Turnover of Adipose Components and Mitochondrial DNA in Humans: Kinetic Biomarkers for Human Immunodeficiency Virus–Associated Lipodystrophy and Mitochondrial Toxicity?

Marc K. Hellerstein
Department of Nutritional Sciences and Toxicology, University of California at Berkeley; and Division of Endocrinology and Metabolism, Department of Medicine, San Francisco General Hospital, University of California, San Francisco

Lipoatrophy (LA)/lipodystrophy and nucleoside reverse-transcriptase inhibitor (NRTI)–associated syndrome are of central importance in human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) care. Neither of these conditions has had a clear pathogenesis or biomarker defined for early detection, prevention research, or patient management. I describe the recent development of kinetic biomarkers for LA and mitochondrial toxicity that involve the measurement of biosynthetic fluxes rather than static concentrations of molecules. The turnover of adipose-tissue components (lipids and cells) and tissue mitochondrial DNA is measured by the incorporation of deuterium from heavy water, using mass spectrometry. Preliminary results in animal models and humans, including the effects of NRTIs on mitochondrial DNA synthesis in rats and adipose-tissue lipid kinetics in HIV-associated LA, are reviewed. The results suggest that the kinetics of adipose-tissue components and mitochondrial DNA are measurable in vivo and that these measurements may prove useful as clinical biomarkers in patients with HIV/AIDS.

Toxicities associated with the antiretroviral treatment (ART) of HIV-1 infection are among the most important problems in contemporary HIV/AIDS care and research [1–4]. Syndromes attributed, at least in part to, agents used in ART regimens include lipoatrophy/lipodystrophy (LA/LD), hyperlipidemia, insulin resistance, and mitochondrial toxicity. In addition to the direct morbidities related to these toxicities, long-term risks for secondary diseases may be increased (e.g., atherosclerotic cardiovascular disease and diabetes mellitus). Equally important, symptoms or concerns about developing secondary diseases may contribute to reduced compliance with life-saving ART regimens. Recent Office of AIDS Research summaries of research needs in HIV/AIDS list toxicities of ART as 1 of the top 3 or 4 priorities [5], for all of these reasons.

Two syndromes, in particular, are relatively novel and are currently without established effective treatment options—LA/LD and a nucleoside reverse-transcriptase inhibitor (NRTI)–associated syndrome often termed “mitochondrial dysfunction” [3, 4]. Because effective treatments for these conditions have not yet been developed, prevention may be the most effective strategy. Prevention requires diagnostic measurements that are both definitive and present early during the course of
the disorder, however, if patients at risk are to be identified before advanced complications have occurred. Unfortunately, objective tests (biomarkers) of neither LA nor mitochondrial dysfunction have yet been identified. Without reliable biomarkers of underlying pathogenesis, the early identification of patients on the path to long-term complications is not possible [6, 7].

The absence of biomarkers for LA or mitochondrial toxicity also constrains research trials that test for therapeutic or preventive interventions. The efficacy of intervention strategies, in general, are difficult to assess without intermediate measurements that can be assessed short of final clinical end points [6–8]. The stratification of patient populations for clinical trials by disease activity, dose adjustments during trials, and post hoc subset analysis of metabolic responders versus nonresponders are also often greatly aided by the availability of accurate biomarkers.

The availability of the biomarkers of disease activity and/or risk would therefore clearly be valuable for these ART-related syndromes. Although neither LA nor NRTI-associated mitochondrial dysfunction currently have a proven pathogenesis, biochemical mechanisms have been proposed for each [4, 9–11]. The measurement of an underlying biochemical process that contributes to disease pathogenesis is often an optimal approach to diagnostic monitoring and risk-factor identification [6–8].

In the present article, recent work toward the development of biomarkers for LA and mitochondrial dysfunction will be discussed. The measurement approach used here involves kinetic biomarkers—measures of biosynthetic fluxes rather than of static levels of molecules. The theoretical basis and early experimental progress for each disorder will be reviewed. I will first review adipose-tissue kinetics in the context of HIV-associated LA, then mitochondrial turnover in the context of NRTI–associated toxicities.

