

Mitochondria-Based Model for Fetal Origin of Adult Disease and Insulin Resistance

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ABSTRACT: Insulin resistance has been recognized as the fundamental underlying metabolic defect in the pathogenesis of metabolic syndrome, a clustering of cardiovascular risk factors such as diabetes, hypertension, dyslipidemia, and obesity. Recent studies established that mitochondrial dysfunction is involved in insulin resistance in general and fetal origin of this state in particular. Because genes are the fundamental molecular basis of inheritance—and thus the cornerstones of evolution—a model explaining insulin resistance is based at the gene level at best. Since a certain mtDNA polymorphism, 16189T>C, is associated with insulin resistance, mtDNA has to be a basic component of the gene-based model. We developed a mitochondria-based model that explains insulin resistance in terms of quantitative and qualitative change of the mitochondrion and its DNA. This model can accommodate several important hypotheses, such as thrifty genotype hypothesis, thrifty phenotype hypothesis, fetal insulin hypothesis, contribution of metabolic imprinting by epigenetic changes, and many other features associated with insulin resistance. We will discuss mechanisms that indicate why the perturbed initial condition of mitochondrial function should lead to the reduced insulin sensitivity.

KEYWORDS: mitochondrial DNA; mitochondrial dysfunction; insulin resistance; fetal malnutrition; thrifty phenotype

THRIFTY GENOTYPE, THRIFTY PHENOTYPE, AND INSULIN RESISTANCE

Insulin resistance has been recognized as the fundamental metabolic defect of metabolic syndrome, a clustering of cardiovascular risk factors such as diabetes, hypertension, dyslipidemia, and obesity.^{1,2} Many epidemiological studies have revealed links between various indices of reduced intrauterine and early postnatal growth, and susceptibility to insulin resistance syndrome in adult life (for review,

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Ann. N.Y. Acad. Sci. 1042: 1–18 (2005). © 2005 New York Academy of Sciences.
doi: 10.1196/annals.1338.001

see Ref. 3). Barker and Hales suggested that fetal origin of this “thrifty phenotype,” a physical condition programmed by poor nutritional condition in early life, leads to insulin resistance in later life.⁴ Altered programming of the body, such as hypothalamo-pituitary-adrenal axis, is suggested to cause decreased insulin sensitivity.^{5,6} Compared to the nutritionally induced changes in the genetic program, genes were suggested responsible for insulin resistance. A contrasting concept, the “thrifty genotype” hypothesis of Neel⁷ was originally proposed to explain the very high prevalence of obesity and diabetes in some American Indians such as the Pima. He suggested that native Indians might have accumulated genes that are beneficial for survival under famine conditions, but are detrimental when society becomes affluent. Both hypotheses are well accepted; the predisposition to insulin resistance is likely to be the result of both genetic and fetal environmental factors. Although Hattersley and his co-workers suggested that altered fetal growth may be a phenotype of a genotype—in other words, the thrifty phenotype is the result of a thrifty genotype^{8,9}—one cannot explain all the thrifty phenotypes with genotypes, because experimental study using the same strain of animals induced insulin resistance in offspring.

The “fetal insulin” hypothesis of Hattersley emphasizes the insulin secreted by the fetal pancreas in response to maternal glucose concentrations as a key factor. There is strong evidence supporting the fetal insulin hypothesis. For example, monogenic diseases that impair sensing of glucose, such as glucokinase gene mutations, lower insulin secretion, or increase insulin resistance, as in IGF-1 gene polymorphism, are associated with impaired fetal growth.^{10,11} Polygenic influences resulting in insulin resistance in the normal population are therefore likely to result in lower birth weight.

Further study is needed to determine whether common gene variants can explain the association between reduced birth weight and increased risk of type 2 diabetes or insulin resistance. Considering recent advances in epigenetics and genomic imprinting, these hypotheses were criticized in that they omitted the important contribution of these mechanisms in the pathogenesis of insulin resistance.^{12,13}

Recent studies established without doubt that mitochondrial dysfunction in general, or dysfunction of mitochondrial genome, might be a major abnormality of this state. Because genes are the fundamental molecular basis of inheritance—and thus cornerstones of evolution—explaining insulin resistance is based at the gene level at best.

As a certain mtDNA polymorphism, 16189T>C, is associated with insulin resistance,^{14–19} mtDNA has to be a fundamental component of the model. Here we present a mitochondria (and its DNA)–based model that is versatile enough to accommodate all the components of previously proposed hypotheses and other unexplained features such as imprinting.

