EFFECTS OF THIAZOLIDINEDIONE TREATMENT ON BONE DENSITY IN HIV-POSITIVE PATIENTS

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INTRODUCTION

The advent of highly active antiretroviral therapy (HAART), including protease inhibitors (PIs), nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs), has dramatically reduced the morbidity and mortality associated with HIV and AIDS. In particular, drug combinations have been shown to slow or reverse both the increase in HIV load and decline in CD4 cells as well as decrease the incidence of opportunistic infections (1). These medications are all associated with significant but relatively harmless acute side effects, usually relating to gastrointestinal discomfort or diarrhea. One of the more serious potential consequences observed with long term use is lipodystrophy and its associated metabolic disturbances.

Lipodystrophy: Lipoatrophy and Visceral Adiposity

The condition of lipodystrophy is generally characterized by the loss of subcutaneous peripheral fat in the arms, legs, buttocks and face with a simultaneous lipo hypertrophy consisting of visceral or central adiposity, breast enlargement, and deposition of dorsocervical fat pads (“buffalo hump”).

These conditions have been observed as a consequence of NRTI therapy, with 63% of stavudine users and 18.75% of zidovudine users developing symptoms within 14 months. The fat wasting was not found to be a consequence of insulin, high triglycerides, or changes in body composition. Furthermore, the lipodystrophy was significantly improved by discontinuing the medication (2).
Protease inhibitors are similarly linked to lipodystrophy, with 64% of PI users compared to 3% of PI-naive HIV-positive subjects showing fat loss (3). As the lipodystrophy resembles body changes associated with Cushing’s disease, it is important to rule out corticosteroid disturbances as a primary cause. One study showed significant increases in visceral compared to total fat after starting indinavir (4) and a significant relationship between dorsocervical fat deposition and antiretroviral treatment including use of a PI which was not related to hypercortisolism (5). Yanovski reports a similar finding that subjects with PI-associated lipodystrophy had normal diurnal cortisol secretion, normal globulin binding levels and normal glucocorticoid receptor numbers and affinity, though their cortisol metabolism was altered, with increased levels of ACTH and a greater excretion of cortisol as 17-hydroxycorticosteroid. These differences were not seen to be indicative of any known form of hypercortisolism, as they were still significantly lower than that seen in patients with Cushing’s syndrome, and the lipodystrophy was attributed to PI use (6). Again, lipodystrophy was significantly improved by discontinuation of PI, with or without a switch to another antiretroviral class (7).

The lipodystrophy syndrome appears to have heterogeneous expression when related to PI or NRTI use. NRTI-related lipodystrophy is associated more often with recent onset fatigue, nausea, elevated lactate, and elevated liver enzymes and also with lower lipid profiles including cholesterol, triglycerides, glucose, and insulin (7). This finding is of particular interest, as finding biochemical markers of early lipodystrophy may allow clinicians to adjust medications in time to prevent the onset of body composition perturbations.
While some researchers attempt to further differentiate this syndrome into distinct sub-categories (lipoatrophy without lipohypertrophy, mixed forms, etc.) in an attempt to further assist clinicians in treatment protocols [8], it is generally agreed that the lack of an objective pathophysiologic test to determine classification barely allows for the diagnosis of lipodystrophy let alone sub-categories. Currently, a mixture of self-reporting, DEXAs (dual-emision x-ray absorptiometry scans), anthropometrics, and abdominal CT slices are being used to assess body fat composition. This lack of consistency in determining the presence of lipodystrophy is probably the cause of wide prevalence ranges (2%-84%) when metanalyses are performed (9). Other sources report a narrower incidence range of 30-50% with a median onset after one year's use of HAART (1).

*Metabolic Consequences Associated with Lipodystrophy*

Numerous studies have found a significantly greater incidence of insulin resistance in subjects with HAART-related lipodystrophy (3), and other studies have found that it precedes the development of lipodystrophy (10). It is not clear whether this is a direct consequence of the medication, or a secondary effect of the loss of peripheral adipocytes.

