examination, the stable rate of $^{13}CO_2$ production in breath was achieved between 5 h and 6 h and maintained until the end of the study. These results suggested that two meals received every 3 h were required to achieve constant $^{13}CO_2$ enrichment, and that the effect of the ^{13}C infusion could be evaluated correctly after the third meal at 15:00.

Experiment 1 demonstrated a similar pattern and a latter steady state ~2.5 h after the start of the stable isotope protocol (Fig. 2), so breath samples for the measurement of the protein metabolism were collected 210 min after the administration of the stable isotope began. Moreover, the protein intake level, the 4.3% or 17.2% casein diets, had a significant effect on breath $^{13}CO_2$ concentration at 18:30, showing that this protocol could detect differences in protein metabolism. These results reflected the supposition that if one indispensable amino acid (limiting) was deficient for protein synthesis, then all other indispensable amino acids (including the indicator amino acid, [^{13}C]phenylalanine) would be oxidized. Therefore, when the rats were fed a low protein diet, the 4.3% casein diet, most of amino acids were oxidized, and the $^{13}CO_2$ concentration in breath increased. By increasing the protein intake with the 17.2% casein diet, the intake of the limiting amino acid also increased, and the values produced by the IAAO method decreased, reflecting the increasing incorporation into protein.

The mean protein intakes for metabolic demands determined by the IAAO method were 13.1 g/kg BW/d for the casein and 18.1 g/kg BW/d for the wheat gluten. Therefore, the protein intakes for metabolic demands based on wheat gluten was higher than that based on casein. The differences between the casein and wheat gluten diets will be a function of the limiting amino acid in the respective protein source. This limiting amino acid will be dependent on both the amino acid profile and the digestibility of the protein. These results also conformed with our hypothesis, that the protein requirement will decrease with good quality (amino acid scoring pattern) protein intake, and increase with poor quality protein intake, validating the concept that the IAAO method could be employed to evaluate the quality of protein.

In regard to the measured phenylalanine oxidation, the enrichment of breath $^{13}CO_2$ differed between the rats fed the casein and wheat gluten diets. The enrichment of breath $^{13}CO_2$ was consistently higher in rats fed the wheat gluten diet, compared with rats fed the casein diet, even at the plateau line with a protein intake more than the metabolic demand for protein. According to intake of protein, specifically, the limiting amino acid, the indicator amino acid is partitioned between incorporation into proteins and oxidation. The quality of the protein also affected the $^{13}CO_2$ volume in the breath. Future extensions of this study to other protein sources will be necessary in order to confirm this relationship.

Hegsted (34) suggested the necessity of taking account of adaptation in their nitrogen balance methods, arguing that prior adaptation is required. The IAAO method can be conducted in short time periods because no period of adaptation to each intake is employed (35). Therefore, the IAAO method could be employed to evaluate the metabolic protein demand for all age groups (infants, children, adolescents, adults, and the elderly), as well as for post-operative patients or patients with injuries or infections that have specific metabolic conditions, such as a widely varying metabolic demand. In a clinical setting, the adequate quality and quantity of protein or amino acid for each specific condition could be estimated using the IAAO method.

The results of this study demonstrated that the IAAO method can be employed to evaluate not only the protein intake for metabolic demands, but the dietary protein quality in freely living rats. Further studies are necessary to assess the viability of the IAAO method in a clinical setting.

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Review

The Optimal Dietary Fat to Carbohydrate Ratio to Prevent Obesity in the Japanese Population: A Review of the Epidemiological, Physiological and Molecular Evidence

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Summary The prevention of obesity, which leads to diabetes and other diseases, is a major concern for public health. There might be an optimal dietary fat to carbohydrate ratio for prevention and treatment of obesity. According to the Japanese Dietary Reference Intakes (RDA) for 2010, the optimal fat intake is 20–30% of energy for ages 1–29 y and 20–25% for ages 30 y and over. Upper boundary values of this recommendation were the median of the percentage of energy from dietary fat in Japanese. In a systematic review to estimate the optimal dietary fat to carbohydrate ratio, it was found that obese subjects with hyperinsulinemia (or insulin resistance) lost more weight on a mild low-carbohydrate (LC) (or low-glycemic load diet; 40% carbohydrate, 30–35% fat) than on a low-fat (LF) diet (55–60% carbohydrate, 20% fat), whereas those without hyperinsulinemia showed the opposite. In non-obese primarily insulin-sensitive subjects, decreasing fat rather than carbohydrate intake is generally more effective to prevent obesity. Physiological and molecular evidence supports this conclusion. Increased carbohydrate intake, especially in high-glycemic food, leads to postprandial hyperglycemia and hyperinsulinemia, which are exaggerated in obese insulin-resistant subjects. Even in an insulin-resistant state, insulin is able to stimulate fatty acid synthesis in liver, activate lipoprotein lipase, and prevent lipolysis in adipose tissues, which all facilitate adipose tissue enlargement. Optimal dietary fat to carbohydrate ratio may differ in populations depending on their prevalence for obesity. Because the prevalence of overweight/obesity in Japanese is low, a LF diet is recommended in the general population.

Key Words low-carbohydrate diet, low-fat diet, RDA, insulin resistance, obesity

Obesity in the United States and in much of the westernized world has increased dramatically over the past several decades: 64.5% of adults in the United States are overweight (body mass index [BMI]≥25 kg/m^2 and <30 kg/m^2) or obese (BMI≥30 kg/m^2) (*1*). Overweight/obesity (BMI≥25 kg/m^2) was the most important predictor of diabetes. In the Nurses' Health Study, during 16 y of follow-up, 3,300 new cases of type 2 diabetes were observed in the baseline population of 84,941 female nurses. The relative risk of diabetes was 38.8 for women with a BMI of 35.0 kg/m^2 or higher, 20.1 for women with BMI of 30.0 to 34.5 kg/m^2, and 7.59 for women with BMI of 25.0 to 29.9 kg/m^2, as compared with women who had a BMI of less than 23.0 kg/m^2 (*2*).

In Japan, the prevalence of overweight/obesity (BMI≥25 kg/m^2) in adults is very low compared with the United States: 30.4% in men and 20.2% in women in 2007, according to Japanese cross-sectional nationwide surveys (*3*). However, a strong positive association between baseline BMI and the incidence of diabetes in the follow-up period was observed similar to that in the United States. In a Japanese cohort of healthy men (n=16,829) and women (n=8,370) followed for 7.4 y, new cases of diabetes were documented in 869 men and 224 women (*4*). The relative risk of diabetes was 5.55 for men with a BMI of 25.2 to 26.3, compared with men who had a BMI of 15.0 to 19.7, and the relative risk of diabetes was 5.70 for women with a BMI of 24.4 to 25.9, compared with women who had a BMI of 14.9 to 19.1. Therefore, in Japan also, the prevention of overweight/obese subjects is a major public issue.

The role of dietary fat and carbohydrate in the obesity epidemic has been a hotly debated topic for decades and remains unresolved. To reduce the incidence of obesity in general populations, public statements on optimal ratios of dietary fat to carbohydrate have been issued. Health organizations have recommended diets that are low in total and saturated fat and high in carbohydrates obtained from vegetables, fruits, and whole grains or fiber-rich foods (*5–7*). Dietary guidelines for Americans published in 2005 emphasized the importance of the amount of energy consumed rather than the proportions of protein, fat, and carbohydrate in the diet, pro-

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vided that the macronutrients are within the AMDR, the acceptable macronutrient distribution range: 10–35% of energy from protein, 45–65% from carbohydrate, and 20–35% from fat (*8*). Dietary reference intakes for Japanese issued by the Ministry of Health, Labour, and Welfare in 2010 indicated that optimal fat intake is 20–30% for ages 1–29 y and 20–25% for ages 30 y and over. Upper boundary values of this recommendation were a median of the percentage of energy from dietary fat in Japanese, a recommendation that most Japanese are able to follow.

The present review was conducted to determine the optimal dietary fat to carbohydrate ratio to prevent obesity in the Japanese population. As a result, it was suggested that a mild low-carbohydrate (LC) diet was effective in reducing body weight in obese subjects with hyperinsulinemia (or insulin resistance), whereas a low-fat (LF) diet favored prevention of obesity in non-obese subjects or treatment of obese subjects without hyperinsulinemia. In addition, to elucidate the molecular mechanisms of obesity in response to a carbohydrate-rich diet, several aspects of insulin actions, namely lipogenesis in the liver, activation of lipoprotein lipase (LPL), and lipolysis under insulin-resistance state were also reviewed.

Methods of Review and Definitions

Selection of publications of epidemiological studies. For epidemiological studies, key words "(Diet, Fat-Restricted [MESH]) AND (dietary OR intake OR consumption) AND ((randomized controlled trial [PTYP] OR random [WORD]) OR (cohort studies [MESH] OR risk [MESH] OR (odds [WORD] AND ratio [WORD]) OR (relative [WORD] AND risk [WORD]) OR case control [WORD] OR case-control studies [MESH]))" with a limitation of "humans" were used in PubMed to select all publications through June 1, 2011 (n=1,004), initially to review the effects of dietary fat on mortality and mobility reported therein. From these publications, those related to changes in body weight were selected and reviewed. Other important topics, such as the effects of dietary fat subtypes, i.e., saturated, mono-unsaturated, n-6, and n-3 fatty acids, on obesity, are not discussed in this review. Because several reviews and meta-analyses have been published since the original search date, publications that appeared after this date are presented in this study with comments relating their findings to those of the previous reviews and meta-analyses. To show a visual representation of the results of the review, findings from representative publications are presented here in figures.

