2 Ala12Pro SNP was found among more than 86% of Mongoloids, and metabolic syndrome-sensitive SNP of β3 adrenergic receptors (β3ARs), uncoupling proteins 1-3 (UCPs 1-3), and adiponectin (AD) were also frequently found. Optimal personalized nutrition based on polymorphism enables not only appropriate treatment but also prediction of the risk for prophylaxis. C677T-TT, an SNP of MTHFR found among 15% of the population, is associated with a 3.5-fold higher risk for stroke as compared to the wild type, and for TT, 400 µg folic acid/day are needed, instead of RDA (240 µg). The individual variation in the values of clinical chemistry is caused by the genetic polymorphisms, and the daily fluctuation of the values of an individual is controlled by clock gene.

In the phase 1 detoxification of drugs, including traditional ones, DNA polymorphisms have been identified in the genes encoding a number of the cytochrome P450 (CYP) enzymes, leading to wide interindividual variation in drug clearance. There are 11 CYP subtypes such as CYP2D6, with total of 388 polymorphisms, the frequencies of which are highly different among ethnic groups. It is clear that traditional drugs that are substrates for CYP2D6 and CYP2C19 can display wide interindividual variation in their metabolism as a result of polymorphisms in CYP2D6 and CYP2C19 gene (Fig. 3). CYP2D6 metabolizes a significant number of clinically used traditional medications, and genetic variants of the CYP2D6 isozyme that result in varying levels of metabolic activity are of clinical importance in some settings. The exact nature of the clinical effect caused by polymorphisms of the gene depends on the drug in question and the specific variant alleles expressed, as individual variants result in differing phenotypes with a range of levels of enzymatic activity. Variation in CYP isoform activity has important therapeutic consequences and can play a significant role in the development of adverse events in susceptible individuals. The CYP polymorphisms may become more important as robust

![Figure 3: Ethnic variation of CYP2D6 and CYP2C19](image-url)
clinical tests become widely available and as the use of multiple combinations of traditional crude drugs and the attendant risk for drug-drug interactions increases.

2. Nutrimetabolomics and Nutribioenergetics

The application of metabolomics to nutritional research is nutrimetabolomics. Classical nutritional assessment depends largely on the analysis of metabolites such as glucose and lipids in serum. But little is known of the extent to which changes in the nutrient content of the human diet and traditional crude vegetable drugs elicit changes in metabolic profiles. Moreover, the metabolomic signal from nutrients absorbed from the diet must compete with the myriad of non-nutrient signals including chemical compounds in crude vegetable drugs that are absorbed and metabolized. In addition to these confounding effects, the potential use of metabolomics for nutritional assessment is hampered by the difficulty in obtaining data from human living cells by biopsy. The non-invasive determination of metabolome data by nuclear magnetic resonance (NMR) is one method to overcome this difficulty, as described in the following section on nutribioenergetics. This potential lies both in more detailed metabolic profiling through the use of pattern-recognition statistics on assigned and unassigned metabolite signals and in the collection of comprehensive data sets of identified metabolites. Both approaches have the potential to distinguish between different dietary treatments, which would not have been possible with conventional techniques. The development of libraries of small molecules will aid in metabolite identification in nutrimetabolomics.

Nutribioenergetics: Bioenergetics based on ATP synthase is the most important reaction of human activity, and this activity may be related to “ki” in traditional medicine. In addition to metabolites, energy expenditure (EE) of the entire body must be estimated. The “gold standard” method of measuring EE is the doubly labeled water (DLW) method using $^2$H/$^{18}$O. Other classical methods include direct and indirect calorimetry systems, and heart rate. The preferred method to determine EE is likely to depend principally on factors such as the number of study participants to be monitored, the time period of measurements and the finances available. In small studies, the EE of the participants may be measured accurately over a short period of time by means of indirect calorimetric methods (stationary and portable systems). For periods longer than 3-4 days, EE should ideally be measured using the DLW method. However, the use of motion sensors is very promising in the measurement of EE, and has a number of advantages over the DLW method. Thermographic measurement of energy emission is also widely used. An excellent example of modern nutribioenergetics is the use of nuclear magnetic resonance (NMR) to estimate metabolism in vivo. In order to assess the sensitivity of liver and muscle to insulin, hyperinsulinemic-euglycemic clamps in combination with infusions of [6,6-$^2$H]glucose in healthy, insulin-resistant offspring of patients with type 2 diabetes and insulin-sensitive control subjects matched for age, height, weight, and physical activity were analyzed. Proton NMR was performed to measure lipid in muscle and TG in liver. Rates of whole-body and subcutaneous fat lipolysis were assessed by measuring the rates of $[^3]$H glycerol turnover in combination with microdialysis measurements of glycerol release from subcutaneous fat. $^{31}$P-NMR was used to assess the rates of oxidative phosphorylation in muscle. The insulin-stimulated rate of glucose uptake by muscle was approximately 60 percent lower in the insulin-resistant subjects than in the insulin-sensitive control subjects ($P < 0.001$) and was associated with an increase of approximately 80 percent in the lipid content in muscle ($P = 0.005$). This increase in lipid content in muscle was most likely attributable to mitochondrial dysfunction, as reflected by a reduction of approximately 30 percent in mitochondrial phosphorylation ($P = 0.01$ for the comparison with controls), since there were no significant differences in systemic or localized rates of lipolysis or plasma concentrations of tumor necrosis factor alpha, interleukin-6 or adiponectin. These data support the hypothesis that insulin resistance in the skeletal muscle of insulin-resistant offspring of patients with type 2 diabetes is associated with dysregulation of fatty acid metabolism in muscle, possibly because of an inherited defect in mitochondrial oxidative phosphorylation. To investigate how insulin resistance arises, we studied healthy, lean, elderly and young participants matched for lean body mass and fat mass. Elderly study participants were markedly insulin-
resistant as compared with young controls, and this resistance was attributable to reduced insulin-stimulated muscle glucose metabolism. These changes were associated with increased fat accumulation in muscle and liver tissue assessed by proton NMR, and with an approximately 40% reduction in mitochondrial oxidative and phosphorylation activity, as assessed by in vivo $^{13}$C/$^{31}$P NMR. These data support the hypothesis that an age-associated decline in mitochondrial function contributes to insulin resistance in the elderly. There have been many traditional drugs that affect human EE, and in near future these new technology of nutribioenergetics will elucidate the mechanism.