Lipid abnormalities have been found in patients receiving HAART without either lipodystrophy or insulin resistance (10), suggesting an even earlier onset in this metabolic syndrome, although it is still unclear if this is a primary effect of the medication. A significant increase in cholesterol and triglycerides has been found with the use of ritonavir, saquinavir and nelfinavir (11). Other studies have found a significant
association between the development of visceral adiposity and hyperlipidemia in indinavir-treated patients (4). Because low DHEA relative to cortisol levels in patients with hyperlipidemia has been found to predict development of lipodystrophy, it has been suggested that steroid supplementation may stabilize or reverse the progression of metabolic disturbances to lipodystrophy (12).

Insulin resistance, hypercholesterolemia, hypertriglyceridemia, and impaired glucose tolerance are associated with increased cardiovascular risk including formation of atherogenic plaques, heart attacks, strokes, and hypertension, as well as type 2 diabetes and its complications (13). Researchers have found PI treatment significantly correlated with a higher rate of diabetes mellitus, impaired glucose tolerance and hyperinsulinemia (14), suggesting that lipid and glucose tolerance testing may be necessary before initiating PI therapy.

**Osteopenia**

Another potential consequence of HAART is bone mineral loss. Although research in this area is limited, one study did show a significantly higher incidence of osteopenia in HIV-positive men taking PIs compared to HIV-positive men not taking PIs. There was no difference between the two groups in the degree of hypogonadism, testosterone levels, CD4 cell count, or viral RNA load (15). It is unclear from this study alone whether the medication is causative, as other factors such as cytokines from activated T-cells are know to interact with osteoclasts.
Theories of Pathophysiology

While it is unlikely that one unifying mechanism will explain the formation of all the symptoms discussed above, the search for possible mechanisms will allow a greater understanding of how to treat these dangerous and unwanted side effects while also empowering pharmaceutical companies to design alternative treatments with a greater likelihood of preventing such problems in the future.

Protease inhibitors have been thought to be relatively safe, as their mechanism of action is to inhibit the cleavage of the HIV gag and gag-pol polyproteins at the Phe-Pro and Tyr-Pro sequences, thus disallowing activation of the growing HIV virion (1). Since mammalian cell proteases rarely recognize this sequence, it was hoped that this inhibition would have relatively little cross-reactivity with human enzymes. In light of the evidence implicating PI treatment in lipodystrophy, an analysis of human protein sequences with homology to the HIV protease-active site was carried out. Cis-9-retinoic acid is required in the formation of the active heterodimer RXR*PPARγ, a transcription regulator of adipocyte differentiation, low levels may cause lipoatrophy in affected tissues. The cis-9-retinoic acid binding protein (CRABP-1) shares 6 of 12 amino acid residues with the inhibited HIV protein. Because CRABP-1 binds and presents retinoic acid for conversion to cis-9-retinoic acid, researchers believe PIs may competitively inhibit CRABP-1 and thus produce lipoatrophy (16). This theory has been questioned by some, as addition of an RXR (retinoid X receptor) agonist does not alleviate the PI-induced lack of adipocyte differentiation (17). However, this finding does not completely rule out low
RXR as a possible mechanism, as additional PI-induced factors may be inhibiting the formation of this complex.

Another protein homologue, sharing 7 of 12 amino acid residues with HIV protease, is the low-density lipoprotein receptor-related protein (LRP). This protein binds VLDL and chylomicron remnants and also interacts with lipoprotein lipase (16). Inhibition of this protein could therefore be responsible for abnormal lipid and cholesterol metabolism, with a decrease in hepatic clearance and loss of inhibition of endogenous generation. Increased blood lipids could then lead to central adiposity and insulin resistance. To date, no study has been done to test this hypothesis, but it is an idea worth investigating.

As stated above, insulin resistance is seen before body composition changes following PI treatment. It has been observed that PIs selectively inhibit GLUT-4, an insulin-responsive glucose transporter (17). GLUT-4 transporters are responsible for muscle and adipocyte glucose entry. Insulin up-regulates the number of membrane-bound GLUT-4 transporters, thus promoting the entry of glucose into fat and muscle cells (18). Inhibition of these receptors would therefore decrease insulin sensitivity. It has also been shown that knockout mice devoid of GLUT-4 transporters have a scarce supply of fat tissue, which leads researchers to speculate that these receptors are somehow related to adipocyte differentiation (17).

