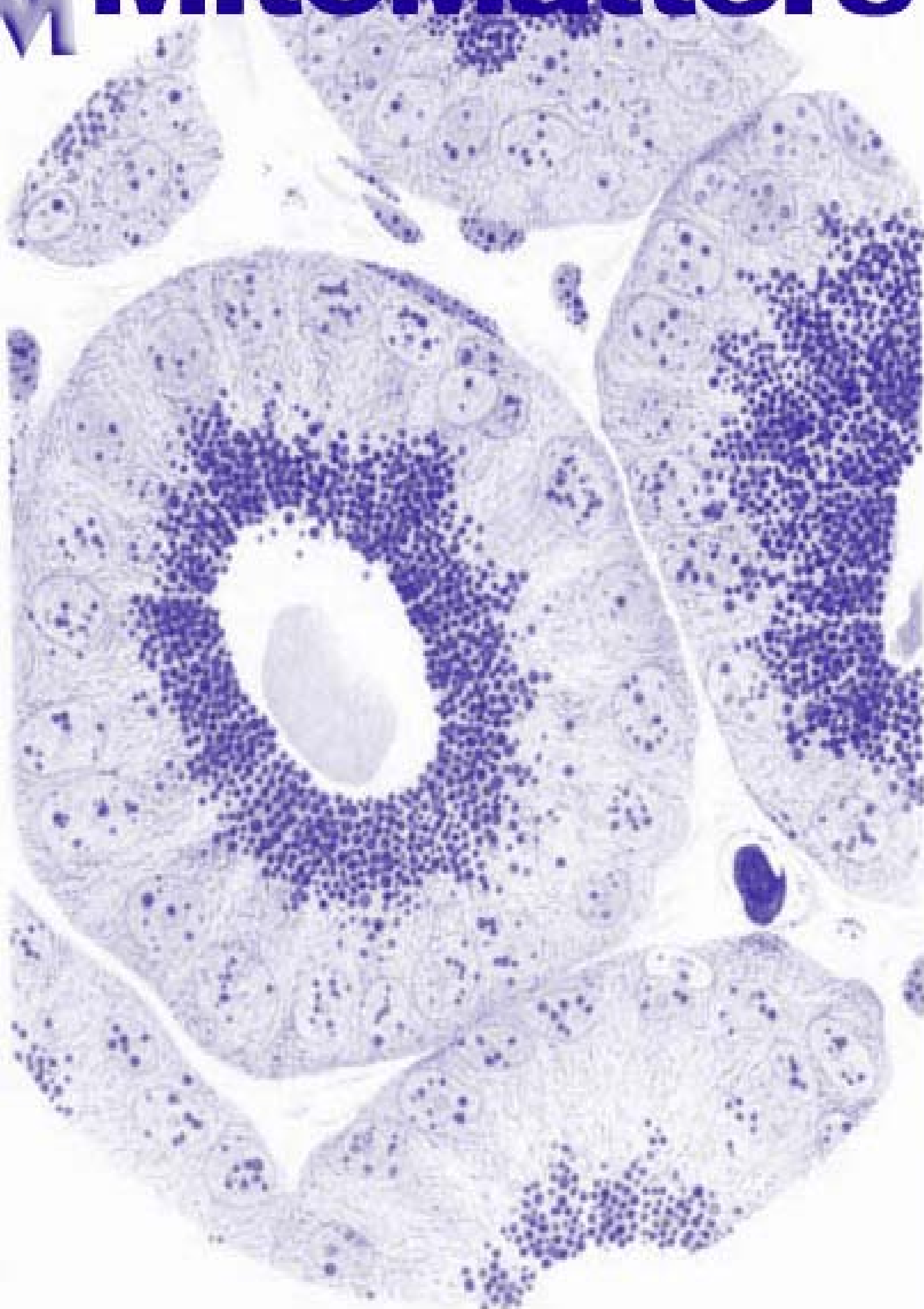

The Official Newsletter of the Mitochondria Research Society,
Volume 1, Issue 2, 2002

Editor's note

It is with great pleasure that we present the second issue of *MitoMatters*. The first issue received an excellent response from our community and we are filled with enthusiasm to continue our mission of disseminating information about mitochondria and mitochondrial diseases.