MITOCHONDRIA-BASED MODEL OF INSULIN RESISTANCE— INTRODUCTION

By expanding three major hypotheses presented above and taking into account several observations that show (1) a certain mitochondrial DNA polymorphism, 16189T>C variant, is associated with insulin resistance, (2) mtDNA density in pe-

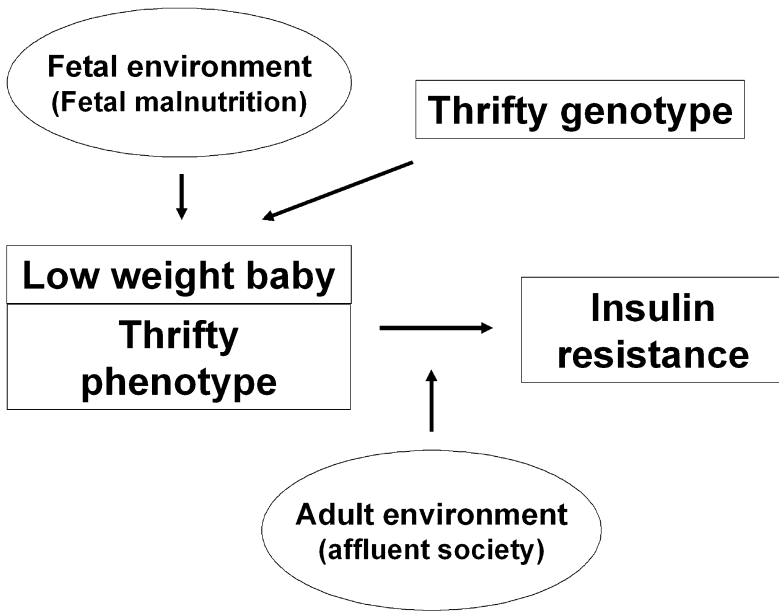


FIGURE 1. Diagram of the thrifty genotype/phenotype hypotheses.

ripheral blood is associated with insulin sensitivity and predictive of development of diabetes at population level, (3) mtDNA density is lower in offspring of a diabetic parent, (4) mitochondrial structure and mtDNA density are altered in offspring of malnutrition induced dams, and (5) much other supporting evidence, we developed a model based on mitochondria or the mitochondrial genome, which is shown in Fig. 1. This model is based on our early idea,²⁰ which was revised recently.²¹

This model accommodates three critical elements newly identified: first, taurine is an important structural component of mitochondrial tRNA, and its deficiency during early life causes poor fetal development and impaired insulin secretion in offspring, and, most importantly, a lowered fetal plasma taurine level from a low-protein diet is the main predictor of the fetal plasma insulin level.²² Second, epigenetic mechanisms play a critical role at the nexus between nutrition and genome; and finally that highly active antiretroviral therapy (HAART) for AIDS patients causes mitochondrial toxicity and insulin resistance.^{23–25} Details on metabolic imprinting will not be discussed here (for two recent reviews, see Refs. 26 and 27).

Since the mitochondrion has its own genome from its symbiotic origin and is under the control of the nuclear genome, it plays the dutiful servant role of generating ATP for the cell. On the other hand, the mitochondrion also plays a role as a commander, to protect and to supply substrates for cell survival. From the mitochondrial perspective, one can incorporate nuclear gene effects without making a model highly complex, which is otherwise inevitable. This synthesis bring us to a view that one of the defects underlying insulin resistance is a quantitative or qualitative change of mi-

tochondria or their DNA, which might result from the thrifty genes the subjects have, as well as the programming effect of poor nutritional conditions during early life and the environmental effect during development. This model considers the mitochondrial genome as a thrifty gene that is rapidly evolving to adapt to the human environment,^{28,29} and (thrifty) nuclear genes are considered as controlling elements controlling mitochondrial function.

BASES OF MITOCHONDRIA-BASED MODEL— BIOCHEMICAL ASPECT

Mitochondrial Abnormalities Are Linked to Diabetes and Insulin Resistance

mtDNA mutations are well known to cause diabetes by affecting insulin secretion from pancreatic beta cells.³⁰ Glucose enters the cell through a specific transporter, followed by phosphorylation of glucose, activation of glycolysis, and stimulation of mitochondrial oxidative phosphorylation, resulting in an increase of intracellular ATP. This leads to the closure of the ATP-sensitive K⁺ channel, the opening of the Ca²⁺ channel, and increased intracellular Ca²⁺, which eventually triggers insulin secretion. It is thus reasonable that pancreatic beta cells with abnormal mitochondria would show a poor insulin-secretory response to glucose stimulation.