**Measurement of Cell Division (DNA Replication) in Adipose Tissue Using Heavy Water ($^2$H$_2$O)**

The differentiation sequence of mature adipocytes from undifferentiated cells is shown in figure 1A. Cell proliferation is involved at several steps during the process. The most direct measurement and characteristic feature of cell division is DNA replication [16, 17]. Thus, the production of mature adipocytes can, in principle, be measured by the fraction of mature adipocytes with newly synthesized DNA (i.e., the fraction of cells that had recently undergone the proliferation/differentiation sequence shown in figure 1A). We recently developed a non-radioactive (stable isotope/mass spectrometric) labeling technique for measuring DNA synthesis, and thus cell proliferation, in living organisms [16–24]. The technique involves the metabolic labeling of the deoxyribose (dR) moiety of newly formed dNTPs with deuterium, administered either in the form of labeled glucose (e.g., [6,6-$^2$H$_2$]-glucose) or heavy water ($^2$H$_2$O or $^2$H$_2$O) (figure 1B), and mass-spectrometric measurement of the deuterium content of dNTPs in DNA. The incorporation of deuterium-labeled dNTPs into replicating DNA reveals the fraction of genomic DNA that was newly synthesized during the period of exposure to label. Purine deoxyribonucleosides, such as deoxyadenosine (dA), have the advantage that the de novo nucleoside synthesis pathway contribution is high and relatively constant, whereas the nucleoside salvage pathway contribution is lower and more variable [25, 26].

This stable-isotope metabolic labeling approach has several technical advantages over traditional DNA labeling methods, such as $^3$H-thymidine or bromodeoxyuridine [17]. In addition, the lack of mutagenicity or toxicity in stable-isotope labels permits their use in humans. A number of cell types have been studied by this approach, including T and B lymphocytes, colonocytes, mammary and skin epithelial cells, vascular smooth-muscle cells, hepatocytes, monocytes, granulocytes, natural killer cells, bone marrow stem cells, breast cancer cells, chronic lymphocytic leukemia cells, myocytes, and others [16–22]. The applications to adipocytes [21, 24] (F. Antelo, K.-H. Kim, R. Hoh, et al., unpublished data; A. Strawford, F. Antelo, M. Christiansen, and M.H.K., unpublished data) and mtDNA [27, 28] will be discussed here (see below).

**Measurement of Lipid Turnover in Adipose Tissue Using Heavy Water**

We recently also described a new technique for measuring the synthesis and breakdown rates of adipose TG, and, concur-
Figure 1.  A, An overview of adipose-tissue maturation sequence (adapted from [12]). Note that steps in the differentiation process from preadipocyte to adipocyte involve cell proliferation. B, Pathways of label incorporation into cellular DNA [19]. Stable-isotope label incorporation from 2H-glucose or 2H2O into replicating DNA occurs through the de novo nucleotide synthesis (DNNS) pathway and can be measured by mass spectrometry. 3H-dT, tritiated thymidine; BrdU, bromodeoxyuridine; dN, deoxyribonucleosides; DNPS, de novo purine and pyrimidine synthesis pathways; G6P, glucose-6-phosphate; Glc, glucose; GNG, gluconeogenesis; NDP, nucleoside-diphosphate; PRPP, phosphoribosylpyrophosphate; RR, ribonucleoside reductase.

Currently, the contribution from the de novo lipogenesis (DNL) pathway [21, 23, 24] (A. Strawford, F. Antelo, M. Christiansen, and M.H.K., unpublished data). These processes can be measured on the basis of the incorporation of deuterium from 2H2O into C-H bonds of the glycerol and fatty acid moieties of TGs, respectively. We have described these techniques in detail elsewhere [21, 24 (A. Strawford, F. Antelo, M. Christiansen, and M.H.K., unpublished data)]. In brief, the number (n) of C-H bonds in TG glycerol that exchange with body H2O during metabolic synthesis of free α-glycerol-phosphate (used for the synthesis of newly formed TG) is determined by combinatorial analysis (mass isotopomer distribution analysis [MIDA]) [29–31] of the derivatized glycerol moiety. MIDA is a mass-spectrometric technique that can be done on essentially any biomolecule that was synthesized from repeating subunits. The fraction of newly synthesized TG glycerol can then be calculated on the basis of the measured enrichment of 2H2O in body water [24, 31]. Similarly, the value of n for nonessential fatty acids synthesized through the DNL pathway is determined by MIDA, and the fractional contribution from DNL is then calculated on the basis of the measured body 2H2O enrichment [21, 24, 32].

