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Linking mitochondrial function to diabetes mellitus: an animal’s tale

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Berdanier CD. Linking mitochondrial function to diabetes mellitus: an animal’s tale. Am J Physiol Cell Physiol 293: C830–C836, 2007; doi:10.1152/ajpcell.00227.2006.—Diabetes mellitus is one of the most common genetic diseases that afflicts humans. It is not a single disease but a collection of diseases having in common an abnormal glucose-insulin relationship and a dysfunctional regulation of glucose homeostasis. Of interest is the diabetic state that results when the mitochondrial genome mutates. Epidemiological studies have shown this to occur in humans. Detailed metabolic studies that are impossible to conduct in humans have been carried out in the BHE/Cdb rat. This rat has a mutated mitochondrial ATPase 6 gene. Strategies to ameliorate the consequences of this mutation have been explored and some of the mechanisms for the transcription and translation of the mitochondrial gene product have been elucidated.

keywords: mitochondrial DNA, retinoic acid, BHE/Cdb rats, diabetes

Diabetes mellitus is a polygenomic disease. More than 200 mutations in the nuclear genome have been found to associate with abnormal glucose metabolism. Of recent interest are the reports that this disease is also associated with mutations in the mitochondrial genome (2–4, 36–38, 53, 58, 60, 64, 71). Several mutations have been reported and are listed in Table 1. Diabetes is characterized by an abnormal use of glucose and an abnormal insulin response to rising levels of blood glucose. In some instances, this is due to autoimmune-mediated destruction of the insulin-producing β-cells of the pancreas. In many instances, however, diabetes develops as a response to mutation in one or more genes encoding components of glucose metabolism that are downstream of the insulin releasing function of the pancreas.

A mitochondrial mutation that phenotypes as diabetes affects not only the downstream use of glucose by many different tissues, it also affects the glucose sensing system of the β-cell and the insulin-releasing system of that cell. Mitochondrial mutation affects the production of ATP and many of the glucose using reactions are ATP dependent. In particular, insulin release by the islet cell is an ATP-dependent process (36, 55). The islet cell senses the rise in blood glucose and glucokinase on the interior aspect of the islet plasma membrane is activated. Glucokinase requires ATP. There is a series of reactions that follow that ultimately result in substrates flowing into the β-cell mitochondria that in turn are metabolized by the TCA cycle releasing CO2 and reducing equivalents. Reducing equivalents are sent down the respiratory chain to make water and the energy released is captured in part by the synthesis of ATP. Of the components of this oxidative phosphorylation system (OXPHOS), 13 are encoded by the mitochondrial genome (Table 2). In addition to these structural genes, the mitochondrial genome also encodes all of the components for protein synthesis: the tRNAs and the ribosomes. Two of the structural genes are subunits of the F1F0ATPase, while the remaining eleven structural genes are components of the respiratory chain. Should there be a mutation in the mitochondrial genome, either in the structural genes themselves or in one of the components for mitochondrial protein synthesis, OXPHOS will be affected. Some of the mutations occur in vital portions of the code such that the resultant gene product is nonfunctional. If this occurs, the affected individual will manifest very serious neurological, neuromuscular disease. Impaired multisystem function is observed (71). In these individuals, the disease state develops early in life. However, if the mutation occurs in a less important part of the code, or the mutation burden is <80–90%, or the mutation occurs in a part that does not translate into the active site of the resultant gene product, then less serious (if any) consequences can follow. Mutation can also occur in nonstructural or noncoding areas of the genome with no consequences to the organism at all (67). In addition, mitochondrial disease can develop if mutations in the nuclear genome occur that encode components of the mitochondrial genomic expression. For example, if mutation occurs in the retinoic acid binding protein that acts as a transcription activator of mitochondrial gene expression then there could be effects on mitochondrial gene expression that in turn affects mitochondrial function (27, 65, 67, 71).