In addition to reducing insulin secretion, mitochondrial abnormalities can also cause insulin resistance. Poulton *et al.* reported that a polymorphism in the first hypervariable region of the mtDNA control region (16189 T>C) is associated with insulin resistance in the English population.¹⁴ The finding was confirmed in other populations, including Koreans.^{15–19}

Our laboratory found that the decreased mtDNA density in peripheral blood preceded the development of diabetes,³¹ suggesting that quantitative mitochondrial abnormalities could be primary events. Inverse correlations were noted between mtDNA content and components of the metabolic syndrome such as blood pressure, fasting glucose level, and waist-to-hip circumference ratio,³¹ suggesting for the first time that mitochondrial abnormality might be associated with insulin resistance. Song *et al.* extended this observation to diabetic offspring and found that mtDNA density was indeed associated with insulin sensitivity in the offspring of type 2 diabetic patients.³²

Petersen *et al.* established that there is deranged mitochondrial function in insulin resistance.^{33,34} They reported that elderly people were insulin resistant compared with young controls matched for lean body mass and fat mass, and this resistance was attributable to reduced insulin-stimulated muscle glucose metabolism. These changes were associated with increased fat accumulation in muscle and with an approximately 40% reduction in mitochondrial oxidative and phosphorylation activity. This group extended the study to the insulin-resistant diabetic offspring, and found that they have a 80% increase in intramyocellular lipid content and a 30% reduction in mitochondrial phosphorylation activity compared with insulin-sensitive, age-, height-, and weight-matched control subjects.³⁴

As peripheral blood mtDNA density was correlated positively with fat oxidation during hyperinsulinemic clamp,³⁵ and as insulin resistance in the elderly is related to

the increase in intramyocellular fatty acid metabolites, these two observations might be corollaries to a common phenotype. This possibility is consistent with the fact that the whole-body oxygen consumption rate correlates with insulin sensitivity.^{36,37}

Park *et al.*³⁸ investigated the effects of mtDNA depletion on glucose metabolism at the cellular level. When the human hepatoma SK-Hep1 cells were treated with repeated sublethal doses of ethidium bromide, they lost mtDNA (ρ^0 cells) and the cells failed to hyperpolarize their mitochondrial membrane potential in response to glucose stimulation. Intracellular ATP content, glucose-stimulated ATP production, glucose uptake, steady-state mRNA, and protein levels of the glucose transporters, and cellular activities of the glucose metabolizing enzymes, including hexokinase, decreased. These results suggest that quantitative reduction of mtDNA suppresses the expression of nuclear DNA-encoded glucose transporters and enzymes of glucose metabolism.

Insulin Resistance and Mitochondrial Dysfunction Are Induced by Malnutrition in Animal Models

Several metabolic abnormalities that lead to insulin resistance in protein-malnutrition models also have been reported. The muscle and liver organ weight were reduced in these models,³⁹ and the activities and gene expression of insulin-sensitive hepatic enzymes changed. In addition, the glucokinase activity was reduced and phosphoenolpyruvate carboxykinase (PEPCK) increased, both resulting in increased hepatic glucose production.^{40,41} The ability of insulin to inhibit glucagon-stimulated glucose output from the perfused liver was lost and indeed reversed.⁴² Proteome analysis of the fetal pancreas to examine the intrauterine programming of β cell gene expression showed that the expressions of 70 proteins were changed by fetal protein malnutrition.⁴³ They include the proteins related to mitochondrial energy transfer, glucose metabolism, RNA and DNA metabolism, protein synthesis and metabolism, the cell cycle and differentiation, cellular structure, and cellular defense. The glucose tolerance of the offspring of low-protein-fed dams was markedly age dependent. The younger of such offspring had improved glucose tolerance at 12 weeks of age when compared with controls,⁴⁴ which is explained by adaptation of the peripheral tissue through increased whole-body insulin sensitivity.⁴⁵⁻⁴⁷ These age-dependent changes are compatible with ever-decreasing mitochondrial function along the aging process.⁴⁸

Ogata *et al.*^{49,50} have developed a rat model using utero-placental insufficiency as a cause of intrauterine growth retardation by inducing ischemia to the fetus by partially ligating the placental blood supply. This model exhibits marked insulin resistance early in life, characterized by blunted whole-body glucose disposal in response to insulin,⁵¹ and impaired insulin suppression of hepatic glucose output.⁵² Mitochondria of intrauterine growth retardation (IUGR) rats have not been well studied but, in skeletal muscle from IUGR, rats exhibited significantly decreased rates of stage 3 oxygen consumption with pyruvate, glutamate, α -ketoglutarate, and succinate. Such a defect in mitochondria leads to a chronic reduction in the supply of ATP available from oxidative phosphorylation, and the authors of the study concluded that impaired ATP synthesis in muscle compromises energy-dependent glucose transport and glycogen synthesis, which in turn contribute to the insulin resistance and hyperglycemia of type 2 diabetes.⁵³

In the offspring of dams fed a low-protein diet during gestation and lactation, the mtDNA content of the liver and skeletal muscle was reduced and did not recover until 20 weeks of age, despite restoration of nutrition after weaning.⁵⁴ The reduced mtDNA content was accompanied by a decrease in mitochondrial DNA-encoded gene expression.⁵⁴ Park *et al.*⁵⁵ found that these rats also have decreased mtDNA levels in the pancreas at 25 weeks of age, accompanied by decreased pancreatic β cell mass, and reduced insulin secretory responses to glucose load. These findings indicate that poor nutrition in early life causes long-lasting changes in the mitochondria and their mtDNA densities.