Current body weight is the result of the accumulated daily balance of energy intake and expenditure over previous days. Therefore, the causes of obesity are multifactorial, including such factors as physical activity level, energy intake, and food availability. It is difficult to assess these factors, and there are strong limitations to examining the effects of dietary macronutrients on obesity in cross-sectional and prospective studies (confounding factors may not be measured adequately). For this reason, carefully conducted intervention studies in which dietary fat to carbohydrate ratios were changed were mostly selected for this review.

Selection of publications of physiological and molecular studies. In a review of the mechanism of lipogenic action of insulin (covered later in this review), key words "insulin AND obesity AND ((lipogenesis AND liver) OR LPL OR (lipolysis and adipose tissues))" were used initially in PubMed to select appropriate publications, including reviews. Additional publications, which were necessary to describe the effects of insulin in an insulin-resistance state, were included from citations obtained from review articles and personal reference lists.

Definitions of LF and LC diets. The term LF diet is used relative to that of a high-fat diet in the literature; therefore, the absolute amounts of fat were diverse. In general, a high-fat diet means fat intake provides more than 30% of energy and a LF diet means less than 30%. The LC diet has been used in two different types of diet: a very LC diet (ketogenic diet) and a mild LC diet (low-glycemic load diet). Glycemic load is the mathematical product of glycemic index and carbohydrate amount. In the ketogenic diet, carbohydrate intake is less than 40 g/d (*9*), whereas in the low-glycemic load diet, the total amount of carbohydrate is decreased by 10–20% of energy, and foods containing carbohydrate with lower glycemic index were used. In Japanese, median intake of energy in adults was 1,856 kcal/d, and median intakes of carbohydrate, fat, and protein were 258 g/d (56% of energy), 51 g/d (24.8%), and 68 g/d (15%), respectively, according to The National Health and Nutrition Survey in Japan, 2007 (*3*). In this review, these two types of LC diets are reviewed separately.

Results and Discussion

A LF diet prevents obesity in general populations

In a meta-analysis of general populations under free-living conditions, weight loss was positively and independently associated with a reduction in the percentage of energy as fat (0.37 kg/%, p<0.005) (*10*). Another meta-analysis of intervention studies also supports this conclusion (*11*). For every 1% decrease in energy from fat, there was a 0.28-kg decrease in body weight.

A large randomized intervention trial including 48,835 post-menopausal women in the United States (The Women's Health Initiative Dietary Modification Trial) also supports a LF diet for the prevention of obesity (*12*). This intervention included group and individual sessions to promote a decrease in fat intake and did not include weight loss or energy restriction goals. Energy from fat was decreased from 38.8% to 29.8% in the intervention group, whereas there was no alteration of fat intake in the control group (from 38.8% to 38.1%). Concomitantly, energy from carbohydrate was increased from 44.5% to 52.7% in the intervention group, whereas there was no alteration of carbohydrate intake in the control group (from 44.5% to 44.7%). Women in the intervention group lost weight in the first year and maintained a lower weight than the control

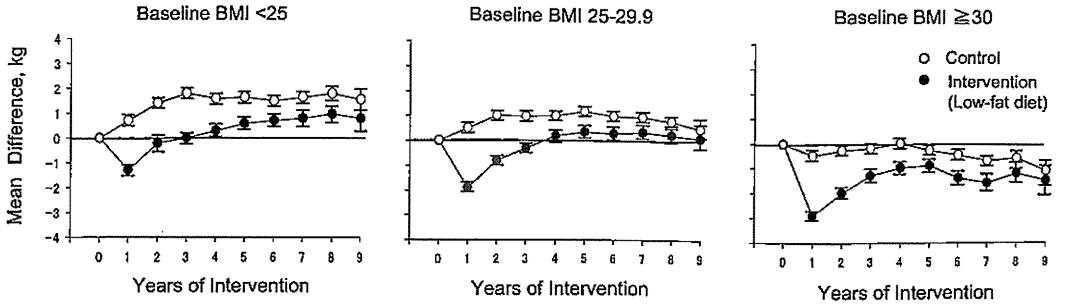

Fig. 1. Differences in body weight by body mass index (BMI) at screening in response to a low-fat (LF) diet. A large randomized intervention trial including 48,835 post-menopausal women during an average 7.5 y of follow-up supports a LF diet (energy from fat decreased from 38.8% to 29.8%) but not energy intake for the prevention of obesity. Women in the intervention groups lost weight in the first year and maintained lower weight than did women in the control groups. No tendency toward weight gain was observed in the intervention groups, whereas body weights in the control groups gradually increased. Error bars indicate 95% confidence intervals. Patient numbers at baseline for the intervention and control groups by BMI: BMI <25 kg/m^2, 5,072 and 7,585; BMI 25–29.9 kg/m^2, 6,940 and 10,446; and BMI≧30 kg/m^2, 7,442 and 11,126, respectively. Reproduced with permission (*12*).

women over an average 7.5 y of follow-up (Fig. 1). No tendency toward weight gain was observed in the intervention group, whereas body weights in the control group gradually increased. In both groups, weight loss was greatest among women who decreased their percentage of energy from fat. Weight loss in response to fat reduction was also slightly greater in subjects with a baseline BMI of <25 kg/m^2.

Several mechanisms for body fat increase in response to a high-fat intake have been proposed (*13*, *14*). Fat is the most energy-dense of the macronutrients and is palatable. Fat produces less of a thermogenic effect than does carbohydrate (*15*, *16*), and fat intake is not regulated, whereas carbohydrate intake is regulated for combustion of carbohydrate substrates (*17*). A prompt increase in glucose oxidation occurs after ingestion of carbohydrate-containing meals, whereas fat oxidation is reduced after food consumption, even when meals provide substantial amounts of fat (*18*). These findings indicate that when energy intake is not intentionally restricted, a LF diet prevents body weight increase in the general population.

A very LC diet (ketogenic diet) decreases body weight in obese subjects

Intervention studies to compare the efficacy of LF and very LC diets to reduce body weight in obese subjects have been conducted and summarized in several meta-analyses (*19–22*). All analyses revealed that a very LC diet is more effective than a LF diet in reducing body weight in obese subjects. In a recent meta-analysis performed by Hession et al., studies comparing the weight loss effects of a very LC diet (less than 60 g/d carbohydrate without intentional energy restriction) against a LF diet with energy restriction (less than 30% fat with 600 kcal/d energy restriction) of more than 6 mo were included (*21*). Among 9 studies analyzed (n=690 in total), 6 studies (*23–28*) showed greater reduction in body weight by LC diet than by LF diet, whereas 3 studies (*29–31*) reported no differences between LC and LF diets in the decrease of body weight when measured at 6 mo of intervention.

However, several adverse effects were observed in a very LC diet. A meta-analysis showed an increase in LDL cholesterol (*22*). Increased blood ketone productions showed unfavorable effects, such as hyperuricemia and orthostatic hypotension (*32*). Recently, even under energy restricted conditions, it was reported that a very LC diet (60% fat/5% carbohydrate) for 6 wk (*33*) or a very LC diet (60% fat/4% carbohydrate) for 1 y (*34*) reduced endothelium-dependent flow-mediated dilation of brachial arteries. A relatively very LC diet (60% fat/20% carbohydrate) worsened the aortic augmentation index (*35*). These adverse effects might be mediated by a large amount of dietary fat. Therefore, a very LC diet was not recommended in the general population.

Mixed evidence that a mild LC diet (low-glycemic diet) decreases body weight in obese subjects

In a Cochrane review, a low-glycemic-index or low-glycemic load diet was compared with a high-glycemic-index or high-glycemic-load diet on different indices of body fat in 6 studies (*36*). Pooled data from 4 studies (*37–40*) showed that weight loss was significantly greater in participants (n=163 in total) receiving the low-glycemic diet (−1.1 kg of difference, p<0.05). Other studies reported a favorable percent change in body mass (*41*) or a favorable change in BMI on a low-glycemic diet (*39*, *42*).

However, two recent intervention studies suggested that reduced-calorie diets resulted in meaningful weight loss, regardless of macronutrient balance. In one study, a total of 34 healthy overweight adults ate a high-glycemic load diet (20% fat, 20% protein, and 60% carbohydrate) or a low-glycemic load diet (30% fat, 30% protein, and 40% carbohydrate) under 30% energy-restricted conditions (*43*). There was no significant change in body weight between the two groups: percentage weight change at 12 mo was −8.04±4.1% in the high-glycemic load diet group and −7.81±5.0% in the low-glycemic load diet group. In the other study, a total of 811 overweight adults (BMI>25 kg/m^2) ate one of four diets for 2 y (*44*). The targeted percentages of energy derived from fat, protein, and carbohydrate in