It is therefore now possible to administer a single nonradioactive isotopic tracer (2H2O) that is extremely simple to use (i.e., given as drinking water) and is relatively inexpensive, to measure the turnover rate of TG, the contribution from endogenous synthesis versus diet to fatty acids in stored TG, and the turnover rate of cells in adipose tissue. Some examples of this technique will be described below.

Application of Multiple Adipose Tissue Dynamics Based on 2H2O Labeling: Role of Leptin Deficiency versus Hyperphagia in ob/ob Mice

The ob/ob mouse is a monogenic model of obesity and insulin resistance caused by the absence of leptin secretion from adipose tissue. Leptin, an adipose-derived circulating protein, has effects on food intake, energy expenditure, and nutrient partitioning. Because serum leptin levels correlate with the whole-
body adipose tissue mass [33], a negative feedback loop or “adipostat” role for leptin has been suggested. Although leptin has gained considerable attention as a mediator of adipose tissue mass, the independent roles of hyperphagia versus direct metabolic effects on the adipose tissue metabolism of ob/ob mice have not been clearly elucidated [34]. We studied 4 groups of C57BL6/j female mice: normal control mice fed ad libitum (con), ob/ob mice fed ad libitum (ob/ob), food-restricted ob/ob mice (ob-r), and ob/ob mice treated with a constant subcutaneous infusion of leptin (2 μg/day) (ob-lep). 3H2O (4%) was administered as drinking water for 21 days, to measure 3 biosynthetic processes concurrently: triglyceride synthesis (total lipogenesis), palmitic acid synthesis (DNL), and adipose DNA replication (cell proliferation). Compared with con mice, the absolute rates of TG synthesis (~1.0 vs. 0.2 g for 21 days) and DNL (0.2 vs. 0.05 g for 21 days) were elevated up to 4–5 times in ob/ob mice (figure 2). A significantly higher rate of adipose-tissue cell proliferation (average, 4.5 vs. 1.7 × 10^6 cells over 21 days) and adipose TG turnover or net whole-body lipolysis (average, 0.4 vs. 0.1 g over 21 days) were also seen in the ob/ob group. Food restriction in the ob/ob mice resulted in only a modest reduction in the rates of DNL, TG synthesis, and adipose cell proliferation, whereas net lipolysis was increased. In contrast, leptin administration reduced all 3 synthetic measurements (DNL, TG synthesis, and adipogenesis). In the ob-lep group, lipolysis was higher than in any other group, whereas serum insulin levels remained elevated. We conclude that leptin deficiency results in abnormal elevations in DNL, TG synthesis, and adipose cell proliferation and that these abnormalities are not driven primarily by hyperphagia but are corrected by leptin administration preceding major changes in body composition or insulin levels. These conclusions were made possible by the availability of techniques for measuring the dynamics of adipose-tissue components in living animals.

Preliminary Results in Healthy Human Subjects and Patients with HIV/AIDS Who Did and Did Not Have ART-Associated LA

Pilot studies have been done in humans that have measured the turnover of adipose-tissue components (A. Strawford, F. Antelo, R. Hoh, and M.K.H., unpublished observations; F. Antelo, K.-H. Kim, R. Hoh, et al., unpublished data; A. Strawford, F. Antelo, M. Christiansen, and M.H.K., unpublished data). For these studies, 3H2O was given to subjects as a daily dose (70 mL/day) for 9–10 weeks, under free-living, outpatient conditions. After 5 and 9–10 weeks of daily 3H2O intake, percutaneous adipose-tissue needle aspirates were obtained (under local lidocaine anesthesia with a 14-gauge needle) from 3 subcutaneous adipose depots (gluteal, thigh, and flank regions). The lipids were extracted, isolated, and derivatized for analysis by gas chromatography/mass spectrometry (GC/MS) of the TG-glycerol moiety (mass to charge [m/z] 159–161 of the glycerol-triacetate derivative) and TG-palmitate (m/z 256–258 of palmitate-methylester). A stromal-vascular–depleted, mature adipocyte–enriched fraction of adipose tissue was also prepared by enzymatic digestion of the collagen matrix of adipose tissue to free cells, followed by differential centrifugation, to isolate the less dense, mature adipocyte fraction [21] (F. Antelo, K.-H. Kim, R. Hoh, et al., unpublished data). DNA was prepared from the cell fractions isolated, and hydrolyzed enzymatically to free deoxyribonucleosides, and the free dA was isolated [16, 18]. The dR moiety of dA was derivatized to pentose-tetraacetate and analyzed by GC/MS (m/z 245–246, chemical ionization) [33]. Body-water enrichment levels were measured by a GC/MS method described elsewhere [19, 33].