Finally, mitochondrial toxicity has been proposed as a central cause of HAART-related lipodystrophy. Researchers noted a striking similarity between this lipodystrophy and
multiple symmetric lipomatosis type 1 (MSL), a genetic disorder characterized by fatty deposits in the cervical and abdominal regions with associated dyslipidemia and impaired glucose tolerance, as well as other symptoms. MSL has been traced to a mutation in the gene coding for part of the mitochondrial respiratory chain, leading researchers to conclude that mtDNA damage could lead to a similar syndrome (19). The finding of lactic acidosis is also consistent with mitochondrial dysfunction.

A recent review summarizes studies showing that AZT-treated patients had reductions in muscle mtDNA and AZT-treated cells showed mitochondrial damage on electron microscopy. Mouse studies showed oxygen damage of mtDNA leading to mitochondrial dysfunction after four weeks of AZT. D4T-treated cells showed a similar reduction in mtDNA viability, and ddC and ddI showed similar mitochondrial toxicity in vitro. It is important to note that the presence of the virus alone may also damage mitochondria, as HIV RNA has been found intercalated into the mitochondrial genome and some HIV proteins are capable of inducing apoptosis and mitochondrial damage (19).

The finding that AZT and other NRTIs can impair mitochondrial function has some intuitive appeal. Not only has AZT been found to inhibit the activity of γ-polymerase (19), an enzyme involved in maintaining mtDNA replication, but the very action of NRTIs is to integrate into a host genome. Mitochondrial DNA is extremely efficient with almost every base pair encoding at least one gene, and in some cases two genes (18). At almost any point, NRTI binding to the mtDNA could cause chain termination, potentially disrupting the coding of enzymes necessary to correct for this change as well as other factors necessary for the normal function of the respiratory chain and therefore mitochondrial function.
Researchers also implicate TNF-α in the indirect damage HAART may cause to mitochondria and adipocytes. In general, PIs and NRTIs both control viral load and activate T-cells, promoting the release of various cytokines including TNF-α. Mitochondria themselves are intracellular targets of TNF-α, and adipocyte metabolism is altered in the presence of this chemical to generate heat (20). This decreases fatty acid uptake by adipocytes, and some studies have shown increased adipocyte regression to preadipocytes as well as enhanced apoptosis of mature cells (19). While these changes can obviously lead to hyperlipidemia, the finding that omental adipocytes are more susceptible to apoptotic stimuli than subcutaneous adipocytes (21) fails to explain how TNF-α could be implicated in the formation of peripheral lipoatrophy with concurrent central adiposity. However, it does indicate that that the two pools of fat cells respond differently to the same stimuli, suggesting a possible mechanism for HAART-related lipodystrophy.

Although a unifying mechanism appears unlikely, further investigation into the synergistic direct effects of HAART with secondary immune system mediators and the body’s attempt at homeostasis may supply researchers with the information necessary to adequately treat the HIV virus without these deleterious side effects.

**Treating HAART-Related Lipodystrophy**

The irony of attempting to treat HAART-related lipodystrophy is that researchers are looking for ways to increase the amount of subcutaneous fat, which seems incongruous
since one of the main risks associated with lipodystrophy is hyperlipidemia. However, the goal is actually to increase the subcutaneous to visceral fat ratio, as subcutaneous adipocytes are relatively metabolically inactive, serving as a storage site for triglycerides and therefore absorbing both esterified and FFA from the blood. This decreases blood lipids and has a positive effect on insulin resistance as well (18).

Another important benefit of redistributing the fat is to address what is often the main concern of patients: body image. While physicians focus primarily on cardiovascular risk factors, patients presenting for services are often more concerned with how they look and feel. Maldistribution of fat is not only an assault on their general appearance, it is also physically distressing. Loss of gluteal fat makes sitting for even short periods of time incredibly uncomfortable, and enlarged breasts can be sore and painful. Patients can feel uncomfortably full with swollen and tender abdomens, leading them to believe that they need to lose weight, especially fat. It can be a challenge to explain the need to find ways to increase fat, and that a sign of successful treatment might be overall weight gain.