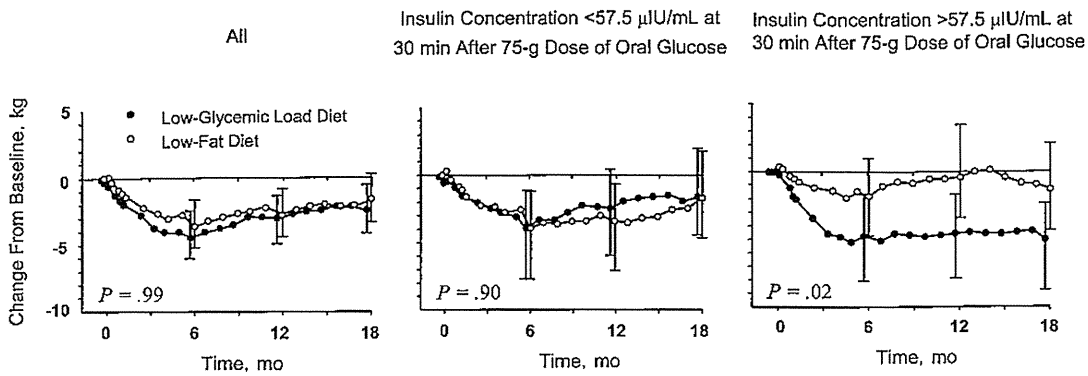

Fig. 2. Changes in body weight in insulin-sensitive and -resistant obese subjects. Obese nondiabetic insulin-sensitive (insulin concentration ≤57.5 μU/mL at 30 min after 75-g dose of oral glucose, n=28) and obese nondiabetic insulin-resistant (insulin concentration >57.5 μU/mL at 30 min after 75-g dose of oral glucose, n=28) young adults were randomized to either a low-fat diet (55% carbohydrate of energy, 20% fat, and 25% protein) or a low-glycemic load diet (or a low-carbohydrate diet; 40% carbohydrate, 35% fat, and 25% protein) for a 6-mo intervention and a 12-mo follow-up period. In the insulin-resistant groups, a low-glycemic load diet produced a greater decrease in weight than did the low-fat diet at 18 mo. Reproduced with permission (47).

the four diets were 20%, 15%, and 65% (LF/low protein [LP] diet); 20%, 25%, and 55% (LF/high protein [HP] diet); 40%, 15%, and 45% (LC/LP diet); and 40%, 25%, and 35% (LC/HP diet). At 2 y, weight loss remained similar in those who were assigned to a diet with 15% or 25% protein (3.0 and 3.6 kg, respectively), in those assigned to a diet with 20% fat or 40% fat (3.3 kg for both groups), and in those assigned to a diet with 65% carbohydrate or 35% carbohydrate (2.9 and 3.4 kg, respectively). There were no differences in reduction of body weights between groups when measured at 6, 12, and 18 mo. When considering the results of recent intervention studies, it is not conclusive that a mild LC diet is preferable for obese subjects.

A mild LC diet preferentially reduces body weights in obese subjects with hyperinsulinemia (insulin resistance)

The studies described above comprised mixed populations of insulin-sensitive and insulin-resistant obese subjects. However, when only the publications that separately examine the effects of LF and mild LC diets on body weight decrease in insulin-sensitive and insulin-resistant subjects were selected, a clear picture appeared. In obese subjects with hyperinsulinemia and insulin resistance, a mild LC diet was more likely than was a LF diet to reduce body weight under energy-restricted conditions (45–47).

In the first intervention study, obese nondiabetic insulin-sensitive (fasting insulin <10 μU/mL, n=12) and obese nondiabetic insulin-resistant (fasting insulin >15 μU/mL, n=9) women were randomized to either a LF diet (60% carbohydrate, 20% fat, and 20% protein) or a mild LC diet (40% carbohydrate, 40% fat, and 20% protein) for 16 wk under a 400-kcal energy deficit/d (45). A marked difference was observed in body weight reduction. Insulin-sensitive women on the LF diet lost 13.5±1.2% (n=6) of their initial body weight, whereas those on the mild LC diet lost 6.8±1.2% (n=6). In contrast, among the insulin-resistant women, those on the mild LC diet lost 13.4±1.3% (n=5) of their initial body weight as compared with 8.5±1.4% (n=4) lost by those on the LF diet. Differences in resting metabolic rate, physical activity, or energy intake between the two dietary groups were not observed (45).

In the second intervention study, obese (BMI 25–29.9 kg/m^2) insulin-sensitive (insulin concentration ≤66 μU/mL at 30 min after 75-g dose of oral glucose, n=16) and obese nondiabetic insulin-resistant (insulin concentration >66 μU/mL at 30 min after 75-g dose of oral glucose, n=16) adults were randomized to either a LF diet (or high-glycemic diet; 60% carbohydrate, 20% fat, and 20% protein) or a mild LC diet (or low-glycemic diet; 40% carbohydrate, 30% fat, and 30% protein) for 6 mo at 30% calorie restriction compared to baseline individual energy needs (46). In the insulin-resistant groups, the mild LC diet produced a greater decrease in weight (−10.2 vs −6.2 kg) than did the LF diet at 6 mo. There were no significant differences in weight decrease between the mild LC and LF diets in the insulin-sensitive groups.

In the third intervention study, obese nondiabetic insulin-sensitive (insulin concentration ≤57.5 μU/mL at 30 min after 75-g dose of oral glucose, n=28) and obese nondiabetic insulin-resistant (insulin concentration >57.5 μU/mL at 30 min after 75-g dose of oral glucose, n=28) young adults were randomized to either a LF diet (or high-glycemic diet; 55% carbohydrate, 20% fat, and 25% protein) or a mild LC diet (or low-glycemic diet; 40% carbohydrate, 35% fat, and 25% protein) for a 6-mo intervention and 12-mo follow-up period (47). Although both the mild LF- and LC-diet groups decreased energy intake similarly by 400 kcal/d, effects of LF and LC diets on body weight reduction were markedly different between the insulin-sensitive and -resistant groups. In the insulin-resistant groups, the mild LC diet produced a greater decrease in weight (−5.8 vs −1.2 kg) and body fat percentage (−2.6 vs −0.9%) than did the LF diet at 18 mo (Fig. 2). There were no significant differences in decreases in weight and body

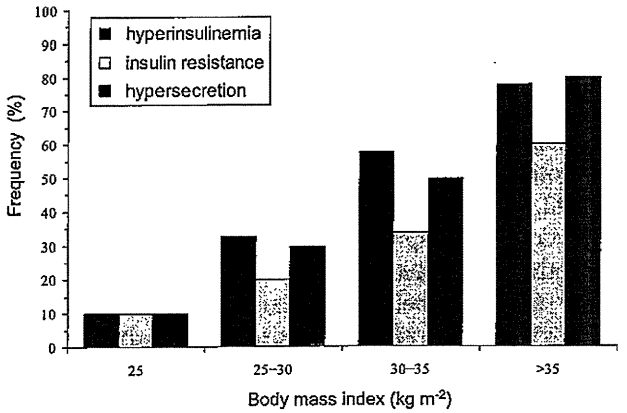

Fig. 3. Prevalence rates of insulin resistance, hyperinsulinemia, and insulin hypersecretion (all defined as the top decile of the respective distributions in lean subjects) as a function of the body mass index (BMI). Black bars, hyperinsulinemia; light gray bars, insulin resistance; dark gray bars, hypersecretion. Reproduced with permission (*51*).

fat between the mild LC and LF diets for any subjects or in the insulin-sensitive group.

Metabolic syndrome is closely associated with hyperinsulinemia (*48*). A recent study examining the effects of LF and mild LC diets in subjects with and without metabolic syndrome under 500-kcal/d energy deficit conditions indicated that a LF diet is preferable in insulin-sensitive obese subjects (*49*). In this study, 202 obese subjects were randomized to either a LF diet (55–60% carbohydrate, less than 30% fat, and 15% protein) or a mild LC diet (or low-glycemic diet; 30–35% carbohydrate, 35–40% fat, and 25–30% protein) for a 12-mo follow-up period. In the subjects with metabolic syndrome, both the mild LC and LF diets were equally effective in reducing waist circumference, whereas in subjects without metabolic syndrome, the LF diet was preferable to that of the mild LC diet: the change in waist circumference was -7.8 ± 7.1 cm in the LF diet group versus -3.8 ± 5.0 cm in the mild LC diet group.

Thus, these four studies suggest that a mild LC diet preferentially reduces body weight in obese subjects with hyperinsulinemia (insulin resistance), whereas a LF diet preferentially reduces body weight in obese subjects without hyperinsulinemia.

Physiological aspects of a mild LC diet making it preferable in obese, insulin-resistant subjects to reduce body fat

It is known that not all obese subjects show insulin resistance (*50*, *51*). In a European study of insulin resistance in the obese, hyperinsulinemia, insulin resistance, and insulin hypersecretion were found to increase linearly with an increase in BMI (Fig. 3) (*51*). In this study, hyperinsulinemia was defined as the upper 10% of fasting plasma insulin concentrations in the lean groups. Insulin resistance was defined as the bottom 10% of glucose disposal estimated by euglycemic insulin clamp technique in the lean groups, and insulin hypersecretion was defined as the upper 10% of the distribution of posthepatic insulin delivery rate. According to these criteria, roughly one-half of the obese subjects (BMI>30 kg/m^2) were insulin resistant. The frequency of insulin resistance was 20% in subjects with a BMI of 25–30 kg/m^2, 34% in subjects with a BMI of 30–35 kg/m^2, and 60% in subjects with a BMI of >35 kg/m^2, relative to 10% in subjects with a BMI of 25 kg/m^2 (*51*). Similar trends were observed in regard to hyperinsulinemia and insulin hypersecretion.

Insulin resistance in liver and skeletal muscles elevates glucose concentrations, by which insulin secretion is increased. Moreover, pancreatic beta cells can acutely assess the body's sensitivity to insulin and translate this information into an insulin response that is precisely balanced to offset the severity of insulin resistance (*52*). In patients with insulin resistance, the increment of insulin secretion from β-cells in response to a fixed amount of glucose is greater than that in normal subjects (*53*). Therefore, the sensitivity of glucose to an increased blood insulin level is augmented in obese subjects. Diets with higher glycemic load resulted in higher postprandial insulin concentration in a dose-dependent manner in lean young adults (*54*). It is well known that obese subjects show hyperinsulinemia after oral glucose tolerance testing (glucose is a substance of high glycemic load) (*55*, *56*). Postprandial hyperglycemia and hyperinsulinemia augmented by an increase in dietary carbohydrate intake in obese subjects may further promote fat cell enlargement (*57*).