In this issue, we are very pleased to welcome the contributions of Dr. Barbara Levin, who writes about standard reference material for mtDNA heteroplasmy and the NIST mtDNA interactive web site. Her article is the first in our new section, *Tools and Technology*, which will inform our readers about technical aspects of mitochondrial research, including where to find various resources and databases on mitochondria.

With this newsletter you will find the MRS 2002 application/renewal form. If you have not already renewed your membership, please do so as soon as possible. With you early renewal you will continue to receive an uninterrupted subscription to the *Mitochondrion* journal as well as other membership benefits.

We hope you enjoy this issue of *MitoMatters.* We invite you contribute short review articles, news reports and your ideas for future issues of the newsletter. You input will make this publication a success.

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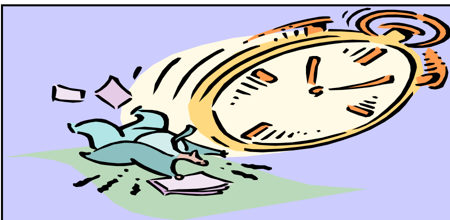

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Mitochondria are Multi-Hit by Antiretroviral Therapy Causing the HIV-Lipodystrophy Syndrome

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The Lipodystrophy

Antiretroviral therapy, composed of a combination of nucleoside reverse transcriptase inhibitor (NRTI), non- nucleoside reverse transcriptase inhibitors (NNRTI), and protease inhibitors, has improved disease progression and mortality in HIV-infected patients. Recently, patients treated with PI and/or NRTI are developing a lipodystrophy syndrome with an estimated prevalence rate ranging from 2%-84% of patients. The syndrome can be characterized by peripheral fat wasting in the face and limbs, accumulation of visceral fat, breast adiposity, cervical fat-pads, hyperlipidemia and insulin resistance. There are four different phenotypes of these lipodystrophies: metabolic abnormalities with peripheral fat loss and abdominal fat accumulation; metabolic abnormalities with fat accumulation but without fat loss; metabolic abnormalities with fat loss but without fat accumulation; and body shape changes without metabolic changes. The metabolic abnormalities include altered lipid metabolism (increased triglycerides, increased total cholesterol, increased low density cholesterol) and altered glucose metabolism (insulin resistance, impaired glucose tolerance, diabetes mellitus). Risk factors for the development of moderate/severe lipoatrophy are increasing age, any use of stavudine, use of indinavir for longer than 2 years, body mass index (BMI) loss, and measures of duration and severity of HIV disease. Whereas, independent risk factors for moderate/severe fat accumulation are increasing age, BMI gain, measures of amount and duration of immune recovery, and duration of antiretroviral therapy.

Mitochondrial Etiologies

The role of mitochondria in the etiology or pathogenesis of the lipodystrophy was first recognized when a similarity was noted with multiple symmetric lipomatosis (MSL) type 1, a disease of fatty tissue which effects men. MSL has been associated with the 8344 point mutation in the t-RNA-lysine gene of mtDNA which is also mutated in myoclonic epilepsy ragged-red fibers (MERRF) patients. Patients with MSL develop large unencapsulated lipomas in the subcutaneous tissue of the cervical, deltoid, thoracic, abdominal, and lumbar areas and is accompanied by hyperuricemia, dyslipidemia, acrocytic anemia, peripheral neuropathy, and impaired glucose tolerance. Also, since NRTIs are known to inhibit mitochondrial DNA-polymerase γ, both *in vivo* and *in vitro*, thus the similarities between MLS and the HIV-lipodystrophy were hypothesized to be drug induced.