Mitochondria-Based Model Accommodates Nuclear Gene Effects

The replication of mtDNA is under the relaxed control of the cell cycle. All of the proteins required for replication and transcription of mtDNA are nuclear encoded, suggesting the importance of mitochondrial and nuclear interaction in mtDNA replication. The detailed process of mtDNA replication is well reviewed by Clayton *et al.*⁵⁶ and Lecrenier *et al.*⁵⁷ The control of mitochondrial biogenesis is extremely complex, involving the coordinated expression of hundreds of genes. The nuclear respiratory factors NRF-1 and NRF-2 are transcriptional regulators for the genes of subunits of the oxidative phosphorylation system, as well as for many genes involved in mtDNA replication such as the Tfam gene.⁵⁸ The fact that numerous genes are regulated by NRFs suggests that the NRF-dependent genes are involved in the control of mitochondrial biogenesis in general, which is a possible link between external stimuli and mtDNA content.⁵⁹ The mechanisms of how malnutrition in early life induces mtDNA reduction that lasts until the adult period without recovery despite restoration of nutrition are not clear yet. Further understanding of nuclear gene effects on insulin resistance could be incorporated to this model as molecular biology advances.

Taurine Is a Key Component of Mitochondrial tRNA, and Taurine Availability Is a Key Determinant for the Development of Insulin Resistance

In an isocaloric, low-protein fetal-malnutrition model, although basal blood sugar and plasma insulin were not modified, the amino acid profile was disturbed in the maternal and fetal plasma as well as in the amniotic fluid.⁶⁰ The levels of essential, branched, and sulfur-containing amino acids were reduced. The most affected amino acid in maternal and fetal plasma, amniotic fluid, and fetal islets is taurine. Surprisingly, supplementation of the maternal low-protein diet by 2.5% taurine in the drinking water completely restored islet cell proliferation as well as the insulin-like growth factor 2 (IGF-2) and vascular endothelial growth factor content in the islets of fetuses and suckling pups. Taurine supplementation also restored fetal islet vascularization by increasing the number of blood vessels.⁶¹ Moreover, taurine supplementation of the mother's diet normalized the insulin secretion of the fetal islets.⁶² Taurine is an amino acid that does not participate in protein synthesis, but has a function in cholesterol excretion, as a neurotransmitter and as a potent antioxidant (for review, see Ref. 63). Taurine is not considered an essential amino acid for humans because it can be synthesized from cysteine in the liver.⁶⁴ However, the plasma concentration of taurine in the fetus is 1.5-fold that of maternal blood, and this level is mostly dependent on transport from the maternal blood through the placenta because

the bioregulatory systems for taurine in the fetus are not fully developed.⁶⁵ Reduced activity of placental taurine transporters results in low fetal-aurine levels and IUGR fetuses.⁶⁶ Most importantly, Bertin *et al.* reported that a lowered fetal-plasma taurine level from a low-protein diet was the main predictor of the fetal-plasma insulin level.²²

A recent report provides unequivocal evidence that taurine critically affects mitochondrial function. Suzuki *et al.*⁶⁷ found two novel taurine-containing modified uridines, 5-taurinomethyluridine and 5-taurinomethyl-2-thiouridine, in mtDNA, and they showed that taurine was a constituent of mitochondrial tRNAs. Modification of the taurine-containing uridines has been found to be lacking in mutant mitochondrial tRNAs for Leu (UUR) and Lys in pathogenic cells of the mitochondrial encephalomyopathies, mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episode (MELAS), and myoclonus epilepsy associated with ragged red fibers (MERRF), respectively. In addition, these modification deficiencies of mutant tRNAs cause defective translation due to weak codon-anticodon interactions, which might significantly contribute to the defective mitochondrial function in mitochondrial diseases. It is likely that low taurine in the fetus may induce a deficiency of modification of nucleosides that leads to defective translation activity and mitochondrial function, and may result in impaired insulin secretion and insulin resistance.

Furthermore, there is an interesting study by Nakaya *et al.*⁶⁸ that reports that abdominal fat accumulation, hyperglycemia, and insulin resistance were significantly ameliorated by taurine supplementation in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a genetic model of spontaneous development of type 2 diabetes, which exhibits hyperglycemia, obesity, and insulin resistance, similar to that seen in humans.

HAART in HIV-Infected Subjects Causes Insulin Resistance Syndrome by Mitochondrial Toxicity

Another example is the nucleoside-analogue reverse transcriptase inhibitors that are used as therapy for human immunodeficiency virus (HIV) infection, which act by inhibiting HIV replication. After phosphorylation in the cell, these agents are internalized into nascent mtDNA by their substitution for the natural base, which leads to mtDNA damage. The human DNA polymerase gamma is also inhibited,²³ thereby inhibiting replication of mitochondrial DNA, thus leading to depletion of mitochondrial DNA.^{24,25} A syndrome almost identical to clinical features of metabolic syndrome, type 2 diabetes, central obesity, increased intra-abdominal fat, hyperlipidemia, and insulin resistance is caused by mtDNA depletion resulting from the toxicity of these drugs.