Increased blood insulin stimulates the synthesis of fatty acid in liver and the preferential uptake of fatty acids in adipose tissues to store fat and prevents lipolysis in adipose tissues, all of which facilitate adipose tissue enlargement. Furthermore, these lipogenic effects of insulin are not impaired in obese subjects, whereas the glucose-lowering effects of insulin (inhibition of gluconeogenesis/glycolysis in the liver and stimulation of glucose uptake in skeletal muscles) is severely impaired. Recently, it was shown that hyperinsulinemia is associated with increased production of intestinal apoprotein B-48, which is one of the causes of postprandial hypertriglycemia (*58*). This effect of insulin also indirectly promotes obesity. In the following sections, the mechanisms of insulin-mediated increases in lipid synthesis and fat accumulation in the insulin-resistant state are reviewed.

Insulin-induced lipogenesis in liver is not impaired in insulin-resistant animals or humans

The insulin signaling pathway is thought to proceed through receptor-mediated tyrosine phosphorylation of insulin receptor substrate (IRS)-1 and/or IRS-2. This leads to activation of phosphoinositide 3-kinase (PI3K) and activated Akt (also known as protein kinase B). In activating hepatic lipogenesis, insulin increases transcription of genes encoding acetyl-CoA carboxylase, fatty acid synthase, and others. These actions are caused by an insulin-induced increase in sterol regulatory element-binding protein-1c (SREBP-1c) mRNA (*59*).

To examine the insulin signaling pathway and lipogenesis in the insulin-resistant state, two different ani-

mal models of insulin resistance and hyperinsulinemia, those of lipodystrophy induced by overexpression of the aP2-SREBP1c transgene in adipocytes and obesity induced by mutational disruption of the leptin gene (ob/ob mice) were investigated (*60*). Both animal models showed a reduction of IRS-2 mRNA and protein and increased gluconeogenesis in livers, whereas they showed an increase in SREBP-1c mRNA and lipogenesis. IRS-1 mRNA in the liver was not altered in these animal models. In addition, prolonged insulin treatment in isolated rat hepatocytes led to a fall in IRS-2 mRNA and protein and an increase in SREBP-1c transcript, suggesting that chronic hyperinsulinemia promotes gluconeogenesis in the liver and hyperglycemia, whereas it stimulates fatty acid synthesis in the liver and hypertriglycemia (*60*). It was shown with IRS-1 and IRS-2 liver knockout mice that IRS-1 could convey signals to increase SREBP-1c mRNA and lipogenesis (*61, 62*). The complete blockage of insulin signaling observed in liver insulin receptor knockout mice showed a decrease in the expression of SREBP-1c (*63*), suggesting that selective insulin resistance may occur in animal models of insulin resistance (*64*). Recently, a branch point in the insulin signaling pathway that may account for selective insulin resistance (in which insulin loses its ability to block glucose production but retains its ability to stimulate lipogenesis) was identified (*65*). In rat hepatocytes, subnanomolar concentrations of rapamycin, an inhibitor of the mammalian target of rapamycin complex 1 (mTORC1), blocked insulin induction of SREBP-1c but had no effect on insulin suppression of phosphoenolpyruvate carboxylase (PEPCK), suggesting that the kinase complex designated mTORC1 was a branch point in the insulin signaling pathway. Therefore, the IRS-1/Akt/mTORC1 pathways are thought to mediate the increase of lipogenesis in the insulin-resistant state.

The finding that insulin-induced lipogenesis in the liver was not impaired in the insulin-resistant state in animal studies could apply to humans. The pattern of stored energy distribution derived from a high-carbohydrate meal is different in young, lean, insulin-resistant individuals (fasting insulin concentration of 12.1±1.2 μU/mL) compared with young, lean, insulin-sensitive individuals (fasting insulin concentration of 7.6±0.6 μU/mL) (*66*). In contrast to the insulin-sensitive subjects, who stored most of their ingested energy in the liver as glycogen, the insulin-resistant subjects had a marked defect in muscle glycogen synthesis and diverted much more of their ingested energy into hepatic de novo lipogenesis, as assessed by incorporation of deuterated water into plasma triglyceride, resulting in increased liver and plasma triglycerides (TGs). Increasing very-low-density lipoprotein-TG secretion from the liver may lead to increased fat accumulation in adipose tissue (*67*). Therefore, insulin activation of the liver IRS-1/Akt/mTORC1 pathway in the insulin-resistant state may lead to obesity.

An increase in lipoprotein lipase (LPL) activity in adipose tissue in response to insulin is not impaired in obese subjects

LPL, located on the capillary endothelium of tissues, catalyses the rate-limiting step in the hydrolysis of TGs from circulating chylomicrons and very-low-density lipoproteins. Most LPL is found in adipose tissues and skeletal muscles, where some of the liberated free fatty acids are taken up and are either stored or oxidized, respectively (*68*). In healthy humans, a combination of stable isotope labeling and arteriovenous difference measurements in adipose tissues showed that in postprandial periods, there is preferential uptake of fatty acids released from chylomicrons by LPL in adipose tissues and also a release of LPL-derived fatty acids into plasma (*69*). Therefore, an increase in LPL activity in adipose tissues may promote fat cell enlargement via increased uptake of fatty acids into adipocytes, in addition to an increased supply of fatty acids to muscle and liver.

Regulation of LPL activity is complex and is controlled by several modulators, such as apoproteins and angiopoietin-like proteins ANGPTL3 and ANGPTL4 (*70*). LPL is active as a dimer, whereas its monomer is inactive. ANGPTL4 inhibits LPL activity by promoting the conversion of active LPL dimers into inactive LPL monomers. Insulin not only increases the level of LPL mRNA but may also regulate LPL activity through both posttranscriptional and posttranslational mechanisms (*71*). The fact that feeding increases active dimeric LPL from inactive monomeric LPL in adipose tissues suggests that insulin may stimulate dimer formation of LPL by an unknown mechanism (*72*). Glucose also increases adipose tissue LPL activity and enhances the stimulatory effects of insulin, possibly by the glycosylation of LPL (*73*).

In humans, feeding or insulin/glucose infusion stimulates LPL activity in adipose tissues, whereas its activity decreases in skeletal muscles (*74*). This divergent response would serve to direct lipoprotein TG-derived fatty acids away from muscle to adipose tissue for storage. A high-carbohydrate diet for 16 d in normal-weight subjects increased postprandial LPL activity in adipose tissue, with elevation of blood glucose and insulin concentrations after meals, relative to a high-fat diet (*75*). Therefore, increased insulin and glucose from a high-carbohydrate diet may promote obesity via activation of LPL in adipose tissues.

The LPL activity in adipose tissues in response to insulin during maintenance of euglycemia was examined in 22 obese and 8 normal-weight subjects (*76*). Basal levels of LPL activity per g of fat tissue in the obese and control groups were 18.7±2.0 and 9.6±2.7 nEq/g/min, respectively. When the responses of LPL in absolute change from basal values were compared between the obese and control groups, no significant differences were found. However, because of the higher baseline LPL activity in the obese subjects, the percent increase in LPL from the basal value was significantly blunted in obese subjects. Basal LPL activity expressed per 10^6 cells correlated positively with cell size, and both the

obese and normal-weight subjects were found to respond similarly to insulin. These data suggest that insulin activates LPL in adipose tissues in obese subjects, irrespective of insulin resistance.

Inhibition of lipolysis in adipocytes in response to insulin is not impaired in insulin-resistant subjects

The concentration of blood free fatty acids (FFA) is determined primarily by their rate of appearance from adipose tissues (lipolysis) and also by their rate of disappearance from plasma. Blood FFA concentrations are elevated during fasting and decreased after feeding. Lipolysis is stimulated by catecholamines during fasting and inhibited by insulin after feeding. If the antilipolytic effect of insulin in obese subjects were impaired due to insulin resistance, fat mass would be smaller in obese subjects. However, most of the studies suggested that insulin resistance is not observed at this step in obese subjects (see following paragraph), although the resistance of insulin to increased glucose oxidation in enlarged adipocytes was clearly shown and is due to a marked decrease in GLUT4 in adipocytes (*77*, *78*).

The antilipolytic effects of insulin on fat cells of different sizes were examined in the 1970s by measuring glycerol release. Basal lipolysis was larger in larger cells (*79*). The antilipolytic effects of insulin on noradrenalin-stimulated lipolysis were more pronounced in the large cells at all tested concentrations (*80*, *81*). Responsiveness and sensitivity to insulin was not altered in adipose tissues of either control or obese subjects (*82*). Rather, a marked resistance to the lipolytic effect of noradrenalin was observed in isolated adipocytes from obese subjects (*83*).

In vivo studies also show that the antilipolytic effect of insulin is not impaired in obese subjects. Both antilipolytic and antiketotic actions occurred at lower insulin concentrations (<90 μU/mL) than those required for hypoglycemic activity (>1,000 μU/mL) (*84*), suggesting that marked insulin resistance might be required to reduce antilipolytic action in adipose tissues. Decreases in blood FFA and glycerol observed during oral glucose tolerance tests were not impaired in obese subjects (*85*). Insulin and glucose infusion rapidly produced antilipolysis in obese and normal groups, as evidenced by large falls in FFA at 20 min after insulin infusion, where FFA was 47% of the basal level in the obese subjects and 31% of the basal level in the normal subjects (*76*).