Subcutaneous fat biopsies have been examined morphologically and analyzed for mitochondrial DNA depletion. Walker et al. (*J. AIDS*, 29: 117-21, 2001) analyzed fat from 24 HIV-infected (19 treated with NRTI and 5 without NRTI treatment) and 8-HIV negative controls for mtDNA content by Southern Blot analysis. The mean mtDNA content was 44% lower in the NRTI-treated group compared to the group without NRTI treatment; and mtDNA levels were decreased by 39% in the group with lipoatrophy compared to the group without lipoatrophy. Electron micrographs of fat from individuals with lipoatrophy demonstrated adipocytes with small lipid droplets in the rim of the cytoplasm surrounding the main lipid vacuole and marked variation in size and shape of the mitochondria with sparse and randomly-oriented cristae. Mallal et al. (4Th International Conference on Nutrition and HIV Infection/2nd European Workshop on Lipodystrophy, 2001, Cannes, France) analyzed fat biopsies from 14 HIV-infected patients and 4 HIV-negative controls for mtDNA content by real-time quantitative PCR. Patients receiving AZT or d4T (n=9) showed mtDNA depletion. Furthermore, Shikuma et al (*AIDS,* 15:1801-09, 2001) measured mtDNA levels by PCR in four different cohorts: 8 lipodystrophic, 7 non-lipodystrophic, 5 antiretroviral naïve HIV-infected, and 7 HIV sero-negative. The lipodystrophic cohort had decreased mtDNA levels

compared to the other groups. Thus, three independent research groups have demonstrated that there is tissue specific-adipose mtDNA depletion.

Another mitochondrial target could be changes in lipid metabolism via an initiating event from either insulin resistance or defective post-prandial lipid metabolism. The physiological features are increased turnover of fatty acids and high levels of systemic fatty acids in the post-prandial phase. Resting energy expenditure has been shown to correlate with insulin resistance in HIV-infected patients taking NRTIs and PI. The hypothesis is that the elevated resting metabolic rate is due to partial 'uncoupling' of mitochondrial respiration induced by the elevated tissue levels of long chain fatty acids. This uncoupling would lead to decreased mitochondrial efficiency leading in a controlled way in response to the presence of increased fatty acid delivery.

Lipid metabolism may also be effected by the PI due to the homology of the catalytic regions of the HIV protease and cytoplasmic retinoic-acid binding protein 1 (CRABP-1) and low density lipoprotein-receptor-related protein (LRP). The hypothesis is that the PI inhibits CRABP-1 and CYP3A-mediated synthesis of cis-9-retinoic acid and peroxisome proliferator-activated receptor type-γ (PPAR-γ) heterodimer. This results in an increased apoptosis of adipocytes and in a reduced differentiation from pre-adipocytes to adipocytes, with the final effect of a reduced trigylceride storage and increased lipid release. PI binding to LRP would impair hepatic chylomicron uptake and endothelial triglyceride clearance, resulting in hyperlipidemia and insulin resistance.

Yet another etiological factor may be tumor necrosis factor-α (TNF-α). It has been demonstrated to be dysregulated in patients taking antiretroviral therapy. TNF-α is closely associated with the synthesis of interleukin-2, a crucial factor for T lymphocytes that allows them to escape apoptosis. TNF-α leads to fat wasting and hyperlipidemia by inhibiting the activity of lipoprotein lipase and the intake of free fatty acids by adipocytes. This increases the hepatic synthesis of triglycerides and lipogenesis. In addition, TNF-α activates the production of leptin that down regulates lipogenic enzymes and results in insulin resistance. Lastly, TNF-α induces adipocytes into apoptosis. Subcutaneous fat biopsies from patients taking NRTIs and PI have shown TUNEL positive adipocytes.

The role of mitochondria in the NRTI and/or PI induced lipodystrophy appears to be a multi-hit phenomenon. Mitochondrial genetics is affected through depletion of mtDNA in the adipocytes that very likely cause changes in oxidative phosphorylation and increased oxidative stress. Lipid metabolism is altered by changes in fatty acid metabolism resulting in uncoupled mitochondria and increased apoptosis. The latter is also induced by TNF-α. Thus, the etiology of this complex lipodystrophy, which includes a morphological phenotype with diabetes, and hyperlipidemia, is due to mitochondrial function being altered.