Mitochondrial disease is quite different from disease due to nuclear mutation. This is because mitochondria have a different dynamic with respect to their DNA (67). There are many mitochondria in each cell. Cell types differ in the number: hepatic cells can have 800–1,200 mitochondria, adipose cells have far less than ~400/cell, while ova can have 20,000 mitochondria. Each mitochondrion has 1–10 copies of its DNA and there can be mixtures of the DNA sequence within the mitochondria and within the cell. That is, there can be some DNA with one base sequence and another with a different base sequence within the same individual mitochondrion. This problem is compounded by the fact that there is a small population of cells that are mitotic. These cells can then pass on their mitochondrial DNA to their children. This is even more complicated when one considers that the membrane of the mitochondrion is impermeable to the passage of molecules larger than 1,000 molecular weight. This means that if a cell is divided, the DNA that is present in the divided cell is likely to be lost to the rest of the cell. This makes the problem of interpreting the effects of mitochondrial mutation in the context of disease quite complex.
sequence at a specific location. If a mixture occurs it is called heteroplasm. If all DNA is identical in sequence, then it is homoplasmy. If a mutation has occurred that is pathogenic it generally can be found in the heteroplasmic individual. Abnormal pathogenic homoplasmic can be lethal. For the devastating mitochondrial diseases, heteroplasm with only a small percentage of normal DNA (~10%) and a large percentage (~90%) of abnormal cells is found. Less devastating conditions can develop where the percentage of abnormal DNA is less. Tissues can differ in the degree of heteroplasm as well. Obviously, those tissues with a high ATP requirement (neural tissue, β-cells, renal cells) will be more affected by an ATP shortfall than tissues such as bone that have a much lower ATP requirement. Hence, the symptoms of mitochondrial disease will be found in high ATP-requiring cell types.

One other difference between genetic diseases due to a mutation in the nuclear genome and those due to mutation in the mitochondrial genome is that mitochondrial disease is maternally inherited (39). This is because almost all the mitochondrial DNA comes from the mother. Very little is contributed by the father. This is because the mitochondrial DNA in the sperm cell is lost during the fertilization of the egg (5, 41, 43, 66). This could include selective elimination of the paternal DNA and/or the DNA is outcompeted or selectively eliminated early in the fertilization process. Hence, females contribute their mutated DNA to their progeny but males seldom do. Both male and female progeny can be affected yet only the female progeny pass this on to the next generation. With respect to mitochondrial diabetes, this means that mothers who are diabetic due to a mutation in their mitochondrial genome will pass this trait to their sons and daughters but their sons will be unlikely to pass the trait on to their progeny. It should be noted, however, that mutation in the mitochondrial genome can occur spontaneously as well as be inherited from one’s mother (2–4, 36–39, 53, 60, 64, 67, 71).

As mentioned earlier, mitochondrial mutation can result in devastating disease. However, milder disease states can develop that are associated with milder impairments of OXPHOS and tissue function. Animal models have been almost nonexistent with respect to the study of these milder conditions. However, there is one that models mitochondrial diabetes, and it is the purpose of this review to describe the development of this model and some of the results of detailed studies of its metabolism. Some of this work shed light on the mechanism for mitochondrial gene transcription so this work is described as well.

### The BHE/Cdb Rat

During and after World War II, scientists at the USDA Nutrition Research Facility in Beltsville, Maryland, were sometimes hard pressed to obtain research animals. They used rats extensively. They decided to breed their own to ensure a ready supply. They crossed some Osborne Mendel rats with those from a colony maintained at the Pennsylvania State College (a colony no longer in existence) and produced a colony that they named BHE for the Bureau of Home Economics. This breeding program crossedbred rats of many coat colors and characteristics. As the generations were randomly bred they were variable in many of their physiological characteristics and in their response to dietary manipulation (68). Eventually, the scientists began to segregate this colony into four subgroups: 1) obese, 2) those that developed hydronephrosis at an early age, 3) very excitable and mean (this group was deleted), and 4) those that developed a fatty liver, especially