Triglycerides in tissues are hydrolyzed in a sequential process involving different lipases. Adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) are necessary for proper hydrolysis of tri- and diglycerides, respectively. The last step in lipolysis is performed by monoglyceride lipase (MGL), which hydrolyzes monoglycerides to form glycerol and fatty acids (*86*). The activity of ATGL and HSL is tightly regulated by catecholamines and insulin. β-Adrenergic stimulation of the G-protein-coupled receptor activates adenylate cyclase to increase cellular cAMP levels. The antilipolytic action of insulin is mediated by lowering cAMP levels via activation of phosphodiesterase 3B (*87*). The IRS-1/PI3K/PDE3IK (an insulin-stimulated protein serine kinase) signaling pathway is involved in PDE3B activation (*88*). cAMP binding to protein kinase A (PKA) induces phosphorylation of HSL and perilipin, a protein coating the lipid droplet. PKA phosphorylation of HSL causes HSL translocation from the cytosol to the lipid droplet, whereas phosphorylation of perilipin by PKA alleviates the barrier function of this protein and promotes lipolysis (*89*). ATGL is phosphorylated on two conserved serine residues (Ser 404 and 428), although PKA does not phosphorylate ATGL (*90*). However, insulin treatment downregulates ATGL mRNA levels in adipocytes (*91*, *92*). To my knowledge, it has not been shown that decreases in cAMP concentration or ATGL mRNA in adipocytes in response to insulin are blunted in adipocytes from obese subjects.

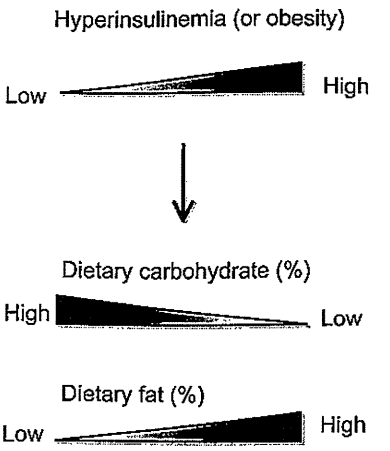

Fig. 4. A proposed model of optimal dietary fat to carbohydrate ratio according to the degree of hyperinsulinemia (or obesity). A key to macronutrient balance in the reduction of body weight is the state of hyperinsulinemia (insulin resistance or obesity); thus, optimal dietary fat to carbohydrate ratios may differ between prevention and treatment of obesity. A mild low-carbohydrate diet (40% carbohydrate) is preferable for obese, hyperinsulinemic, insulin-resistant subjects, whereas a low-fat diet (20–25% fat) is preferable for normal-weight, normoinsulinemic, insulin-sensitive subjects.

Shift from a mild LC diet to a LF diet during obesity treatment (hypothesis)

When a mild LC diet is given to obese subjects, body weights might decrease with improvement in hyperinsulinemia and insulin resistance. Data from the National Weight Control Registry of people who were successful in losing weight and maintaining reduced body weight show that despite wide variation in the methods used to lose body weight, there was remarkable similarity in how they maintained the weight loss, including a diet that was, on average, 24% fat (*93*). Therefore, fat intake might be gradually decreased with a concomitant increase in carbohydrate intake with improvement in obesity (Fig. 4).

Conclusions

In terms of epidemiological, physiological, and molecular aspects, the optimal dietary fat to carbohydrate ratio varies due to the amount of body fat present and

to hyperinsulinemia (insulin resistance). No evidence was found that the lipogenic effects of insulin were impaired in subjects with insulin resistance. In general, in non-obese subjects, most of whom are insulin sensitive, decreasing fat intake is more effective than decreasing carbohydrates to prevent obesity. However, for obese subjects with insulin resistance, a mild LC diet favors a reduction in body weight. The optimal dietary fat to carbohydrate ratio may differ depending on whether the goal is prevention or treatment of obesity, and public guidelines on macronutrients should either be based on the prevalence of obesity in the target society or individualized.

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たんぱく質・アミノ酸の必要量に関する研究

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摂取エネルギーは十分であっても，摂取たんぱく質が不足した時にクワシオコールが発症し，感染症などを併発しやすい。たんぱく質必要量は，身体の構造と機能を正常に維持するために必要な摂取量（代謝要求量）であり，食事たんぱく質必要量は，それらの要求量を満たす量である。たんぱく質必要量は，窒素出納法によって決定されてきた。しかし，窒素出納法にはその方法上様々な問題がある。指標アミノ酸酸化（IAAO）法は，窒素出納法とは原理が大きく異なり，窒素出納法の代替法として動物とヒトにおいて開発された。私たちは，指標アミノ酸酸化法を用いてラットと健康成人男性の食事たんぱく質必要量とたんぱく質の質を再評価した。その結果，指標アミノ酸酸化法は全てのライフステージ（幼児，小児，学童，成人，高齢者）の食事たんぱく質必要量の評価だけでなく，代謝要求量が大きく変化している術後，傷害，感染症などいろいろな病態時の食事たんぱく質必要量の推定，また，たんぱく質の質の評価にも利用できることがわかった。

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はじめに

たんぱく質必要量に関する議論は1955年に「タンパク質必要量に関するFAO委員会」[1]で行われた。このFAO委員会[1]では，人の不可欠アミノ酸必要量パターンを重視し，これと同じ理想的なアミノ酸組成を持つたんぱく質（比較基準たんぱく質）の必要量が決められた。

1963年の「タンパク質必要量に関するFAO/WHO合同専門グループ」[2]では，たんぱく質必要量は無たんぱく質食摂取時に身体から失われる不可避窒素損失量によって規定されるという新しい概念が導入された。

1971年の「エネルギーとタンパク質の必要量に関するFAO/WHO合同特別専門家委員会」[3]では，エネルギーとたんぱく質が初めて一緒に検討された。この特別専門家委員会では，生物価の高いたんぱく質であっても，窒素平衡維持のための最小必要量は，不可避窒素損失量よりも大きいとした。また，集団に対する必要量を決定する場合，エネルギーとたんぱく質とでは考え方が異なることも明確にした。

1981年の「エネルギーとタンパク質必要量に関する協議会」[4]では，個人のたんぱく質必要量を最適レベルの身体活動を行ってエネルギー平衡を維持している人の，身体から失われる窒素と等しい最小の食事たんぱく質摂取量と定義された。子どもや妊婦，授乳婦では，良好な健康状態を維持しながら，組織の増殖肥大，あるいは乳汁分泌に必要なたんぱく質も含まれる。すべての必要量の算定値は，適当な期間続けて求められた要求量を参考に決められた。このような期間の摂取量は，ある特定の1日の摂取量と区別するために，「習慣的」あるいは「日常の摂取量」といえる。習慣的摂取量を「1日当りの摂取量」で表しているが，これらの量が毎日摂取しなければならない量であることを意味しているわけではない。

2002年の「タンパク質とアミノ酸の必要量に関するWHO/FAO/UNU合同専門家協議会」[5]では，成人のアミノ酸必要量の算定根拠が窒素出納法の成績から^{13}C-指標アミノ酸を用いたトレーサー実験に変わった。たんぱく質必要量については，引き続き窒素出納法の成績が用いられたが，皮膚などからの損失は1981年の8 mgN/kg/日より低い5 mgN/kg/日の値が採用され，安全摂取量が0.75から0.83 g/kg/日に改定された。

このように，たんぱく質とアミノ酸の必要量に関する研究は，確実に進歩してきた。しかし，窒素出納法と^{13}C-指標アミノ酸を用いたトレーサー実験の結果の解釈等，議論を必要とする課題は山積している。本稿では，私たちの最新のデータを示すとともにたんぱく質とアミノ酸の必要量の考え方について概説する。

Ⅰ．たんぱく質欠乏症

たんぱく質欠乏症は，イギリス領黄金海岸（現ガーナ共和国）で1933年にCicely D. Williams[6]によって最初に報告され，クワシオコール（kwashiorkor）と命名された。クワシオコールの主原因は，エネルギーは足りているがたんぱく質が不足することである。浮腫，毛髪の変

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色，ペラグラ様皮疹，下痢，低たんぱく質血症，発育障害などが特徴である。1990年から1年間，国際協力事業団（現国際協力機構）の専門家として，ガーナ共和国ガーナ大学医学部野口記念医学研究所で，現地の乳幼児の栄養調査と栄養改善プログラムの開発に関わることができた。この時はじめて，途上国における栄養問題の重要性を実際に感じることができた。現地では，ガーナ共和国保健省，WHO，UNICEFなど関係組織とともに2回のセミナーを開催した[7~9]。

Ⅱ．たんぱく質必要量の考え方

食事中のたんぱく質必要量とは，生体が必要とする量，すなわち代謝要求量を満たすために必要な摂取量である（図1）。代謝要求量は，アミノ酸を消費する代謝経路を維持するために必要な量と成長，妊娠，授乳など特別な必要量の和として求めることができる。維持必要量とは，アミノ酸を消費し，尿，糞便，皮膚，毛髪，分泌物など生体から排泄される全ての損失を補完できる量をいう。