As the metabolic consequences of HAART-related lipodystrophy resemble those faced in type II diabetes, researchers have tried using antidiabetic medications to treat this condition. Biguanides have been shown to decrease postprandial and fasting glucose levels in diabetic populations. While the mechanism is currently unknown, it is hypothesized that the medication may work by any combination of inhibiting hepatic gluconeogenesis, stimulating peripheral glycolysis, reducing GI absorption, and possibly reducing plasma glucagon levels (1).
Metformin, the biguanide most commonly used in the United States, was tested for three months in HIV-positive patients with lipodystrophy in a randomized double-blind study. The metformin-treated subjects demonstrated significant reductions in insulin area under the curve at 120 minutes after oral glucose tolerance testing compared to placebo, indicating that the medication reduced insulin resistance. Significant reductions in weight and blood pressure were also obtained compared to placebo. Perhaps most importantly, metformin therapy was associated with a decrease in visceral abdominal fat, with a proportional reduction in subcutaneous abdominal fat. The S/V ratio remained stable (22).

Although further studies are needed to confirm these results, it appears that metformin is successful in ameliorating some of the metabolic abnormalities associated with HAART, which may serve to decrease related cardiovascular risks. However, the medication does not appear to help redistribute fat back to the periphery, and hence it may not be able to address some of the psychological and physical comfort issues associated with this condition. Furthermore, metformin had no effect on blood lipid levels, leaving hyperlipidemia and its possible CV risk factors intact. One might also assume that decreasing fat overall leaves even fewer possible depots for blood lipids, and thus could conceivably increase the level of hyperlipidemia. This possibility remains open to investigation.
Another class of antidiabetic medication being considered in the treatment of HAART-related lipodystrophy is the thiazolidinediones. Thiazolidinediones (TZDs) have been found to increase tissue sensitivity to insulin, increase glucose uptake in muscle and adipose tissue, inhibit hepatic gluconeogenesis, and have positive effects on lipid clearance and redistribution of body fat (1). While the mechanism is not entirely understood, several studies have confirmed that this class of drug is an agonist at the peroxisome proliferator-activated receptor-\( \gamma \) nuclear receptor (PPAR-\( \gamma \)). PPAR-\( \gamma \) is expressed primarily in adipocytes, and forms the aforementioned heterodimer with retinoid X receptors (RXR). This heterodimer binds to promoter domains of target genes, and acts as an inhibitor of translation when no ligand is bound by deacetylating the local histones. When a PPAR-\( \gamma \) ligand binds to the complex, it promotes the exchange of the deacetylating enzyme for a histone acetylator, which facilitates active gene transcription (23). Activation of PPAR-\( \gamma \) has been shown to promote differentiation of preadipocytes into adipocytes.

Researchers used chimeric mouse models to inject a 50/50 mixture of wild type and knockout cells with no gene for PPAR-\( \gamma \) into blastocysts, allowing developing organs to select for the most functional cell. This technique answers the question of which organs/tissues require a given gene for functional development. In this murine model, the heart, spleen and small intestine incorporated the mixture in a 1:1 ratio, showing no preference and therefore no requirement of PPAR-\( \gamma \) for formation. In fat tissue, the ratio was 5:1 favoring the wild type – showing a requirement for formation. Additionally,
after controlling for the stromal-vascular cells in this sample, the adipocytes derived almost exclusively from wild-type cells.

This incorporation was found in subcutaneous fat as well as epididymal fat, indicating that PPAR-\(\gamma\) activation is required for adipogenesis, regardless of depot. The only other tissue type showing a favoring for wild-type cells was sebaceous glands, although the significance of this finding is still elusive. The experimenters confirmed these findings with in vitro studies, showing that embryonic stem cells lacking both copies of PPAR-\(\gamma\) do not develop fat. Heterozygotes (+/-) showed an intermediate amount of fat compared to wild type, indicating a dose-response relationship. Lastly, the wild-type cells in vitro had normal hormonal sensitivity while the knockout and heterozygotes did not, indicating that PPAR-\(\gamma\) activation is required for most steps of adipocyte differentiation (24).