### Table 1. List of mutations in the human mitochondrial genome that phenotype as diabetes mellitus

<table>
<thead>
<tr>
<th>Mutated Gene</th>
<th>Position of Mutation</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATPase 6</td>
<td>8993, 8869, 8894</td>
<td>Also phenotypes as Leigh’s syndrome and NARP; two base substitutions at this position have been identified</td>
</tr>
<tr>
<td>tRNALeu</td>
<td>3243, 3252, 3256, 3271</td>
<td>Deafness has also been associated with mutation in this gene</td>
</tr>
<tr>
<td>ND1</td>
<td>3316, 3348, 3394, 3396, 3423, 3434, 3438, 3474, 3483, 4216</td>
<td>LHON also phenotypes with mutation in this gene</td>
</tr>
<tr>
<td>ND2</td>
<td>4917</td>
<td>LHON also phenotypes with mutation in this gene</td>
</tr>
<tr>
<td>tRNAVal</td>
<td>5780</td>
<td>MERRF also phenotypes with this mutation</td>
</tr>
<tr>
<td>tRNAAsp</td>
<td>7476</td>
<td>LHON also phenotypes with this mutation</td>
</tr>
<tr>
<td>COXII</td>
<td>8245, 8251</td>
<td></td>
</tr>
<tr>
<td>tRNAα</td>
<td>8344</td>
<td></td>
</tr>
<tr>
<td>ND3</td>
<td>10,398</td>
<td></td>
</tr>
<tr>
<td>ND4</td>
<td>11,778</td>
<td></td>
</tr>
<tr>
<td>tRNAγ</td>
<td>14,709</td>
<td></td>
</tr>
<tr>
<td>tRNAβ</td>
<td>15,904, 15,924, 15,927, 15,928</td>
<td></td>
</tr>
<tr>
<td>D loop</td>
<td>16,069, 16,093, 16,126</td>
<td></td>
</tr>
</tbody>
</table>

NARP, neurogenic weakness, ataxia, retinitis pigmentosa; MELAS, mitochondrial encephalomyopathy, lactic acidosis, stroke-like syndrome; LHON, Leber’s hereditary optic neuropathy; MERRF, myotonic epilepsy and ragged red fiber disease; ND, NADH-ubiquinone oxidoreductase (seven of the subunits are encoded by the mitochondrial genome and are labeled ND 1, ND 2 etc.); COX: cytochrome c oxidase has three mitochondrial encoded subunits appropriately referred to as COX I, II, or III.

### Table 2. Components of oxidative phosphorylation that are encoded by the mitochondrial genome

<table>
<thead>
<tr>
<th>Component</th>
<th>Nuclear DNA</th>
<th>Mitochondrial DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1F0ATPase</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Complex I</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>Complex III</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Complex IV</td>
<td>10</td>
<td>3</td>
</tr>
</tbody>
</table>

Component refers to the F1F0ATPase synthesizes ATP using the energy released by the respiratory chain. Complex I includes the NAD-linked enzymes that “collect” electrons released by the mitochondrial reactions, mainly the citric acid cycle, transfer them to FMN and then transfer them to Complex II. Complex II has no mitochondrially encoded subunits. Complex III transfers these electrons via cytochrome c to Complex IV that in turn gives them to molecular oxygen the terminal electron acceptor. At each transfer step protons are pumped across the inner mitochondrial membrane producing a proton gradient.

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when fed a sugar-rich purified diet. Adams (1) prepared an extensive summary of all of the work from the 1940s until 1966 that used these rats.

The inconsistent responses of the BHE rats prompted the scientists to put selection pressure on certain characteristics. Marshall bred a line (called IN-BHE) that had an increased biotin requirement, a fatty liver, and hydronephrosis (7). In our hands, after a series of experiments examining the lipemic trait as well as the insulin-glucose relationship, we put selection pressure on the fatty liver, the abnormal glucose tolerance trait at maturity and the lipemic trait of animals fed a 65% sugar diet. Through selective breeding the trait of hydronephrosis was eliminated as was the obese trait and all coat colors except black and white. With continuous inbreeding and back breeding we developed a line of animals that developed abnormal glucose tolerance at 300 days of age. We tested all the breeding animals at this age and any that did not have this trait were deleted from the breeding pool. Their progeny were also deleted. Because of ease in record keeping we kept this closed colony as a maternal line of animals. Males were bred to several females so it was easier to keep track of the female lines than to track the male lines. This was a serendipitous choice as will be seen later.