たんぱく質の必要量がエネルギー摂取量に大きく影響を受けるにもかかわらず，健康づくりのための運動基準相当の運動を行った時のたんぱく質必要量については全く検討されていなかった。そこで，厚生省（現厚生労働省）の健康づくりのための運動所要量（現健康づくりのための運動基準2006）に相当する運動施行時にたんぱく質必要量が変動するかを検討した結果，運動所要量に相当する運動を行ってもたんぱく質必要量を増加させる必要がないことを明らかにした[10,11]。窒素出納法を用いたこの研究では，尿，糞便，だけでなく皮膚など生体から排泄される窒素損失量を測定した。表1に示したように，皮膚などから排泄される窒素を測定していれば，「健康づくりのための運動（200～400 kcal/日のエネルギー消費）をしても摂取するたんぱく質を増やす必要はない」と結論できる。しかし，皮膚などから排泄される窒素を測定しないと，「健康づくりのための運動（200～400 kcal/日のエネルギー消費）を実施すると，摂取するたんぱく質を増やさなくても体たんぱく質の蓄積が増加する」という結論になる。すなわち，窒素出納試験を用いてたんぱく質代謝を評価するためには，尿，糞便，皮膚，毛髪，分泌物など生体から排泄される全ての損失を測定しなければならない。実際に，尿，糞便，皮膚，毛髪，分泌物など生体から排泄される全ての損失を測定することは非常に困難である。

食事たんぱく質必要量とは，代謝要求量を満たし，窒素平衡を維持するために食事として摂取すべきたんぱく質またはその成分であるアミノ酸，またはその両者である。したがって，食事たんぱく質必要量は次式で示すことができる。

食事たんぱく質必要利用＝代謝要求量÷利用効率

食事からの窒素摂取量がゼロで，エネルギーとその他の栄養素が十分量摂取されている場合に，尿中に排泄される窒素量は徐々に減少し，一定の値となる。尿中に排泄される窒素量が一定となるためには5～7日間を要することが報告されている（図2）[12]。すなわち，食事からのたんぱく質摂取量を変化させた時には，少なくとも7日間の適応期間を要し，このようにして得られた窒素平

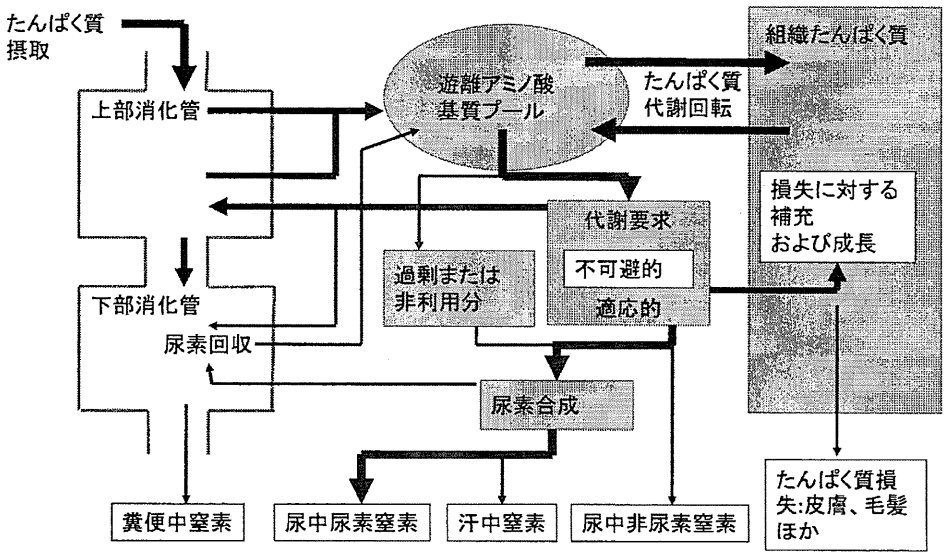


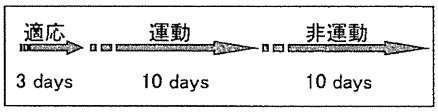
図1　アミノ酸の代謝要求の概略図

成人の不可欠アミノ酸必要量は，窒素平衡をもたらすために必要なアミノ酸摂取量として測定される。

表1　1.08 g/kg/日のたんぱく質摂取量時の窒素出納値に及ぼす運動（400 kcal/日）の影響[10]

Subjects	Non-exercise period						Exercise period					
	IN	FN	TD	DN	UN	NB	IN	FN	TD	DN	UN	NB
L	181.9	15.5	98.3	7.6	150.6	8.1	181.0	15.5	98.3	17.6	149.3	−1.4
M	177.2	19.4	96.0	3.5	124.8	29.5	177.8	16.1	97.9	9.7	123.5	28.5
N	181.9	12.6	99.9	10.3	145.9	13.1	180.0	17.5	97.2	18.4	134.0	10.0
O	179.0	16.8	97.6	8.2	147.9	6.1	179.2	17.8	97.0	9.8	145.5	6.1
P	177.7	20.7	95.3	8.4	152.0	−3.4	178.3	24.0	93.5	9.5	136.8	8.0
Q	180.5	17.7	97.1	5.6	142.2	15.0	179.2	16.8	97.6	7.4	135.6	19.5
Mean	179.7	17.1	97.4	7.3	143.9	11.4	179.3	18.0	96.9	12.1*	137.5*	11.8
SD	2.0	2.9	1.6	2.4	10.0	11.0	1.2	3.1	1.7	4.7	9.1	10.6

($p<0.05$ vs non-exercise period)



摂取たんぱく質量：1.08 g/kg/日
エネルギー摂取量：42.8〜43.8 kcal/kg/日
代謝性糞中排泄量：12.4 mgN/kg/日
運動強度：65% VO_2max
運動によるエネルギー消費量：400 kcal/日

略語：IN，摂取窒素：FN，糞中窒素：TD，真の吸収率：DN，経皮窒素：UN，尿中窒素：NB，窒素出納値

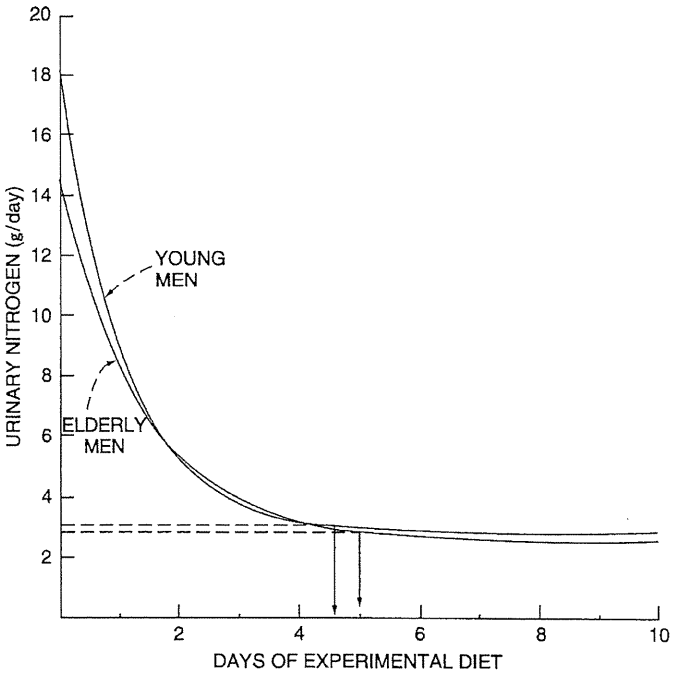


図2　無たんぱく質摂取後の尿中窒素排泄量の変化

（J Nutr; 108, 97（1978）を改変）

衡維持に必要な食事からのたんぱく質摂取量は，最小たんぱく質必要量と定義できる。

私たちは，たんぱく質代謝には，適応現象が存在することに着目した。習慣的なたんぱく質摂取状態に適応しており，低たんぱく質代謝適応が成立していない状態（たんぱく質摂取レベルを変更した実験日）で，たんぱく質代謝を推定できる指標アミノ酸酸化（indicator amino acid oxidation: IAAO）法を用いることにより，習慣的なたんぱく質摂取状態でのたんぱく質代謝要求量の推定を試みた。たんぱく質の摂取量を習慣的な摂取量よりも少ない摂取量に変化させた時に，その少ない摂取たんぱく質量でのたんぱく質代謝状態を反映する期間は，少なくとも7日間を要する（図3）。つまり，一過性のたんぱく質代謝応答は，その時の習慣的なたんぱく質摂取時の代謝を反映していることを意味する。習慣的に十分量のたんぱく質を摂取している時に，生体内で合成されるたん

ぱく質と分解されるたんぱく質はほぼ一定であり，たんぱく質代謝回転が定常状態であると考えられる。この時たんぱく質合成に必要なアミノ酸は，体内の遊離アミノ酸プールから供給される。この遊離アミノ酸プールのアミノ酸の供給源は，食事，体たんぱく質の分解，および体内合成である（図4）。

たんぱく質必要量とたんぱく質代謝要求量は，その意味するところが異なる。窒素出納法で求めるたんぱく質必要量は，低たんぱく質栄養状態に適応した状態での最小たんぱく質摂取量を意味する。この最小たんぱく質必要量を下回る摂取量が続くと，たんぱく質欠乏症が発症すると考えられる。一方，IAAO 法で求めるたんぱく質代謝要求量は，その時の習慣的なたんぱく質摂取時の代謝を維持するために必要なたんぱく質摂取量を意味する。このたんぱく質代謝要求量を下回る摂取量が続いてもたんぱく質欠乏症が発症することはなく，その時のたんぱく質摂取量でのたんぱく質代謝に適応していくと考えられる。