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A new NIST mitochondrial DNA interactive website

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Every human cell has a few dozen to several thousand mitochondria, each of which contain mitochondrial DNA (mtDNA). The sequence of the entire human mtDNA (16,569 base pairs) was determined and published by Anderson *et al*[1]. in 1981. A mtDNA standard reference material (SRM 2392) is now available from the National Institute of Standards and Technology to provide quality control when sequencing mtDNA for forensic identifications, medical diagnosis or mutation detection (Levin *et al.*, 1999). We are currently in the process of adding DNA from the cell culture HL-60 to SRM 2392 and hope to have the new enhanced SRM by December 2002. A number of human diseases are known to be associated with specific mutations and deletions of mtDNA. Many of these diseases have been correlated with heteroplasmic mutations (i.e., only a certain percentage of the mtDNA molecules have the mutation). The disease causes no symptoms until the percentage of the mtDNA carrying the mutation exceeds some threshold. Thus, many mtDNA diseases appear to be age-dependent (i.e., the patient shows the symptoms of the disease only after reaching a certain age which may indicate that the number of mutant mtDNA molecules increase with age). With present state-of-the-art techniques, low-frequency heteroplasmic mutations scattered throughout the DNA, are almost impossible to detect. We are currently developing a new heteroplasmic mtDNA SRM (SRM 2394), which consists of mixtures of various concentrations of two mtDNA PCR products (285 bp) differing in one base pair. This SRM is designed for medical, forensic and toxicological scientists who wish to determine the sensitivity of their techniques to detect low-frequency mutations, polymorphisms or heteroplasmic DNA sites. MtDNA mixtures containing a polymorphic/wild-type site in different percentages (1, 2.5, 5, 10, 20, 30, 40 and 50%) have been constructed. With automated sequencing techniques (ABI 373 or 310), we were able to unambiguously detect the polymorphism at the 30% level. At 20%, the heteroplasmy was detectable if you knew where it was located; otherwise, the polymorphism was difficult to distinguish from the background. The use of Denaturing Gradient Gel Electrophoresis (DGGE) increased our resolution to the 5% level. The addition of a Peptide Nucleic Acid (PNA) complementary to the majority fraction allowed the selective amplification of the polymorphic minority fraction and also increased the detection resolution to 5%. We recently completed an interlaboratory evaluation in which 12 laboratories were provided with PCR products containing 100% of one of the PCR products used to make the mixtures, 100% of the other PCR product, and all of the mixtures (the mixtures were coded before being sent to the laboratories). Each laboratory was instructed to determine the percent heteroplasmy in all the tubes using their preferred method. Based on their results, we hope to determine the best methods for detecting low-frequency mutations. When completed, this SRM will enable investigators to determine the sensitivity of their mutation detection techniques and provide them with a tool to perfect even more sensitive methods. In this way, mitochondrial DNA diseases may become detectable, treatable, and perhaps even preventable prior to the appearance of any symptoms. A full manuscript on this heteroplasmic mtDNA is in preparation.

In addition, we have developed an interactive human mitochondrial DNA web site to enable investigators to determine the reported effects of any single nucleotide polymorphism, mutation, insertion or deletion in mtDNA[2]. The investigator provides information as to whether the change is a deletion, insertion or substitution, then enters the Cambridge Reference Sequence[1] number and the identity (A, C, G, T) of the changed nucleotide. The program compares that to the Cambridge Reference Sequence and provides information on which base in the codon has been changed, where the change occurs (i.e., whether it is in the non-coding region, the HV1 or HV2 region, whether it affects a ribosomal RNA, a transfer RNA, or DNA coding for a protein), which protein is affected, whether it causes a change in an amino acid in that protein, and specifies the new amino acid as well as the one noted by the Cambridge Reference Sequence. If an amino acid has changed, it specifies the position of that amino acid change in the protein (e.g., amino acid # 25 in a protein containing 200 amino acids). This program will also provide the entire amino acid sequence of the

affected protein and allow one to compare the new amino acid sequence to the sequence generated from the original Cambridge Reference DNA.

The web site is available without charge and can be found at: http://www.cstl.nist.gov/biotech/strbase/mitoanalyzer.html. A paper has recently been published describing this interactive web site[2].

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1. Anderson, S. *et al.* (1981) Sequence and organization of the human mitochondrial genome. *Nature* **290**: 457-465.