Many experiments (6–27, 30, 31, 44–52, 54, 61, 62, 72, 73) were conducted to collect basic metabolic data about this inbred line. By the fifth generation of inbreeding and selection, rats were found to have an elevated lactate:pyruvate level in blood and hepatic tissue (11, 12). Their tissue ketone levels were also elevated. By the seventh generation, rats were found to have elevated blood glycerol levels (9) and elevated rates of lipolysis as well as hepatic triacylglyceride and cholesterol synthetic rates (52). Incidentally, these traits were sometimes found in the heterogeneous BHE rats. Mitochondria from the 10th generation were somewhat inefficient and by the 12th generation we were able to detect a reduction in the efficiency of mitochondrial ATP production. It took many generations however, before the variability in response to dietary or hormonal treatment was reduced. However, by the 60th generation such was achieved. Table 3 provides a summary of some of the more important metabolic characteristics of this strain. Note that many of these characteristics are similar to those found in humans with Type 2 diabetes mellitus (2–4, 36–38, 53, 58, 60, 64, 71). By the time it was recognized that the diabetes in this colony was a maternally inherited trait, it was in its 80th generation. Full homogeneity was achieved after 86 generations and the rats were identified as the BHE/Cdb strain.

As a model for maturity onset diabetes mellitus, The BHE/Cdb rat was considered useful because few animal models for this type of diabetes exist. Few could have the time course of their disease influenced by diet. This rat was therefore unique because although it had a fatty liver, it was not obese. Genetically obese rats, such as the Zucker diabetes rat and the ob/ob mouse, have a fatty liver but are obese regardless of diet and these animals develop a form of diabetes that is related to their body fat stores. In this respect, they model the obese or overly fat human with diabetes (called diabesity by some clinicians) rather than the maturity onset diabetes without excess fat stores (42). The BHE/Cdb rat can have a brief period of hyperinsulinemia early in life (6) but this does not always occur. Perhaps this is because it is so transient that we were unable to detect it or it may not occur at all. We have no explanation for this inconsistent feature. This trait soon disappears and the islet cells become increasingly less responsive to a glucose signal (23, 54). The fatty liver characteristic is especially prominent when the animal is fed a sugar-rich diet (8, 11, 22, 24, 30, 59, 61, 62, 72, 73) and when the diet also contains saturated fat (17, 31, 45, 47, 48, 51, 73). Even when fed a standard stock diet, hepatic lipogenesis, and cholesterologenesis is twice that of a normal rat and of course, this increase in hepatic lipid synthesis explains its fasting lipemia (52). Interestingly, the elevated lipogenic characteristic is not associated with an increase in body fat stores primarily because this elevation is also accompanied by an increase in lipolysis (9, 52). Thus the obesity seen in other models of obesity does not occur because there is an active and futile energy-wasting lipogenesis-lipolysis cycle.

**METABOLIC STUDIES**

Studies of oxidative phosphorylation (OXPHOS) by isolated hepatic mitochondria from BHE/Cdb rats compared with Sprague-Dawley or Wistar rats revealed that there was a reduction in the efficiency with which the energy released through the synthesis of water (respiration) could be trapped in the high-energy bond of ATP (16). Efficiency could be reduced by feeding a sugar-rich diet (59, 72); further decreased if a saturated fat (hydrogenated coconut oil) was used (31) and improved if a very unsaturated fat (menhaden oil) was used (26, 44, 48). Efficiency decreased as the animals aged (12). All of these treatments had effects on the inner mitochondrial membrane composition that in turn affected the fluidity of this membrane. The fluidity of this membrane is almost entirely due to the ratio of unsaturated fatty acids to saturated fatty acids since the membrane contains very little cholesterol. An unsaturated dietary fat will increase the amount of unsaturated fatty acids in this membrane whereas a very saturated dietary fat will have the reverse effect. An increase in fluidity facilitates the motion of the ATP synthetase (F,F/ATPase) that moves within the membrane as it synthesizes ATP (63). Any hindrance in this motion will reduce ATP synthesis efficiency, whereas an increase in fluidity will improve ATP synthesis efficiency.