Genomic Imprinting Is Changed in Malnutrition

The long-term programming effect of early malnutrition can be partly explained by an epigenetic mechanism, because inheritance of the thrifty phenotype shows characteristics of genetic imprinting. Once an offspring is affected by malnutrition, it takes several generations to fully recover from it.⁶⁹ This phenomenon can only be explained on the basis of imprinting of the genes. Genomic imprinting is an epigenetic phenomenon in which only a single allele of a gene is expressed in a parent-of-

origin-dependent manner (for review, see Ref. 26). In mammals, the imprinted genes are particularly implicated in the regulation of fetal growth and development, cancer, and aging. The DNA modification for imprinting includes DNA methylation, histone modifications, and differences in chromatin structure. The mice that lack methylation because of a deficiency in DNA-methyltransferase-1 die at early post-implantation. Some genetic imprints are already lost at this stage, suggesting a crucial role for DNA methylation in determining the imprinted status of genes.⁷⁰

Transient nutritional stimuli at critical ontogenic stages can yield lasting influences on the expression of genes by interacting epigenetic mechanisms.²⁷ If certain genomic regions such as imprinted domains are especially labile to such influences, early nutritional influence on these genomic components could have a long-lasting impact on phenotype. Indeed, because nutrition can affect imprinted growth factors such as IGF-2, insulin has been established in animal experiments.⁷¹ Several studies revealed that early nutritional changes, by changing the culture media used during *in vitro* manipulations of early mouse embryos, alter allelic methylation and expression of imprinted genes,^{72,73} and lower birth weight.⁷³ Recent observations have identified that *in vitro* manipulation of human embryos also induces imprinting alterations that lead to congenital disorders such as Angelman's syndrome⁷⁴ and Beckwith-Wiedemann syndrome.⁷⁵ Data from animal models have indicated that the epigenetic lability of imprinted genes is not limited to the early embryonic period. Hu *et al.*⁷⁶ treated mice with 5-azacytidine—an inhibitor of DNA methylation—at postnatal days 11 and 14, and found dramatic alteration in allelic expression of the IGF-2 gene. Waterland and Garza reported that two of 10 differentially expressed genes in the islets of undernourished rats during the suckling period were imprinted genes.⁷⁷

Mammalian one-carbon metabolism, which ultimately synthesizes S-adenosyl-methionine, providing the methyl group for all biological methylation reactions, is highly dependent on dietary methyl donors and cofactors.⁷⁸ Dietary methionine and choline are the major source of one-carbon units, and folic acid, vitamin B12, and pyridoxal phosphate are critical cofactors in methyl metabolism. The availability of dietary methyl donors and cofactors during critical ontogenic periods therefore might influence DNA methylation patterns.⁷⁹ For example, the coat-color distribution of A^{vy}/a offspring (the A^{vy} mutation resulted from the insertion of retrotransposon into an exon of the agouti gene; a is the loss of function mutation) was shifted when their mothers' diets were supplemented with methyl donors and cofactors.⁸⁰ The coat-color shift in the offspring caused by methyl donor supplementation of the dam was revealed to be caused by altered methylation status of the agouti promoter in the offspring,⁸¹ suggesting the epigenetic effects of dietary nutrition.

Last, it will be important to note the unpublished data of Ng *et al.*, who found that mtDNA density is more affected by the paternal history of diabetes,⁸² suggesting that imprinting in paternal genes might be directly involved in mtDNA density determination and inheritance of diabetes mellitus.

Vicious Cycle of Mitochondrial Dysfunction and Oxidative Stress

Protein malnutrition is associated with depressed antioxidant defense systems and increased oxidative stress.⁸³ Proteome analysis of fetal protein-malnourished pancreas revealed that antioxidant protein 2, which protects the pancreas against ox-

oxidative injury⁸⁴ by reducing hydrogen peroxide (H_2O_2), was downregulated.⁸⁴ Because of the role of oxidative stress in mtDNA damage,⁸⁵ we can speculate that oxidative stress might be involved in malnutrition-associated mitochondrial changes.

The mitochondrion is a major organelle for free radical production and also most vulnerable to free radical damage. Once mitochondrial function is deranged, there is more free radical production and more rapid mitochondrial function decline. Thus, it is likely once the mitochondrial capacity of the whole body is set low, a vicious cycle will operate, leading to early exhaustion. This mechanism is one of the most plausible hypotheses explaining aging.⁸⁶ Because the antioxidant system is also deficient in offspring of malnourished animals, one can explain the development of insulin resistance or the metabolic syndrome in later life by persistent decline of mitochondrial system through a vicious cycle, a feature of the mitochondria-based gene model.