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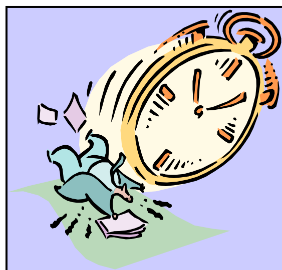

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Telomere and Mitochondrial Connection

Telomeres are repetitive sequences at the end of chromosomes. With age, these sequences become smaller and this process is believed to underlie cellular senescence. Telomerases are enzymes that specifically prevent this telomere loss and therefore are believed to be anti-senescence agents. However, the linkage between telomere shortening and the cellular dysfunction that accompanies senescence is still obscure. Through gene expression profiling, Nautiyal and co-works have demonstrated that loss of telomerase is followed by an up-regulation of energy production genes and mitochondrial proliferation in *Saccharomyces cerevisiae*. These results suggest that senescence may be associated with a change in the cellular metabolic program and the triggering of a stress-response involving oxidative metabolism.

- Nautiyal, S.; DeRisi, J.L. & Blackburn, E.H. (2002). The genome-wide expression response to telomerase deletion in *Saccharomyces cerevisiae*. *PNAS*, **99** (14): 9316.

Nitroglycerin and mitochondria

Nitroglycerin (GNT)) has been used as a treatment for angina and heart failure for over a century. However, it was still largely unclear how nitroglycerin was metabolized in our bodies to generate the molecular species involved in the protective action. Recently, Chen and co-workers purified a nitrate reductase that specifically catalyses the formation of 1,2-glyceryl dinitrate and nitrite from GNT. This enzyme was identified as the mitochondrial aldehyde dehydrogenase (mtALDH), demonstrating that mitochondria play a central role in the biotransformation of this drug and in the mechanism leading to relaxation of vascular smooth muscles.

- Chen, Z.; Zhang, J. & Stamler, J.S. (2002). Identification of the enzymatic mechanism of nitroglycerin biotransformation. *PNAS*, **99** (12): 8306.

Twinkle and mitochondrial disease

A large number of diseases associated with mutations in genes involved in mitochondrial metabolism have already been identified. But the recent discovery of the Twinkle gene by Spelbrink and co-workers added a new player to the wide range of proteins involved in mitochondrial disorders. Twinkle encodes for a DNA helicase that is believed to be critical for the maintenance of mtDNA integrity. Mutations in the Twinkle gene have been identified in 12 pedigrees of autosomal dominant progressive external ophthalmoplegia (adPEO). Many diseases and syndromes had been previously associated with mutations in DNA helicases involved in nuclear DNA maintenance; the discovery of Twinkle adds mtDNA to the expanding area of targets for helicase diseases.

- Spelbrink, J.N.; Tirani, V.; Nikali, K. Et al. (2002). Human mitochondrial DNA deletions associated with mutations in the gene encoding Twinkle, a phage T7 gene 4-like protein localized in mitochondria. *Nat. Genetics*, **28** (3): 223.

Mitochondrial methods

The expanding field of mitochondria poses a real technical challenge for those starting in this field, or even for those that are trying to keep up with all the developments in this area. How to better answer our questions and what are the most appropriate methods to use are questions that first come to mind. In this regard, the publication of methodological reviews is always welcome. The journal Methods has just come out with an issue dedicated to mitochondria research. Check it out.

- **Methods**, April 2002, **26** (4).

Notes and News

The Mitochondria Research Society sponsored a workshop in Dallas on June 6, 2002 on Laboratory Methods For the Diagnosis of Mitochondrial Diseases. It was attended by more than 100 investigators and was a great success. Several methods including respiratory chain biochemistry, immunodiagnosis, DNA analysis, pathology, neuroimaging and non-respiratory chain biochemistry were discussed. A panel discussion was held to develop uniform standard for diagnosis of mitochondrial disease. Pictured below are some of the faculty who were present at the workshop..

Some of the faculty of mitochondrial standard workshop

Upcoming Meetings

August 24-29, 2002	Gordon Research Conference on Mitochondria and Chloroplast. Visit http://www.grc.org
October 9-11, 2002	Mitochondria Research Society meeting in Moscow, Russia, Visiit:http://www.pediatr/mtu-net.ru/News-eng/News.html

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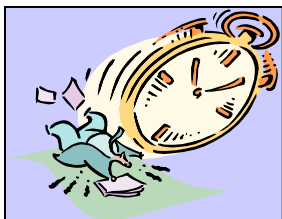

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