Corresponding changes in glucose homeostasis were observed with the above described dietary manipulations. Gluconeogenesis was more active in the BHE/Cdb rats than in

Table 3. Summary of important characteristics of the BHE/Cdb rat

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATPase 6 base substitution</td>
<td>at positions 8204 and 8289</td>
</tr>
<tr>
<td>Age-related reduced responsiveness of β cell to glucose stimulation</td>
<td>Aggressive hyperinsulinemia</td>
</tr>
<tr>
<td>Age-related impaired glucose tolerance</td>
<td>Poor glucose tolerance</td>
</tr>
<tr>
<td>Early death due to renal disease</td>
<td>Glucose intolerance</td>
</tr>
<tr>
<td>Fatty liver especially when fed a sugar-rich-coconut oil diet</td>
<td>Elevated levels of lipolysis</td>
</tr>
<tr>
<td>Elevated lactate-pyruvate level</td>
<td>Elevated levels of cholesterol and glycerol levels</td>
</tr>
<tr>
<td>Elevated fasting triacylglycerol, cholesterol, and glycerol levels</td>
<td>Twice normal rate of hepatic lipogenesis</td>
</tr>
<tr>
<td>Twice normal rates of hepatic fatty acid and cholesterol synthesis</td>
<td>Elevated levels of lipolysis</td>
</tr>
<tr>
<td>Elevated rates of lipolysis</td>
<td>Gestationally diabetic</td>
</tr>
<tr>
<td>Gestationally diabetic</td>
<td>Poor reproductive capacity</td>
</tr>
<tr>
<td>Poor reproductive capacity</td>
<td>Inefficient ATP synthesis</td>
</tr>
<tr>
<td>Inefficient ATP synthesis</td>
<td>ATP synthesis efficiency improved with vitamin A supplements</td>
</tr>
</tbody>
</table>

BHE, Bureau of Home Economics.
control rats (14); sucrose feeding as well as hydrogenated coconut oil in the diet increased gluconeogenesis (51, 45–47, 61, 62, 73). Tracer studies using tritiated and [14C]glucose confirmed that the saturation of the fat and the source of the carbohydrate affected not only glucose oxidation but also glycogen synthesis and mobilization, glucose turnover and gluconeogenesis (45–47). In all instances where there were dietary effects on OXPHOS, there were corresponding effects on glucose homeostasis. That is, if mitochondria were less well coupled, liver fat rose, gluconeogenesis rose, lipogenesis rose, glycogen breakdown fell and glycogen synthesis and stores rose, and glucose oxidation fell. These changes are typical features of the glucose dysregulation that occurs in diabetics mellitus.

Although we had a plethora of data characterizing the BHE/Cdb rat and although we knew that these traits were genetically associated, we did not have a genetic signature identified. We attempted to obtain this after we conducted a number of studies characterizing the mitochondrial response to exogenous thyroid hormone. In normal rats, this hormone upregulates mitochondrial activity and if given within the normal range of thyroid hormone (not toxic levels), mitochondrial respiration will increase as will ATP synthesis (40, 70). We observed that this did not occur in the BHE/Cdb rat (19). We did observe an increase in F1ATPase activity, an increase in α-glycerophosphate shuttle and malate aspartate shuttle activity and an increase in gluconeogenesis but no increase in ATP synthesis. This then suggested that the genetic problem probably involved the F0ATPase.

GENETIC STUDIES

The F1F0ATPase is a multiunit enzyme complex. It can dissociate into the F1 and F0 parts. The F0 part is embedded in the inner mitochondrial membrane while the F1 protrudes out into the mitochondrial matrix. As mentioned, the ATPase rotates in a clockwise direction as it synthesizes ATP and this movement is influenced by the fluidity of the inner membrane. Two of the subunits of the F0ATPase are encoded by the mitochondrial DNA. They are referred to as the ATPase 6 and the ATPase 8 genes. The ATPase 6 encodes subunit a of the F0ATPase and overlaps the ATPase 8 gene.