BASES OF MITOCHONDRIA-BASED MODEL—BIOPHYSICAL ASPECT

Mitochondrial Function of Unit Cell and Tissue Is Quantitatively Related to Whole-Body VO_2max and Insulin Resistance

In 1956, Smith reported that the mitochondrial density of liver correlated with whole-body energy expenditure, and the relative amount of mitochondria in any given tissue was suggested to be the controlling factor in determining the regression of oxygen utilization on total body size of the species.⁸⁷ Recently, Rasmussen *et al.*⁸⁸ examined whether parameters of isolated mitochondria could account for the *in vivo* maximum oxygen uptake (VO_2max) of human skeletal muscle. VO_2max and work performance of the quadriceps muscle of six volunteers were measured in the knee extensor model (range 10–18 $\text{mmol O}_2 \times \text{min}^{-1} \times \text{kg}^{-1}$ at work rates of 22–32 W/kg). Mitochondria were isolated from the same muscle at rest. Strong correlations were obtained between VO_2max and a number of mitochondrial parameters (mitochondrial protein, cytochrome aa3, citrate synthase, and respiratory activities). The activities of citrate synthase, succinate dehydrogenase, and pyruvate dehydrogenase measured in isolated mitochondria corresponded, respectively, to 15, 3, and 1.1 times the rates calculated from VO_2max . Fully coupled *in vitro* respiration, which is limited by the rate of ATP synthesis, could account for, at most, 60% of the VO_2max . It is thus not surprising that mtDNA density correlates with oxidative capacity in animal and man, as discussed previously.⁸⁹ Furthermore, because this capacity is related to insulin sensitivity,⁹⁰ one can appreciate that mitochondrial function at the cell or tissue level is quantitatively related to the whole-body insulin resistance.^{20,91}

Biophysical Law between Metabolic Power and Body Mass— Allometric Scaling Law

Recent reformulation of allometric scaling laws by West and his group⁹² provides reasons why mitochondrial density at cell or tissue level should correlate with whole-body insulin sensitivity. This group generated a general model for the origin of allometric scaling law from three broad principles required in the living organ-

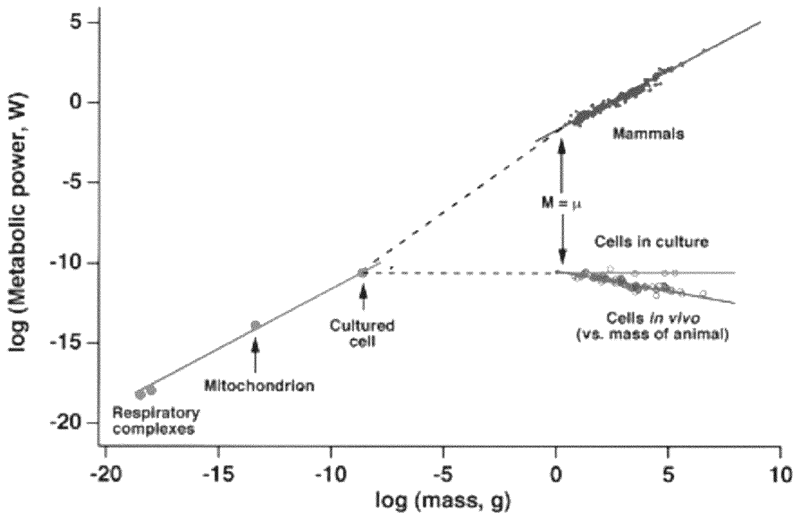


FIGURE 2. A logarithmic plot of metabolic power as a function of mass (from West *et al.*⁹⁴). The entire range is shown, covering 27 orders of magnitude from a cytochrome oxidase molecule and respiratory complex through a mitochondrion and a single cell *in vitro*, up to whole mammals. The solid lines through the corresponding dots are the $M^{3/4}$ predictions. The dashed line is the linear extrapolation from $M = \mu$, the approximate mass predicted and observed for the smallest mammal to an isolated mammalian cell. (From West *et al.*⁹⁴ Reprinted, with permission, from the *Proceedings of the National Academy of Sciences USA*.)

isms: (1) a space-filling, fractal-like branching pattern is necessary to supply the organism with what it needs; (2) the final unit of this branching pattern is a size-invariant unit; and (3) the energy required to distribute resources is minimized. The metabolic rate of organisms is known to be proportional to body mass raised to the power of three-quarters developed from Kleiber's original analysis.⁹³ West *et al.*⁹⁴ extended the relation between body mass and metabolic power, which covers 27 orders of magnitude of body size, from elephant, mice, and mitochondrion to electron transfer chain enzymes (Fig. 2).