たんぱく質摂取量を窒素出納法で求めたたんぱく質必要量に適応させた時のたんぱく質代謝要求量は，たんぱく質必要量と一致すると考えられる。すなわち，窒素出納法で求めた最小たんぱく質必要量にたんぱく質代謝が適応すると，体内の遊離アミノ酸プールもその時のたんぱく質代謝に見合ったサイズになると推定される。このため，この低たんぱく質状態に適応したたんぱく質代謝を維持するために必要なたんぱく質代謝要求量は，最小たんぱく質必要量と一致すると考えられる。

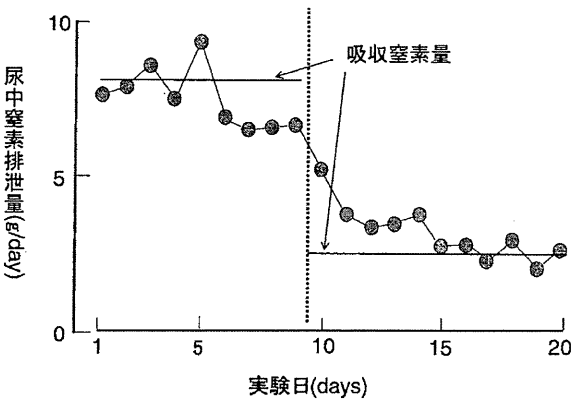


図3　たんぱく質の摂取量を変化させた時の尿中窒素排泄量の変化

Ⅲ．指標アミノ酸酸化（IAAO）法の原理

IAAO 法の理論は，食事に含まれているあるアミノ酸がたんぱく質代謝要求量以下であれば（すなわち，制限アミノ酸），他のすべての不可欠アミノ酸（^{13}C-標識アミノ酸を含む）はたんぱく質合成には利用することができず，この余分の不可欠アミノ酸は酸化されて，不可逆的に重炭酸塩プールに遊離され，呼気中に排泄される，というものである。例えば，図5に示したように，遊離アミノ酸プール中の制限アミノ酸（ここではリシン）がたんぱく質要求量よりも少ないと，たんぱく質合成量は低下し，余った指標アミノ酸（ここでは［1-^{13}C］フェニルアラニン（^{13}C-Phe））の酸化量が増加し，その炭素骨格は $^{13}CO_2$ として排泄される。この $^{13}CO_2$ 排泄量は，摂取するたんぱく質量が増加し，遊離アミノ酸プール中の制限アミノ酸（ここではリシン）がたんぱく質要求量たんぱく質要求量と等しくなるまで減少する。制限アミノ酸（ここではリシン）が合成すべきたんぱく質に必要な量以上に供給されると，たんぱく質をそれ以上合成する必要がないので，指標アミノ酸由来の呼気 $^{13}CO_2$ 排泄量は一定となる（図6）。この屈曲点が食事たんぱく質必要量と考えられる。この条件を満たすためには，指標アミノ酸として ^{13}C-Phe を利用する場合に，組織や血液中の ^{13}C-Phe と ^{12}C-Phe 濃度の割合と量が一定であることが必要である。

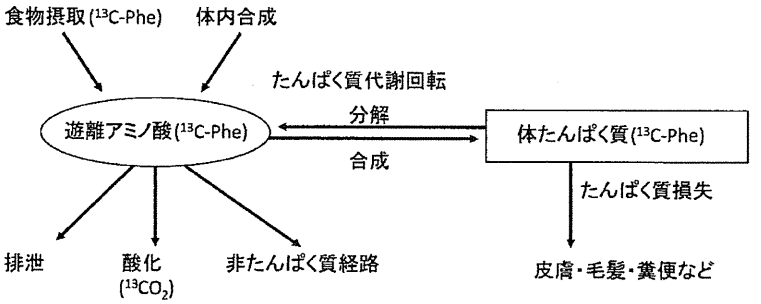


図4　たんぱく質必要量の考え方

成人のたんぱく質必要量は，体外に失われる窒素量を補い，体たんぱく質量を維持するために必要な食事たんぱく質の最小摂取量である。

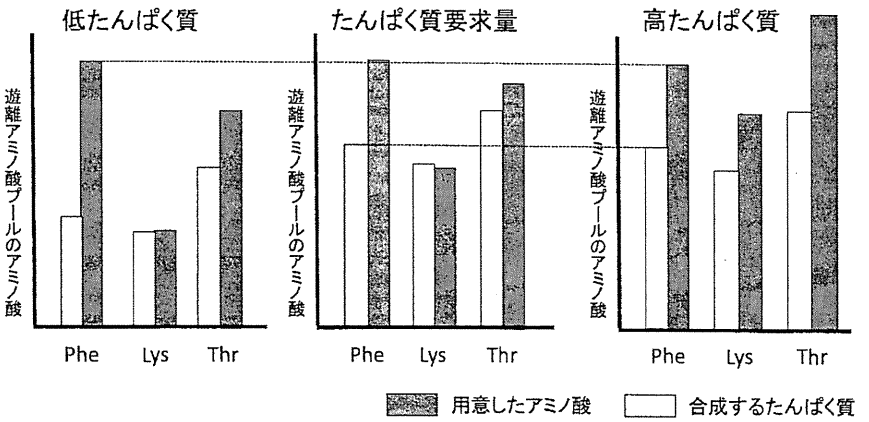


図5　指標アミノ酸酸化法の原理

リシンが第一制限アミノ酸と仮定すると，合成するたんぱく質よりも用意したアミノ酸の量が少ない状態（低たんぱく質）では，合成すべきたんぱく質に必要なリシン量が供給されないので，たんぱく質合成量は低下し，他の余った指標アミノ酸は分解され，その炭素骨格は呼気 CO_2 として排出される。しかし，合成すべきたんぱく質に必要な量以上にリシンが供給される（高たんぱく質）と，たんぱく質はそれ以上合成する必要がないので，指標アミノ酸由来の呼気 CO_2 の排泄量は一定となる。

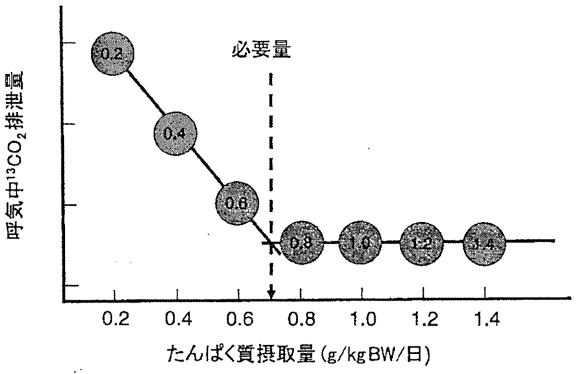


図6　指標アミノ酸酸化法（IAAO 法）

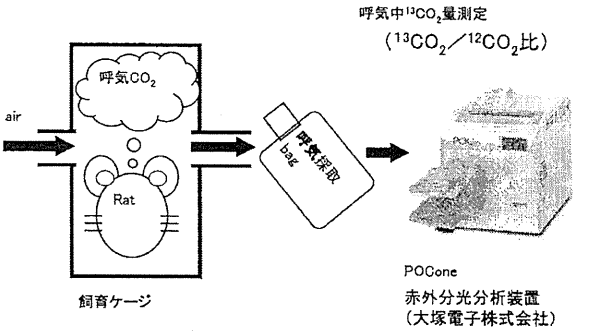


図7　呼気の採取と呼気分析の方法

Ⅳ．たんぱく質代謝研究における IAAO 法の利点

たんぱく質代謝研究における IAAO 法の利点は次の3つが考えられる。

第1に，トレーサーが試験たんぱく質とは別なので，栄養学的にかなりの量のトレーサーを与えても問題がないことである。指標アミノ酸の摂取量は一定に保たれているので，試験たんぱく質よりも指標アミノ酸のほうが濃度の変化が小さい。指標アミノ酸としては，^{13}C-Phe が最も高い頻度で利用されてきた。今後，様々なアミノ酸を指標アミノ酸として利用し，たんぱく質代謝に用いる指標アミノ酸としての評価も必要である。

第2に，出納試験を必要とせず，異なるたんぱく質摂取レベルに対して事前に実験食に適応させる必要がないことである。習慣的な食生活の条件でたんぱく質代謝要求量を求めることが可能である。個々人に見合ったたんぱく質代謝要求量が算出でき，体調や生活スタイルが変化すれば，その都度，最適なたんぱく質代謝要求量を算出することができる。また IAAO 法は，成人だけでなく成長期から高齢者まで同じ方法でたんぱく質代謝要求量を再評価できると考えられる。

第3に，アミノ酸酸化測定の精度や正確さについて高いレベルが要求されないことである。屈曲点は，試験たんぱく質摂取量が十分であることの操作上の指標であり，それは指標物質の酸化率が正確に測定されているか否かに依存しない。私たちは，一定速度で空気を送り込んでいる飼育ケージにラットを入れ，飼育ケージ内の気体を呼気採取バッグに採取し，赤外分光分析装置（大塚電子株式会社）を用いて，呼気 $^{13}CO_2$ 量を ^{12}C との割合として測定している（図7）。

V．ラットにおけるIAAO法によるたんぱく質代謝要求量の測定[13)]