We knew that we had developed a maternally inherited genetic trait so we reasoned that if a mutation had occurred, it might be in the ATPase genes encoded by the mitochondrial genome. Thus we then set about sequencing these genes. We extracted the DNA from isolated mitochondria from both the control strain and the BHE/Cdb rats and sequenced the ATPase region (56). We found two homoplasmic base substitutions in the ATPase 6 gene and no base sequence differences in the ATPase 8 gene. One base substitution was found at bp 8204 and the other at bp 8289. We also found some other substitutions; three were silent because they resulted in a codon that would have translated to the same amino acid as the codon in the normal rat. Two more were heteroplasmic with a low level of the substituted codon present. This heteroplasmia probably has no effect on gene product function.

The substitutions at bp 8204 occurred in the region of the protein that forms the proton channel while the substitution at 8289 translates into the part of the protein that forms a hinge in the molecule that seems to play a role in the flexibility of the molecule within the membrane. Recall the effects of dietary fat on membrane fluidity described above. A mutation that would affect flexibility would thus be more responsive to changes in fluidity than a normal protein. The mutation at 8204, on the other hand, could explain the reduced ATP synthesis efficiency. It meant that an asparagine was substituted for aspartic acid and this affected the polarity of the proton channel. In turn, a change in the proton channel polarity would reduce the efficiency of energy capture into the high-energy bond of the ATP. This is what we had reported earlier (16).

We needed to know whether coupling efficiency was related to lipogenesis. We hypothesized that a reduced ATP synthesis would mean an increase in lipogenesis. To test this hypothesis, we treated control and BHE/Cdb rats with a very low level of (3-(3,4-dichlorophenyl)-1, 1-dimethylurea (DCMU), a mild uncoupler. We studied de novo lipogenesis using tritiated water and found that normal rats responded with an increase in fatty acid synthesis (35). Treatment of the BHE/Cdb rats further elevated their lipogenic capacity. We thus concluded that the fatty liver and elevated lipogenic characteristic of the BHE/Cdb rat was due to its inefficient ATP synthesis.

With a cross-breeding experiment, we showed that the ATPase 6 mutations and the phenotype of impaired glucose tolerance and impaired mitochondrial function were maternally inherited traits (57). We also showed that the mutation was present in a variety of tissues: tail tip, muscle, liver, and pancreas. Progeny of female BHE/Cdb rats mated to male Sprague-Dawley rats had the same base sequence as full-breed BHE/Cdb rats while progeny of female Sprague-Dawley rats mated to BHE/Cdb male rats had the same base sequence and glucose tolerance as full blood Sprague-Dawley rats. This meant that future studies did not have to use two control groups: cross-bred and full bred Sprague-Dawley rats. This of course assumes that all the differences between the strains were due to the differences in the mitochondrial genome. This may not be a completely correct assumption but we have no evidence to the contrary at present.

The mutation in the mitochondrial genome had effects on the responsiveness of the mitochondria to oligomycin and calcium
Mitochondria from BHE/Cdb rats were more susceptible to calcium mediated death than mitochondria from control rats (49) and also more responsive to oligomycin (50). This can probably be explained as being due to an increased leak rate due to the mutation in the ATPase 6 protein that in turn affects membrane potential. This is consistent with the observations in humans with either a T8993G or a T8993C point mutation in the ATPase 6 gene (69). The human mutation is within 30 bp of the rat mutation but since the human genome is larger than the rat genome, the mutation is in roughly the same location. In each one there is a change in the polarity of the proton channel. In the human with the T8993G mutation, the substitution is arginine for leucine. In the rat, it is asparagine for aspartate. In both species, ATP synthesis is impaired, and in both species, there is an increased sensitivity to oligomycin inhibition of OXPHOS. Calcium-mediated cell death is also characteristic of humans with mitochondrial disease (74).