It is illuminating that each cultured cell has identical metabolic power (universal metabolic rate), and *in vivo* the metabolic power decreases as weight of the animal increases. Body temperature is a key constraint of metabolic scale law.⁹⁵ They argued that this relationship is inevitable to keep core body temperature optimal, to which all the enzyme systems were adaptively evolved. If heat production of each cell or tissue is not changed according to body size, core temperature will go up too high or too low and damage cellular function. As West *et al.* discuss in their findings, thus if one knows the scale of power generation at the molecular level, it will be sufficient to predict the metabolic rate of individual mitochondria and cells (whether *in vitro* or *in vivo*) as well as intact mammals. Since this biophysical law of metabolic scaling is true, it predicts that the reverse statement will be true. In other words, if the unit cellular metabolic rate is decreased, increased body mass will occur adap-

tively; in people who were to develop obesity, metabolic power of the unit cell should be lower. This prediction is compatible with data collected on Pima Indians (to be discussed below).

Physiologic Mechanisms for Allometric Scale Law—Adaptive Thermogenesis

Adaptive thermogenesis is the physiological process whereby energy is dissipated in the form of heat in response to external stimuli such as exposure to cold and ingestion of high-calorie diets. Adaptive thermogenesis has been regarded as a physiological defense against obesity,⁹⁶ where brown fat plays a major role.⁹⁷ In humans, there are great differences in how individuals metabolize an intentional caloric overload; some people store most of this energy as fat, while others dissipate much of it through altered energy expenditure, including adaptive thermogenesis.^{98,99} Adaptive thermogenesis occurs primarily in the mitochondria of brown fat and skeletal muscle. Since brown fat is hardly found in large animals including humans, skeletal muscle is thought to be the site of primary importance for this process.

The uncoupling proteins (UCPs) are small intramembranous mitochondrial proteins that are expressed in a tissue-selective manner and play a key role in thermogenesis.^{100,101} Cold and high-calorie diets stimulate the sympathetic nervous system leading to the release of norepinephrine. Norepinephrine triggers the activation of the β -adrenergic receptors (AR) resulting in the elevation of intracellular cAMP and inducing the expression of PGC-1. PGC-1 activates the expression of the subunits of the respiratory chain and mtTFA through the induction of the expression of NRFs and the coactivation of NRF-1-mediated transcription. mtTFA subsequently translocates into the mitochondrion and directly activates the transcription and replication of mtDNA. PGC-1-induced mitochondrial biogenesis is accompanied by an enhanced capacity for energy production, which is made possible by the increased expression of enzymes acting in fatty acid oxidation, the tricarboxylic acid (TCA) cycle, and oxidative phosphorylation.^{102–105} Interestingly, PGC-1 can modulate mitochondrial activity in a cell-type-specific manner. In adipocytes, it induces the inner mitochondrial membrane UCP-1, which can uncouple fuel oxidation from ATP production and generate heat.^{102–105} In muscle, it induces the UCP-2 and stimulates both uncoupled (heat-producing) and coupled (ATP-producing) respiration.¹⁰³ In cardiac myocytes, PGC-1 does not activate the UCPs and stimulates only coupled, ATP-producing respiration.¹⁰⁴ These findings suggest that PGC-1 gears mitochondria to meet tissue-specific metabolic needs. It is possible that, within a given tissue, the PGC-1-induced response (e.g., coupled versus uncoupled respiration) also depends on the physiological state of the organism. The suggested reason involves the need to increase the overall rate of fuel oxidation, allowing for the preservation of normal cellular ATP/ADP ratios, while “wasting” a significant fraction of metabolic energy in the form of heat.

The compensatory mechanism to the decreased metabolic power was studied in detail in human. Leibel *et al.*¹⁰⁶ found maintenance of a body weight at a level 10% or more below the initial weight was associated with a mean (\pm SD) reduction in total energy expenditure of 6 ± 3 kcal per kilogram of fat-free mass per day in the subjects who had never been obese ($P < 0.001$) and 8 ± 5 kcal per kilogram per day in the obese subjects ($P < 0.001$). Maintenance of body weight at a level 10% above the usual weight was associated with an increase in total energy expenditure of 9 ± 7 kcal