TZDs have been shown to decrease the levels of leptin and TNF-\(\alpha\) in obese rats, which may help to decrease future damage to existing adipocytes. In the same study, TZDs caused an increase in the number of small adipocytes with a decrease in the number of large adipocytes when evaluated histologically. This finding was true of both obese and lean rats. The mechanism appears to be promotion of apoptosis of the large adipocytes, as more apoptotic nuclei were found in the TZD-treated fat. It is plausible to conclude from these findings that either the large adipocytes are more likely to express TNF-\(\alpha\) and leptin, or that TZDs directly inhibit the expression of these chemicals in large adipocytes.
This shift from large to small adipocytes promotes better cardiovascular health because small adipocytes can oxidize more glucose than larger cells, they take up more glucose than larger cells at the same levels of insulin, and they are more sensitive to the antilipolytic action of insulin. This should lower serum levels of esterified and FFA, which may also alleviate insulin resistance. Interestingly, these researchers also found that TNF-α was elevated only in visceral, not subcutaneous, fat, again indicating a depot-specific behavior which may help lead to an understanding of HAART-related lipodystrophy (25).

TZDs have been studied in human subjects for the treatment of lipodystrophy. As the assumed mechanism of action requires preadipocyte targets, truly lipoatrophic subjects were not included, and thus the findings cannot be extended to that population. Troglitazone, a TZD not commonly used due to rare but serious liver complications, was shown to significantly decrease hemoglobin A1c levels and triglyceride levels and significantly increase body weight. MRI analysis confirmed a selective increase in subcutaneous fat and a decrease in visceral fat with a redistribution of body fat toward normal. Interestingly, the respiratory quotient was decreased, suggesting a shift from carbohydrate to fat oxidation – the opposite of the effect of insulin. This rules out increased insulin sensitivity as a possible mechanism of action and it would also explain the decrease in FFA levels (increased utilization via oxidation), which indirectly increases insulin sensitivity. The secondary effects of insulin sensitization would then decrease blood glucose levels and the levels of glycosylated hemoglobin as well (26).
These effects were confirmed in a study of type 2 diabetes patients in which troglitazone treatment significantly decreased hemoglobin A1c levels, increased BMI, and significantly redistributed fat from visceral to subcutaneous tissue. The most interesting finding was that troglitazone stimulated the differentiation of preadipocytes into adipocytes selectively in subcutaneous tissue, even though the same level of PPAR-\(\gamma\) expression was found in visceral fat tissue (27). While it is unclear why this would occur, it explains how TZDs can promote the shift from visceral to subcutaneous fat deposition.

As TZDs address both the lipodystrophy as well as its metabolic complications, they are a promising treatment for HAART-related lipodystrophy. However, the necessity of some residual preadipocytes will need to be investigated, and the possibility of prophylactic use might be worth consideration if true prevalence rates are as high as some studies suggest. Additionally, the potential protective effect on bone density may serve to combat osteopenia, making prophylactic use a more desirable choice.

A metanalysis of current treatments available for HAART-related lipodystrophy also includes plastic surgery, fat transplantation, medication holidays, and statins as ways to treat isolated symptoms of this disorder. While none of these alone can address all the problems inherent in lipodystrophy, they may independently serve to treat the problems of greatest concern for the patient and may be worthwhile alternatives to TZDs (28).
TZDs and Bone Density

A growing body of research has shown that PPAR-γ ligands act as modulators of inflammation. Additionally, they decrease resorption of bone both indirectly through suppression of TNF-α and directly through inhibition of osteoclast differentiation (29-32). When activated macrophages bind ligand to their PPAR-γ receptors, a resting phenotype is assumed which inhibits the release of proinflammatory mediators and down-regulates iNOS (29). Interleukin 4, an antiinflammation cytokine, induces PPAR-γ expression in macrophages. Lipoxygenase metabolites 12-and 15-hydroxyeicosatetraenoic acid (HETE) are produced by interleukin 4 treated macrophages and are potent naturally occurring PPAR-γ ligands. This suggests a positive-feedback, anti-inflammatory role for this receptor. Additional studies have found a decrease in iNOS, gelatinase B, scavenger receptor A, interleukin-6, interleukin-1β, and TNFα in macrophages and monocytes following administration of a PPAR-γ agonist (30). The TZD inhibition of macrophages appears to result from apoptosis induction that follows interference of the NF-κB mediated antiapoptotic pathway (29), most likely by antagonizing its binding site (30, 32).