LONGEVITY STUDIES

Although we conducted several acute studies with mitochondria, we wanted to know whether there were long-term characteristics or long-term responses to dietary manipulation so we conducted several longevity studies. In brief, what we learned was that lifespan was diet responsive in that rats fed a beef tallow diet lived longer than rats fed a corn oil or menhaden oil diet and that rats fed an egg-enriched diet lived longest of all (18, 20, 21). The cause of death was usually renal disease. The results of the egg feeding study were curious. These rats had a delay in the appearance of impaired glucose tolerance and a delay in the appearance of the typical renal disease often observed in this strain of rat. This diet also improved OXPHOS efficiency. Several studies (27, 28, 32, 33) were designed to determine possible mechanisms, yet none were as exciting as those that tested the hypothesis that the egg effect was due to its vitamin A content. A feeding study that used graded levels of dietary vitamin A fed to vitamin A-deficient rats revealed that the BHE/Cdb rats had an optimal OXPHOS at an intake three times that recommended for the maintenance of normal rats (32). This is the intake that is usually reserved for growing rats or pregnant/lactating rats.

MITOCHONDRIAL TRANSCRIPTION AND TRANSLATION STUDIES

Mitochondrial transcription is under the control of a single promoter region, the D loop. In this region, binding elements for a variety of promoters have been identified. There are vitamins A and D binding sites, thyroid hormone binding sites, steroid hormone binding sites, insulin binding sites as well as sites for several nuclear-encoded mitochondrial transcription factors, such as transcription factor A (67). Several hormones and nutrients appear to be involved in transcription activation. Figure 1 illustrates this promoter region of the mitochondrial DNA.

A vitamin A dose response study showed that an increase in vitamin A intake increased the amount of ATPase 6 gene product as well as increased the number of mitochondria and the amount of mitochondrial transcription factor A (27, 28, 32–34). A dose response study using primary hepatocyte cultures showed that 10–9 M retinoic acid added to the culture medium likewise increased ND1 and ATPase 6 mRNA as well as the ATPase 6 gene product.

The presence of a retinoic acid binding protein in the mitochondrial compartment confirmed the supposition that vitamin A as retinoic acid stimulated mitochondrial transcription (33, 34). Other investigators have also reported on the retinoic acid effect with respect to mitochondrial transcription (65). Retinoic acid has been shown to upregulate the transcription of NADH dehydrogenase subunit 5 mRNA as well as cytochrome c oxidase subunit 1 and 16 S rRNA (33). These reports suggest that retinoic acid binds via a retinoic acid binding protein to the promoter region of the mitochondrial DNA, the D loop, and promotes the transcription of all of the mitochondrial encoded structural genes. In addition, retinoic X receptor α-knockout mice were shown to have alterations in mitochondrial gene expression. Retinoic acid works in two ways to increase mitochondrial transcription. One is through increasing mitochondrial transcription factor A gene expression, that in turn, increases the steady-state levels of this protein in the matrix. Because mitogenesis is linked to this factor, an increase in the number of mitochondria would be expected. We have reported that this occurs (32, 33). Second, the vitamin acts as a mitochondrial transcription agent promoting the transcription and translation of vital components of the OXPHOS system. Again, we have reported that this occurs. Now we have an explanation for the effect of the egg diet on the health and progression of symptoms in the BHE/Cdb rat. By providing extra vitamin to these rats they were able to increase their mitochondrial number as well as increase the amount of OXPHOS proteins in their mitochondria. Although their proteins were still aberrant, the fact that they then had more of them working would then compensate for the decreased efficiency of the system.

As mentioned in the introduction, mitochondrial mutation has been linked to diabetes mellitus in humans (2–4, 29, 36–38, 53, 60, 64, 69). Several different mutations have been reported and as mentioned above, mutation in the ATPase gene is analogous to the mutation in the BHE/Cdb rat. Clearly, our studies of this rat have shown that the subtle differences in OXPHOS can be related to its mitochondrial mutation and that these subtle differences can eventually lead to abnormalities in the regulation of glucose homeostasis. Because the islet cell as well as the renal cell has a very high requirement for ATP (4, 36) any shortfall in ATP production by the mitochondria in these cells will result in eventual failure. Thus with age, the pancreatic islet cell becomes less responsive to glucose as a signal for insulin release and the renal cell progressively fails as part of the filtration/excretion system. Both of these age-related changes have been documented in the BHE/Cdb rat making this rat valuable as a tool to study mitochondrial diabetes as it occurs in humans.

REFERENCES