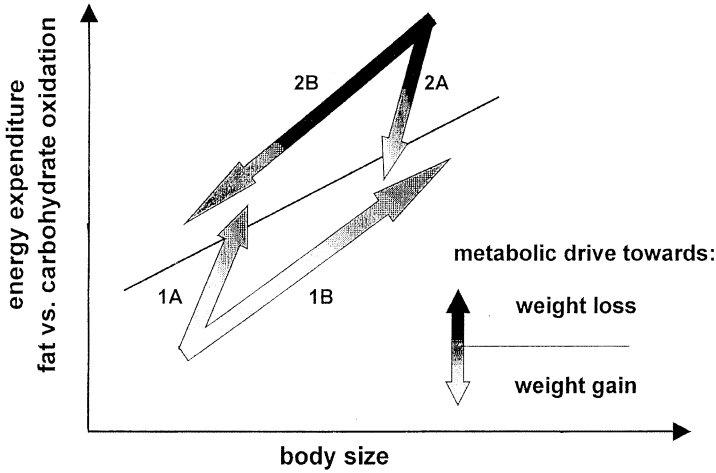


FIGURE 3. Energy expenditure increase after the weight gain in the Pima Indians. (From Weyer *et al.*¹⁰⁷ Reprinted, with permission, from the *Journal of Clinical Endocrinology & Metabolism.*)

per kilogram of fat-free mass per day in the subjects who had never been obese ($P < 0.001$) and 8 ± 4 kcal per kilogram per day in the obese subjects ($P < 0.001$). The thermic effect of feeding and non-resting energy expenditure increased by approximately 1–2 and 8–9 kcal per kilogram of fat-free mass per day, respectively, after weight gain. Maintenance of a reduced or elevated body weight is associated with compensatory changes in energy expenditure, which oppose the maintenance of a body weight that is different from the usual weight. These compensatory changes are consistent with the prediction of allometric scaling law.

Consistent with this law, the metabolic rate of individuals increased after a certain period in Pima Indian subjects whose rate was initially low (arrow 1A and 1B in FIG. 3).¹⁰⁷ Resultant changes in body scale and metabolic rate fall to the line of scale law relation. The weight gain was highest among those subjects with lowest metabolic rate.¹⁰⁸ These findings support the concept that lowered metabolic rate is compensated by increased body mass.

CONCLUSION

Many epidemiologic findings suggest that intrauterine growth retardation is linked to the risk of developing both type 2 diabetes and insulin resistance syndrome. In the light of advances made in the last few years, including development of the thrifty animal model, the thrifty phenotype hypothesis as a possible explanation for these links has been clarified. However, its explanation remains conflicting. Then, the vast amount of work from mitochondrial research and in the area of the pathophysiology of type 2 diabetes provided evidence that mitochondrial dysfunction may

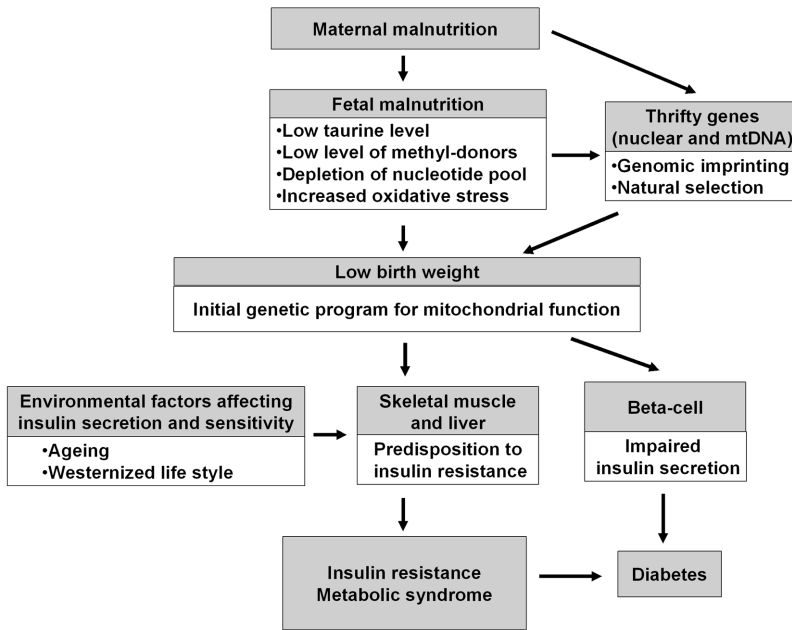


FIGURE 4. General outline of the mitochondrial hypothesis.

be a link between malnutrition in early life and insulin resistance syndrome in adult life. However, its explanation remains conflicting. Recent works on the pathophysiology of type 2 diabetes provided evidence that mitochondrial dysfunction may be a link between fetal malnutrition and insulin resistance syndrome. Here we propose a mitochondria-based model for the fetal origin of adult diseases or insulin resistance syndrome, in which a set point of mtDNA content might change according to the nutritional status of early life and might be inheritable by an imprinting mechanism (FIG. 4).

The mitochondria-based model is compatible with a biophysical law of allometric scaling. It might be simply thought as a special case of this law existing between the metabolic power of mitochondrial unit and the body mass. Among the many predictions this model makes, it is suggested it will be fruitful to identify genes whose expression is persistently altered by early malnutrition, both in genomic and epigenetic approaches, especially the genes that control mitochondrial replication.

ACKNOWLEDGMENTS

This study was supported by a grant (02-PJ1-PG1-CH04-0001) from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea.

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