3. Chrononutrition

Chronobiology is a field of science that examines periodic (cyclic) phenomena in living organisms. These cycles are known as biological rhythms. “Chrono” pertains to time and “biology” pertains to the study of life. RDA does not specify the timing of meals, but regular circadian rhythm in both nutrition and physical activity is essential for health. In fact, the principles underlying traditional medical theory were those of the opposing qualities of “yang” (sunlight) and “yin” (darkness), respectively. The chief cause lay in their imbalance or disharmony. The recent discovery of clock gene elucidated the regulatory mechanism of circadian rhythm. The central clock gene located in suprachiasmatic nucleus is reset by morning light, and peripheral clock genes located in liver and intestine are reset by the intake of breakfast. The effects of breakfast on our vitality and health have been studied by chronobiologists. Despite the superficial “health fad,” the number of people who omit breakfast has increased, the rate of obesity has grown, and daily walking steps have decreased. Thus, our health policy “Healthy Japan 21” has failed. According to the National Health and Nutrition Survey in Japan in 2004, 10.6% of all Japanese skipped breakfast. Only 45% of 20-29 year olds eat breakfast every day. The reason Japanese give for omitting breakfast is that they are staying up about 1.5 hours later than Americans and Chinese. Owing to the irregular lifestyle, the personality development of infants is impaired, students are less active, and lifestyle-related diseases in middle-aged individuals are increased. In fact, breakfast improves academic performance irrespective of subject or grade, as shown by the domestic school survey and exact psychological cognition tests. Breakfast supplies the brain with glucose, which is its sole energy source. Glucose can be supplied from liver glycogen, but it is used up within 16 hours. Hypoglycemia lowers brain activity and the rate of traffic accidents is increased as a result. For human beings, the healthiest lifestyle is to get up in the morning, work in the daytime, and sleep at night. In suprachiasmatic nucleus is a master clock gene where a protein called Clock-Bmal1 binds to a control site, E-box, and expresses a protein called Per-Cry. Per-Cry inhibits Clock-Bmal1. Thus Per-Cry is decreased and there is a circadian oscillation of these proteins. Morning light wakes up the master clock gene (adjusts the phase of cycle), changes its circadian rhythm into day rhythm, and thus starts psychosomatic activity. Peripheral clock genes (slave oscillators) are found in most tissues, and their rhythm is adjusted by eating breakfast. The synchronization of master and peripheral clock genes is essential for a healthy life. E-box is distributed among many genes and controls physiological and behavioral activities of the whole body. The disturbance of the clock gene rhythm causes lifestyle-related diseases. Strong morning light and daily breakfast can correct an irregular daily rhythm. The PPARs/RXR system is closely related to the function of CLOCK/Bmal1 in circadian rhythm, the disturbance of which induces metabolic syndrome. The “yang” and “yin” states of human body can be monitored by infrared thermography of subjects after breakfast or its omission.

4. Bioactive substances and epigenetics

Traditional herb drugs contain many bioactive substances. Nutrigenomics includes study of bioactive substances such as anticancer or hypotensive agents in functional foods, while RDA does not. The effects of functional foods must be established by analyzing gene expression and epigenetics. Epigenetics can be defined as all the meiotically and mitotically inherited changes in gene expression that are not encoded in the DNA sequence itself. Epigenetic modifications of chromatin and DNA have been recognized as important permissive and
suppressing factors in controlling the expressed genome via gene transcription. Two major epigenetic mechanisms are the posttranslational modification of histone proteins in chromatin and the methylation of DNA itself, which are regulated by distinct, but coupled, pathways. It is clear that the epigenetic state is a central regulator of cellular development and activation. The epigenome is influenced by environmental factors throughout life. Nutritional factors and traditional crude drugs can have profound effects on the expression of specific genes by epigenetic modification, and these may be passed on to subsequent generations with potentially detrimental effects.

The NAD+-dependent protein deacetylase family, human SIRT1 (or sirtuins), is important for many cellular processes including gene silencing, fatty acid metabolism, cell cycle regulation, and life span extension. Resveratrol, a polyphenol found in wines and thought to harbor major health benefits, was reported to be an activator of the deacetylase family in vivo and in vitro. In addition, resveratrol was shown to increase life span in three model organisms. In fact, a novel drug called SRT1720 that is 1000 fold more effective than resveratrol was synthesized. The life span extending drug has been the most precious imaginary herb in the traditional Chinese medicine.

In conclusion, the traditional medicine is marked by an empirical development of expert knowledge of vegetable crude drugs that have been scientifically elucidated by randomized control study for EBM, and will be developed by nutrigenomics considering interindividual variation, metabolomics and epigenetics.

References