A recent study found that troglitazone was a potent suppressor of chronic inflammation and pannus formation in adjuvant-induced arthritis. In that study, TZD activation caused a similar apoptotic result in synovial and endothelial cells, leading the authors to conclude that a combination of inhibition of macrophages, synovial cells and endothelial cells was responsible for the improvement (32). A similar study found that treatment of chondrocytes with PPAR-γ ligands decreased interleukin-1β induction of nitric oxide and
matrix metalloproteinase 13 (MMP-13) in a dose-dependent manner. TNF-\(\alpha\) and interleukin-17 induction of nitric oxide and MMP-13 were also inhibited, again through interference of NF-\(\kappa\)B (31). As these cytokines and enzymes are believed to be responsible for the proteolytic destruction of articular tissue, it is presumable that this inhibition is also responsible for the suppression of adjuvant-induced arthritis.

Bone homeostasis is also affected by PPAR-\(\gamma\) ligands. Researchers have found that TZD treatment suppresses bone destruction in adjuvant arthritic models (33). There are several theories about the mechanism behind this finding. TNF-\(\alpha\) and interleukin-1 are mitogens for osteoclasts, the cells responsible for the resorption of bone (32). With suppression of TNF-\(\alpha\), osteoclastic differentiation and therefore the number of active osteoclasts should decrease. Furthermore, osteoclasts express PPAR-\(\gamma\) receptors and ligand binding should inhibit differentiation through inhibition of NF-\(\kappa\)B (29). Mba\'aviele et al (30) conducted a series of experiments to determine the validity of both theories. Osteoclasts share a common progenitor cell as monocytes and are therefore stimulated to differentiate by granulocyte/macrophage-colony stimulating factor (GM-CSF), as well as interleukins-1 and -6, and TNF-\(\alpha\) mainly through transcriptional activators including NF-\(\kappa\)B. It is an intuitive finding that mice deficient in one of the genes encoding for NF-\(\kappa\)B exhibit osteopetrosis (thick bones resistant to normal pathways of osteoclast resorption) (34).

Osteoclasts evolve from CD34+ hematopoietic stem cells (CD34+HSC), in part due to cytokines released by human mesenchymal stem cells (hMSCs). Interestingly, hMSCs
are multipotent and can give rise to adipocytes, chondrocytes, or osteoblasts — the cells that form bone. It is primarily through the actions of hMSCs that bone density is determined (whether the homeostatic concentrations are greater in osteoclasts, favoring bone resorption, or osteoblasts, favoring bone formation). Mbalaviele (30) found that CD34+HSCs but not hMSCs expressed PPAR-γ, and that activation of these receptors inhibited the hMSC activation of CD34+HSCs. In other words, PPAR-γ ligands were able to bind to pre-osteoclasts and overcome the message from hMSCs to differentiate, resulting in fewer active osteoclasts. The terminal cells of hMSCs including adipocytes and chondrocytes only expressed PPAR-γ after differentiation. The data suggest that inhibition of osteoclast formation occurs through the osteoclast progenitor cells.

The authors then assayed the effect of PPAR-γ ligands on fully differentiated osteoclasts by looking at bone resorption in vitro. Ligand binding inhibited bone resorption by osteoclasts stimulated by either hMSC-released cytokines or M-CSF, leading them to conclude that PPAR-γ ligands inhibit both the formation and the function of osteoclasts. Finally, it was determined that the hMSC cytokine, as well as TNF-α and interleukin-6, stimulated differentiation and function of osteoclasts through NF-κB. PPAR-γ ligands were found to be potent inhibitors of NF-κB binding, which suggests a mechanism of action in this setting.

**Hypothesis**

The purpose of the present study was to determine whether thiazolidinedione would have beneficial effects on bone density in a population with HAART-related lipodystrophy.
The current literature suggests that a PPAR-γ activator such as a TZD should decrease the number and activity of osteoclasts, resulting in less bone density loss during a given period of time in HIV-infected patients at risk for bone loss who are also receiving TZD therapy than in a similar group of individuals not receiving TZDs.

METHOD

The basic method was to do a case-control assessment of bone density changes in HIV-positive individuals with HAART-related lipodystrophy. Cases were patients receiving TZDs and controls were patients treated in the past who had not received TZDs and who were identified from computer records.