Adipose-TG turnover. The normal turnover rate of adipose-tissue TG was quite slow, consistent with previous indirect estimates [34]. Fractional TG replacement after 9 weeks of 3H2O intake was 19%–22% in the 3 depots in weight-stable healthy human subjects. The adipose TG half-life was calculated to be in the range of 180–200 days, although there was considerable interindividual variability. Net synthesis/breakdown (i.e., net lipolysis, under weight-stable conditions) was 50–60 g/day (<0.5–0.6 mg/kg/min). These are the first long-term direct measurements of adipose-TG turnover in humans and are congruent with previous indirect estimates [35].

There were no kinetic effects of HIV infection without LA (figure 3) on adipose TG dynamics. Remarkably, patients with LA who had a greatly reduced subcutaneous adipose mass exhibited no difference in the fractional replacement rate or apparent half-life of adipose TG (figure 3). Thus, the TG stores present in subcutaneous adipose tissue from all 3 of the fat depots sampled exhibited very long residence time (>6 months), rather than a hyperlipolytic, high-turnover state that might have been expected if LA were caused by direct catabolic actions on mature adipocytes or by the stimulation of catabolic hormones that act on mature adipocytes. These results demonstrate directly that the absolute rate of TG synthesis (and breakdown) was greatly reduced in patients with HIV/LA. The impaired deposition of new TG therefore represents the kinetic explanation for depletion of subcutaneous fat in ART/HIV-associated LA.

In a subset of subjects, repeat adipose-tissue samples were collected 5–8 months after discontinuing the intake of 3H2O. Die-away curves revealed long life spans of stored adipose TG in patients with LA similar to those of healthy controls, consistent with the label incorporation estimates of TG half-life in adipose tissue.

Adipose-tissue DNL. DNL contributed 4% of adipose TG-palmitate after 9 weeks of 3H2O intake in healthy human subjects. After correction for tissue TG replacement, the fraction of newly deposited TG that derived from the DNL pathway was approximately 20%. These are the first direct measurements...
Figure 2. Effects of leptin administration and pair-feeding on adipose lipid metabolism and cell proliferation in ob/ob mice. (A), Triglyceride (TG) synthesis. (B), Absolute palmitate de novo lipogenesis (DNL). (C) Absolute cell proliferation. Four groups are compared, as described in the text (control, ob/ob, ob/ob-restricted, and ob/ob-leptin). Values not sharing a superscript are significantly different (P<.05).

of long-term adipose tissue DNL in humans and are roughly consistent with previous shorter term measurements or indirect estimates [35].

There were no significant effects of HIV infection without LA on the DNL contribution in adipose TG, nor were any significant differences in the fractional DNL contribution seen in patients with HIV/LA. Because absolute TG synthesis was substantially lower in the HIV/LA group than in the other groups (see above), the absolute flux through DNL into stored adipose TG was proportionately lower in patients with HIV/LA. There was not a specific deficiency in this pathway of lipid accrual, however.

Adipose DNA replication (cell proliferation). Cell proliferation in subcutaneous adipose tissue could only be measured in the healthy controls and HIV/no LA groups, because the tissue yields of cells were too low for the measurement by GC/MS of DNA enrichment in the HIV/LA group. In the healthy subjects, the cell turnover rate was slow (half-life, 280–420 days) in the mature adipocyte–enriched cellular fraction. The stromal vascular fraction of cells exhibited a faster turnover rate (A. Strawford, F. Antelo, M. Christiansen, and M.H.K., unpublished data). Patients with HIV but no LA exhibited no significant differences in the fractional turnover rates of cells in either the mature adipocyte–enriched/stromal-vascular–depleted fraction or the stromal-vascular fraction (half-lives also in the range of 9–12 months).