実験食のたんぱく質源としてカゼインと小麦グルテンを用い，IAAO法によるたんぱく質代謝要求量について，実験食のたんぱく質源により違いが見られるかを検討した。ラットは，小麦グルテンを実験食として用いる場合も含めたすべての実験について，実験前24時間以上，20%カゼイン食を自由摂取とした。実験日，ラットは，6段階のカゼインを含む実験食（4.3，8.6，12.9，17.2，21.5，25.8%カゼイン食），または，6段階の小麦グルテンを含む実験食（7.2，10.8，14.4，18.0，21.6，25.2%小麦グルテン食）のうち一つを09：00から18：00まで3時間ごとに4回摂取した。1回の給餌量はラットの1日摂食量の1/8量ずつとした。^{13}C標識物質投与は3回目の給餌時の15：00（$NaH^{13}CO_3$，0.88 mg/kg BW; $NaHCO_3$，7.92 mg/kg BW; ^{13}C-Phe，3.3 mg/kg BW; Phe，29.7 mg/kg BW）に開始し，16：00，17：00，18：00（^{13}C-Phe，6.0 mg/kg BW; Phe，54.0 mg/kg BW）まで続けた。^{13}C標識物質経口投与後ただちにラットをチャンバーに入れた。15：00から19：00まで30分ごとに，チャンバー内の気体を呼気サンプルとして呼気採取バッグに採取し，赤外分光分析装置（POCone：大塚電子株式会社）により呼気中$^{13}CO_2$量を測定した。

たんぱく質含量が6段階のカゼイン食を実験食とする実験（n=8）と小麦グルテン食を実験食とする実験（n=8）それぞれ6回のIAAO法は，実施日は2日間間隔とし，2週間以内に完了した。実験食の組成を表2に示した。

IAAO法においては，低たんぱく質食から十分なたんぱく質食に食事内容を変化させても，食事中の^{13}C-Pheと^{12}C-Pheの量および［1-^{12}C］チロシン（^{12}C-Tyr）の量を一定に保ことが必要である。このように調整された食事を摂取した時に，組織や血漿中の^{13}C-Pheだけでなく，^{13}C-Tyrと^{12}C-Tyrの量も一定であることを確認することが必要である。4.3%カゼイン食と17.2%カゼイン食を摂取した時のラットの血漿PheとTyr濃度を表3に示した。カゼイン食のたんぱく質レベルを4.3%から17.2%に変化させても，血漿^{13}C-Pheと^{12}C-Phe濃度の割合と量が一定であった。また，血漿^{13}C-Tyrと^{12}C-Ty濃度の割合と量も一定であった。さらに，^{12}C-Tyに対する^{13}C-Tyrの割合は，^{12}C-Pheに対する^{13}C-Pheの割合よりも小さく，このことは，PheからTyrへの代謝は亢進していないことを示唆している。また，肝臓および腓腹筋の遊離アミノ酸について測定した結果，血漿と同様にカゼイン食のたんぱく質レベルを4.3%から17.2%に変化させても，血漿^{13}C-Pheと^{12}C-Phe濃度の割合と量および血漿^{13}C-Tyrと^{12}C-Ty濃度の割合と量も一定であった（表3）。

たんぱく質代謝要求量は，18：30の$^{13}CO_2$量を特異的回帰法（2段階線形交差）[14)]により解析し，段階的なたんぱく質摂取量に対する呼気中$^{13}CO_2$が最小値となる屈曲点として算出した。本研究では，ラットにおいてIAAO法により，カゼインをたんぱく質源とした時のたんぱく

表2　実験食の組成

Protein	Casein diet						Wheat gluten diet					
	4.3%	8.6%	12.9%	17.2%	21.5%	25.8%	7.2%	10.8%	14.4%	18.0%	21.6%	25.2%
	g/kg diet						g/kg diet					
Casein	50	100	150	200	250	300	−	−	−	−	−	−
Wheat gluten	−	−	−	−	−	−	100	150	200	250	300	350
Cornstarch	557	523	490	457	423	390	527	498	470	440	411	383
Sucrose	278	262	245	228	212	195	265	250	235	221	206	190
Rapeseed oil	35	35	35	35	35	35	31	27	22	18	14	9
Soy bean oil	15	15	15	15	15	15	12	10	8	6	4	3
Vitamins	10	10	10	10	10	10	10	10	10	10	10	10
Minerals	35	35	35	35	35	35	35	35	35	35	35	35
Cellulose	20	20	20	20	20	20	20	20	20	20	20	20
L-Phenylalanine	11	9	7	5	2	−	9	7	5	3	1	−
L-Tyrosine	13	10	8	5	3	−	13	11	10	9	8	6
Energy (kJ/g)	15.4	15.4	15.5	15.5	15.5	15.6	15.5	15.5	15.5	15.5	15.6	15.6

カゼインのたんぱく質含量は86.2%（N×6.38），小麦グルテンのたんぱく質含量は72.0%（N×5.70）である。食事中のフェニルアラニン含量は，全ての食事で13,500 mg/kg dietとした。ただし，25.2%小麦グルテン食の場合には，14,350 mg/kg dietとした。また，食事中のチロシン含量は，全ての食事で15,000 mg/kg dietとした。

表3　血漿，肝臓，腓腹筋のフェニルアラニンおよびチロシン濃度

Diet	Phenylalanine			Tyrosine		
	^{13}C-Phe	^{12}C-Phe	Total	^{13}C-Tyr	^{12}C-Tyr	Total
			Plasma (nmol/mL)			
4.3% casein	13.2 ± 2.9	47.2 ± 4.3	60.4 ± 7.0	7.5 ± 2.0	113.0 ± 29.4	120.6 ± 30.7
17.2% casein	12.1 ± 2.5	50.8 ± 10.0	62.9 ± 11.8	8.5 ± 1.4	119.8 ± 15.2	128.3 ± 16.2
			Liver (nmol/g)			
4.3% casein	10.6 ± 0.4	40.9 ± 5.1	51.5 ± 4.9	7.4 ± 1.3	99.4 ± 32.0	106.9 ± 33.2
17.2% casein	10.4 ± 2.1	43.1 ± 10.5	53.6 ± 12.3	8.8 ± 2.8	92.5 ± 7.5	101.4 ± 9.2
			Gastrocnemius muscle (nmol/g)			
4.3% casein	13.0 ± 1.7	46.6 ± 4.5	59.6 ± 5.7	8.4 ± 0.8	91.9 ± 8.7	100.3 ± 8.5
17.2% casein	11.6 ± 1.9	48.2 ± 2.5	59.8 ± 3.6	7.0 ± 1.1	84.7 ± 5.8	91.7 ± 5.0

平均値±SE（4.3% casein, $n=5$; 17.2% casein, $n=5$）．全てのデータに4.3% casein 群と17.2% casein 群との間に Student's t-test にて有意差を認めなかった。

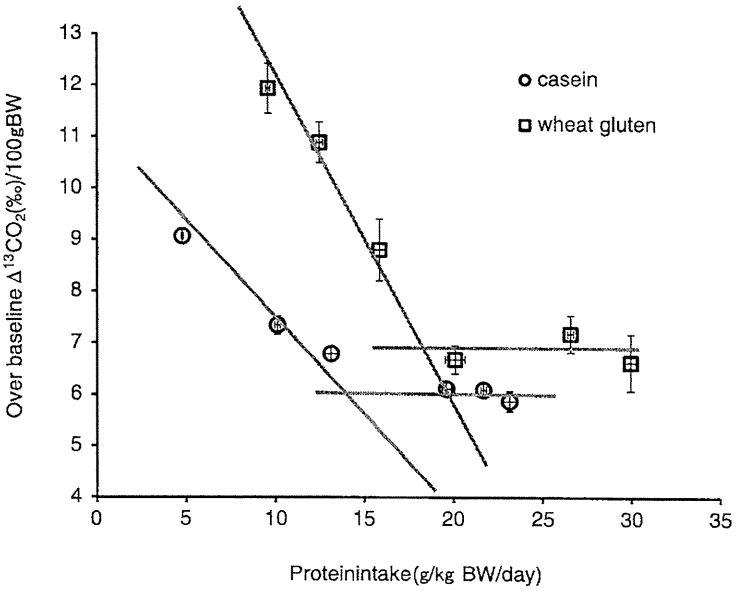


図8　たんぱく質代謝要求量の算出

カゼイン食（$n=8$）と小麦グルテン食（$n=8$）のたんぱく質摂取量を変化させた時の呼気 $^{13}CO_2$ 産生量の変化を平均値±標準偏差で示した。カゼイン食の回帰直線式は，$y=10.73-0.35x$ と $y=6.17$ であり，小麦グルテン食の回帰直線式は，$y=18.87-0.66x$ と $y=6.92$ であった。屈曲点は，カゼイン食が 13.1 g/kg BW/日，小麦グルテン食が 18.1 g/kg BW/日であった。

質代謝要求量は 13.1 g/kg BW/day に相当すると推定された（図8）。

小麦をたんぱく質源とした IAAO 法では，たんぱく質代謝要求量は 18.1 g/kg BW/day と算出され，カゼインをたんぱく質源とした時よりも高い値であった。たんぱく質必要量は良質のたんぱく質摂取で低く，劣質のたんぱく質摂取で高くなったという結果は，我々の仮説に合致し，IAAO 法はたんぱく質の質評価に利用することができると考えられた。

Ⅵ. ヒトにおける IAAO 法によるたんぱく質代謝要求量の測定

1日の総窒素必要量は，不可欠アミノ酸の適切な摂取レベルとバランス，それに α-アミノ窒素源となる十分な可欠アミノ酸を供給することを満たすものである。2007年に Humayun ら[15]は，IAAO 法を用いて成人のたんぱく質必要量を再評価している。彼らによると成人男性のたんぱく質必要量は，0.93 g/kg BW/日であった。我々も，IAAO 法を用いて日本人成人男性のたんぱく質代謝要