Subjects:

The cases were volunteers drawn from patients with lipodystrophy/lipoatrophy who were participating in a study of the effects of thiazolidinediones on adipocyte kinetics and differentiation and body fat stores. The parent research was being conducted by Dr. Marc Hellerstein at San Francisco General Hospital as part of a study approved by the UCSF Committee on Human Research (approval number H3049-18738-02). All subjects enrolled in the study who had an initial dual-energy x-ray absorbiometry (DEXA) scan and a follow-up scan after six months of TZD treatment were included.

Of the 15 subjects initially enrolled in Dr. Hellerstein’s study on adipocyte kinetics, eight completed the protocol. Of the seven who did not complete the protocol, four dropped out because of health concerns unrelated to the study, one was asked to withdraw by his
primary care provider after safety screening showed elevated liver enzymes, one dropped out because of scheduling difficulties related to starting law school, and one had distorted DEXA scans because of a metal hip replacement. Subjects would have been excluded if they had been taking medications known to interfere with bone density such as bisphosphonates or glucocorticoids; however, none of the eight subjects who completed the protocol were taking such medications.

The eight subjects who served as cases included two women and six men. Two were Hispanic and six were white. Their ages ranged from 38 to 57 with the mean age being 49.5 years. They had been diagnosed with HIV for a mean of 11.8 years (range 4-15 years) and had been taking NRTIs or PIs for a mean of 4.9 years (range two months-8 years).

Control data was obtained from stored data on the DEXA computer. Controls were matched with cases for age (within two years), ethnicity (same), gender (same), body composition (weight within 5 Kg, % body fat within 2%), and years on anti-retroviral (ARV) medications (within two years). ARVs were not matched for subtype such as NRTIs, PIs or NNRTIs as most controls were scanned previous to the development of PIs.

Materials

All subjects were scanned on the same Lunar DEXA machine located at San Francisco General Hospital. For all subjects who served as cases, the same technician aligned the
scanning quadrants based on standard placements, i.e. the iliac spine for the pelvic region. Reports generated by the machine express bone density as a Z-score compared to age- and ethnicity-matched populations. Bone density scores were collected for the pelvis, spine, and total body and recorded as final minus initial score for each case and each control with six months’ time elapsing between the final and initial scan.

Procedure

In keeping with the protocol established for Dr. Hellerstein’s study of the effects of TZDs on adipocyte kinetics and differentiation and on body fat stores in patients with lipodystrophy/lipoatrophy, subjects underwent DEXA scanning during the initial eight weeks of the study as part of the baseline studies to determine initial body compositions. The scan was scheduled at a time convenient to the subject during this time frame. This scan provided the initial bone densities used in this analysis. At the end of the initial eight weeks of baseline studies, subjects were given either open-label rosiglitazone or pioglitazone for a total of 24 weeks. Assignment to the two medications was randomized.

If a patient was taking rosiglitazone, the dose was started at 2 mg every morning and increased to 4 mg after four weeks. The dose was then increased to 8 mg after eight weeks. If a patient was taking pioglitazone, the dose was started at 15 mg every morning and increased to 30 mg after four weeks. The dose was then increased to 45 mg after eight weeks. If there was no indication of negative side effects such as elevation of liver enzymes, subjects were kept at their respective doses of 8 mg rosiglitazone and 45 mg pioglitazone until the completion of the study (i.e. they had 24 total weeks of
medication). At the end of the six-month treatment phase a second DEXA scan was performed which provided the final bone density reading used in this analysis.

This research on bone densities was found to satisfy the UC Berkeley Committee for Protection of Human Subjects requirements under Exemption #6, page 5 of CPHS Guidelines of January 1998 (Exemption #4 of the Federal Regulations) and to be exempt from full Committee review. The project number was 2003-1-20.

RESULTS

The following table summarizes the findings of this study, using DEXA measurements for the pelvis, spine and total body, comparing the group means of the subjects taking TZDs (cases) and their matched controls (controls). The summary of the inferential statistics comparing these means is included.