Gene expression in adipose tissue. We also measured the expression of genes related to the adipose-tissue differentiation status (F. Antelo, K.-H. Kim, R. Hoh, et al., unpublished data). RT-PCR was used on adipose tissue samples from the subjects, after the isolation of RNA from samples. Tissue mRNA levels of peroxisome proliferation activator receptor–γ (PPARγ), leptin, and adipocyte-P2 protein (aP2) were measured (as markers of differentiated mature adipocytes) [11]. In addition, the tissue expression of a specific marker of preadipocytes (preadipocyte factor 1, or pref-1) [11] was also measured. The results to date have been striking (K.H. Kim, H.S. Sul, and M.K.H., unpublished observations). Patients with HIV/LA have exhibited a
nearly complete absence of pref-1 expression in adipose tissue, which indicates a reduction in or absence of preadipocytes. In contrast, the tissue expression of PPARγ, leptin, and aP2 tended to be higher when normalized for whole-tissue glyceraldehyde-phosphate dehydrogenase expression (an enzyme expressed approximately equally in all cells in the tissue). Thus, the adipocytes that are present express differentiation markers normally, but there appears to be a deficiency of undifferentiated cells, particularly preadipocytes, in the tissue.

**Tentative conclusions regarding HIV/LA.** On the basis of these preliminary results, we conclude that the lipid present in the subcutaneous adipose tissue of patients with HIV/LA exhibits normal kinetics and a normal lifespan and that the mature adipocytes present in the tissue exhibit the normal expression of differentiation markers. However, the tissue is characterized by a depletion of preadipocytes and by a greatly reduced absolute synthesis rate of TG, despite the presence of a prolipogenic hormonal milieu (i.e., hyperinsulinemia and low serum leptin levels). As was discussed above, leptin suppresses total lipid synthesis, DNL, and cell proliferation in adipose tissue, independent of food intake, and several studies have documented low serum leptin concentrations in patients with HIV/LA [36, 37]. The absence of high rates of lipid synthesis or DNL in patients with HIV/LA therefore implies end-organ insensitivity (i.e., an inability to respond to the low leptin signal).

**TURNOVER OF mtDNA AND MITOCHONDRIAL TOXICITY SYNDROME**

A leading hypothesis of the etiology of many ART-related adverse effects is the mitochondrial toxicity model [4]. This hypothesis postulates that NRTIs inhibit the mitochondrial-specific, evolutionarily primitive mtDNA polymerase γ [4, 38]. mtDNA replication is necessary for the biogenesis of new mitochondria, which can turn over independently of cells [27, 38, 39]. As a consequence of inhibiting DNA polymerase γ, key tissues, such as liver, muscle, adipose, and nerve tissues, may become slowly depleted of functioning mitochondria, which results in tissue dysfunction [4, 38]. It has been difficult to test this hypothesis directly, however, because no method for measuring mtDNA replication directly has been available.

The technique for measuring the synthesis of genomic DNA in cells with ³H₂O (see above) can be applied with equal validity to mtDNA [27] (A. Strawford, F. Antelo, M. Christiansen, and M.H.K., unpublished data). We have done studies to develop the mtDNA synthesis method in rodent tissues and in human blood platelets [27]. These will be described next.

**Measurement of mtDNA turnover in tissues of rodents.** ³H₂O was given to male rats (4% drinking water), and tissue samples were harvested after different periods of labeling. Mitochondria were isolated from skeletal muscle and heart tissue by differential centrifugation [27], and DNA was isolated. The absence of nuclear DNA was confirmed by PCR. The incorporation of deuterium into mtDNA increased over time (figure 4). The half-life was calculated to be 150–300 days for cardiac and skeletal-muscle mtDNA, respectively [27]. Significant differences were apparent for skeletal-muscle versus cardiac mtDNA turnover [27]. Because male rats grew during the labeling period (50% increase in weight over the period of ³H₂O intake), a portion of the mtDNA synthesis could reflect somatic growth, as opposed to a true turnover rate of mitochondria. This issue was addressed in 2 ways. Somatic growth, or cell division, requires the biogenesis of new mitochondria, including new mtDNA, to maintain a constant mitochondrial mass in daughter cells. Cell division and somatic growth also involve nDNA replication. Accordingly, the replication of mtDNA was compared with that of nDNA in the muscle tissues of growing rats (figure 4). The results showed that nDNA synthesis could...
Figure 4. The differentiating somatic growth from mitochondrial turnover in rats given $^2\text{H}_2\text{O}$ in drinking water is shown. nDNA synthesis was compared with that of mtDNA synthesis in muscle from normal male rats. The excess fractional synthesis ($f$) of mtDNA compared with nDNA signifies mtDNA replacement independent of cell proliferation and somatic growth.

Table 1. Fractional synthesis ($f$) replacement rate constants of cardiac and hind-limb skeletal-muscle mtDNA in weight-stable female rats (animals per time point).

<table>
<thead>
<tr>
<th>Week labeled</th>
<th>Cardiac</th>
<th>Hind limb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.6 ± 1.1</td>
<td>2.2 ± 1.4</td>
</tr>
<tr>
<td>2</td>
<td>5.4 ± 1.2</td>
<td>2.6 ± 0.8</td>
</tr>
<tr>
<td>5</td>
<td>12.9 ± 0.8</td>
<td>2.9 ± 1.4</td>
</tr>
<tr>
<td>6</td>
<td>16.4 ± 1.0</td>
<td>7.7 ± 1.9</td>
</tr>
<tr>
<td>7</td>
<td>18.3 ± 5.1</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>20.9 ± 0.8</td>
<td>7.9</td>
</tr>
</tbody>
</table>

NOTE. Data shown are mean ± SD. ND, not done.

Human studies of mtDNA turnover. We have done pilot studies of mtDNA turnover in the blood platelets of humans [27]. Subjects received $^2\text{H}_2\text{O}$ by mouth for 6 weeks (70 mL/day), and blood was drawn at serial time points (10 mL/draw). Platelets lack nuclei and are rich in mitochondria; they therefore represent an ideal and readily accessible tissue for testing the effects of agents or genes suspected to alter mtDNA turnover. Mitochondria were isolated from platelets by centrifugation, and the DNA was isolated. The absence of contamination by nDNA was confirmed by real-time PCR. The time course of incorporation into platelet mtDNA was roughly linear. Of importance, plateau enrichments of mtDNA from platelets were identical to enrichments of nDNA from fully turned over cells (blood monocytes [19, 31]). The results of preliminary studies have suggested a reduced incorporation rate of deuterium into mtDNA platelets in patients with AIDS who received NRTI agents (M. Collins and M.K.H., unpublished observations). Analogous measurements of mtDNA turnover in platelets or other tissues of humans (e.g., lymphocytes and muscle, adipose, and liver tissue) are now possible, with the $^2\text{H}_2\text{O}$ labeling approach described here allowing the direct testing of the effects of NRTI agents on mitochondrial biogenesis.

SUMMARY AND CONCLUSIONS

These results using the $^2\text{H}_2\text{O}$ labeling technique represent a proof of principle that the turnover rate of adipose-tissue components and of mtDNA in muscle or blood cells can be measured in vivo. The techniques are applicable in human subjects as well as in experimental animal models.

It should be emphasized that the direct measurement of the synthesis and breakdown rates of any molecular component of an organism that exhibits very slow turnover is difficult, a priori. This methodological fact of life concerning the kinetics of long-lived molecules has constrained the measurement of bone collagen, muscle protein, and other clinically important biomolecules for many years [40]. Unfortunately, all of the key molecular elements believed to be involved in ART toxicities explain only a portion of mtDNA replication. Weight-stable adult female rats were also studied: their body weight increased by only 10% during the $^2\text{H}_2\text{O}$ labeling period in these animals, but >10% replication of cardiac muscle mtDNA was seen (table 1). After discontinuing $^2\text{H}_2\text{O}$ administration, the die-away curves of mtDNA labeling gave similar slow turnover rates [28].

An NRTI agent, 3'-azido-3'-deoxythymidine (AZT), was added to water (1 mg/mL) and administered ad libitum to female Sprague-Dawley rats for 1–8 weeks. Neither body weight nor food intake was affected by AZT intake. Untreated controls and AZT-treated rats were labeled with 4% $^2\text{H}_2\text{O}$ for 2 weeks. AZT intake produced a significant ($P < .05$) decrease in cardiac and hind-limb muscle mtDNA fractional synthesis compared with control groups (from 13.8 ± 4.2% to 7.0 ± 4.8% and 7.6 ± 1.8% to 4.5 ± 0.4%, respectively) after 4 weeks (figure 5A). The cytochrome c oxidase content in hind-limb muscle was also decreased by 50% compared with controls after 4 weeks of AZT treatment ($P < .07$). The absolute mitochondrial biogenesis rate was significantly reduced by week 2 of AZT treatment ($P < .05$) (figure 5B). We concluded that mtDNA synthesis is measurable by this $^2\text{H}_2\text{O}$ labeling technique and allows the rapid testing or screening of potentially mitochondrial-toxic ART agents in animal models (M. Collins and M.K.H., unpublished data).
Figure 5. Effects of 3′-azido-3′-deoxythymidine (AZT) on mtDNA synthesis in muscle of normal rats. A, Fractional synthesis (f) of mtDNA during 4 weeks of AZT treatment. Significant differences were present between baseline and week 4 treatment values in both skeletal (left) and cardiac (right) muscle (P < .05). B, Absolute rates of mitochondrial biogenesis during 4 weeks of AZT treatment are shown. Significant differences from baseline were present by week 2 of AZT treatment (P < .05).