<table>
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<tr>
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<th>PELVIS</th>
<th>SPINE</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORMAL RANGE g/cm²</td>
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<td>1.10-1.30</td>
<td>1.10-1.30</td>
</tr>
<tr>
<td>CASES (MEAN BONE DENSITY CHANGE) g/cm²</td>
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<td>0.021142</td>
<td>-0.00957</td>
</tr>
<tr>
<td>CONTROLS (MEAN DENSITY CHANGE) g/cm²</td>
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<td>POOLED VARIANCE</td>
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<td>T-STATISTIC</td>
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<td>BORDERLINE SIGNIFICANCE</td>
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</tbody>
</table>
As indicated above, the mean change in bone density for the individuals receiving TZDs increased more than the mean change in the control group for the spine and pelvic measurements. The change in spine density has a p-value of 0.07, indicating a borderline significant result. There was no significant difference between the two groups for the measurements taken at the pelvis or total body.

DISCUSSION

DEXAs are typically evaluated by using the bone density of the lumbar vertebrae, both because this skeletal area is highly prone to compression fractures in osteoporosis (indicating an area of metabolic activity) and because the instrumentation has been developed to best assess bone mass in this area of the body.

The results of this study indicate that TZD treatment for ARV-induced lipodystrophy might have a positive impact on bone density measured in the spinal area. The finding is not significant at $\alpha=.05$, but would be significant at the less stringent value of $\alpha=.10$.

The values at the pelvis show a trend toward increased bone density in the TZD-treated group compared to bone loss in the control group, although the findings are not statistically significant. The unexpected result of increased total body bone density in the controls and decreased bone density in the TZD-treated group is also not statistically significant and may be explained by a single anomalous control who showed a marked increase in total bone density (see control #2 in the graph below). When case and control #2 are removed, the group means become nearly identical, and non-significant. These
findings are mostly supportive of the hypothesis that TZDs may decrease the rate of bone loss in HIV-positive patients with lipodystrophy/lipoatrophy. They are also consistent with research showing that TZDs inhibit osteoclastic activity (29-34).
The total bone density change for cases is represented by the left bar, while the total bone density changes for a matched controls is represented by the bar immediately to the right. The summation of the group means are represented as subject #9 above.

**Study Limitations**

The greatest limitation of this study was the sample size. The most significant finding, increased bone density in the spine, fell short of the standard power value of 0.80, being only 0.47. Assuming the measured means were true representations, it would have taken an additional 78 cases with their 2:1 matched controls to have power = 0.80. Another limitation was the amount of time between readings. DEXAs are sensitive enough to measure a change of 0.01 g/cm2, and both populations had changes greater than this over six months. However, a better study design would have allowed for serial measurements over 12 to 18 months. The natural degeneration of bone in this population is not a rapid event. It stands to reason that assaying possible changes over a longer period of time would allow more sensitive measurement of that change.

Finally, a matched case-control study is always limited by the variables the experimenter chooses to match. In this case, every effort was made to match subjects on variables that are highly correlated with the dependent variable, bone density. The most obvious difference between the groups was ARV history. As much of the control data was collected prior to the advent of PIs, this group had far less PI exposure. Additionally, the controls were not selected to participate in their original study of the life history of HIV
based on lipodystrophy. Attempts were made to match body fat distribution according to DEXA measurement, but a better design would have included defining body fat distribution by athropometrics and electrical resistance (BIA). Finally, factors unaccounted for, rather than the TZD treatment, may be responsible for the changes in bone density that were seen. A better design would have been a randomized, double-blind, placebo control study with several treatment groups on varying levels of medication. However, since this study was added to an existing project focusing on measurement of a different variable, its design was to some extent preordained.

**Directions for the Future**

While this study did not find a definitive, statistically significant effect of TZDs on bone density, the trend toward protection of bone loss in this vulnerable population warrants further investigation. For TZDs to affect lipodystrophy, preadipocytes bearing PPARγ must be present. Initial, as yet unpublished, results of the original parent study suggest that the lipodystrophic population is deficient in preadipocytes and that TZDs are not increasing peripheral fat levels. While this is fascinating in that it suggests a pathogenesis for the formation of ARV-induced lipodystrophy, it also speaks to the inability of TZDs to treat lipodystrophy once it has become established. Considering that 70% of patients on HAART eventually develop lipodystrophy, it is worth considering investigating the use of TZDs in the primary prevention of this disorder, perhaps before pre-adipocytes are depleted. If TZDs are found to have an additional, beneficial effect on bone density the decision to use the medication becomes even more compelling. The results of this study
suggest that TZDs hold much promise for the treatment of HIV-positive patients with lipodystrophy, at risk for bone loss.
REFERENCES


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