have exactly this kinetic characteristic, including adipose TG, adipocytes, and mtDNA in cells such as muscle. Indeed, it may be the case that most slowly evolving toxicities occur as a result of an alteration in the dynamics of long-lived molecular or cellular components. These toxicities will, by their nature, escape the acute toxicity studies that are currently used to test and approve drugs. Biosynthetic processes that take months or years to manifest as a clinical pathology are therefore prime targets for ART toxicities. Indeed, toxicities of other classes of agents that need to be taken for years in the treatment of chronic disease may also follow this rule.

It is therefore important and very encouraging that direct kinetic-measurement techniques could be developed to measure changes in the synthesis or breakdown of slow-turnover components in humans and experimental animals and exhibited excellent sensitivity and accuracy. The half-life of adipose TG and cells in humans is 6–12 months; that of muscle mtDNA in rats is ~3 months. Even in the face of such slow turnover, differences between tissues or physiological settings were measurable, because of the unique features of 2H2O labeling (easily administered for extremely long periods of time without toxicities, simply by giving as drinking water) and MS analysis (which is extremely sensitive and reproducible). The fact that parameters can be measured by the same methods in an animal or a human subject represents an important practical advantage of this approach.

Although the results presented here are preliminary, it is clear that large-scale, systematic studies can easily be done using these tools. A number of applications can be envisioned. Adipose dynamics might be worth measuring during early exposure to ART (when adipose-tissue components, including cells, are still abundant and the early inhibition of lipid deposition may be apparent), to identify patients on the path to LA. Trials of either modified ART regimens or anti-LA interventions could then be tested. Drug screening for mitochondrial or adipose-tissue toxicities, in preclinical animal studies or in US Food and Drug
Administration phase I–III trials, is another potential use. The testing of putative therapies for LA or mitochondrial toxicity syndromes (e.g., thiazolidinediones and exercise training) is, of course, a potentially useful feature of clinical biomarkers. Finally, the management of individual patients may some day prove possible if kinetic tests are used as outcome measures. As in all clinical disorders, the presence or absence of reliable biomarkers that reflect underlying pathogenesis can be the key to successful therapeutics [6–8]. This principle is exemplified by cardiovascular disease prevention, where serum lipid concentrations and blood pressure are recognized as treatable outcome measures in their own right, because of their relationship to hard clinical outcomes, to the point that the largest selling class of drugs in the world (the statins) treats a biomarker (cholesterol), not a symptomatic condition. Similar statements can be made regarding diabetes (e.g., hemoglobin A1C or blood glucose concentrations), HIV-1 infection (plasma virus load), and other conditions. On the other hand, cancer therapy and prevention have been recognized as being constrained by the lack of reliable biomarkers of disease status and treatment response [7, 8]. Transforming HIV/ART toxicities, such as LA and mitochondrial dysfunction, from the latter class (no reliable biomarkers to guide management) to the former class (effective biomarkers available to guide management) could have profound implications for these important clinical conditions. The results summarized here suggest that $^{2}$H$_2$O label incorporation into adipose tissue components and into mtDNA may represent useful kinetic biomarkers for HIV/ART toxicities.

Acknowledgments

I thank the many colleagues and students who participated in the work. In particular, Dr. Alison Strawford, Rebecca Hoh, Fernando Antelo, Michelle Collins, Dr. Mark Christiansen, Dr. Richard Neese, Denise Cesar, and Meghan Lane all made important contributions without which this work could not have been done. The nurses at San Francisco General Hospital General Clinical Research Center also contributed to these studies, for which I am grateful. It should be noted that some of the techniques described here have been patented (by the University of California at Berkeley and M.K.H.) and licensed for potential commercial use (to KineMed, Inc., Emeryville, CA).

References

mitochondrial proliferation in vivo using $^2$H$_2$O incorporation into mitochondrial DNA. FASEB J 2000;14:A620.


