Mitochondrial function, inflammation, fat and bone in HIV lipoatrophy: randomized study of uridine supplementation or switch to tenofovir

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Abstract

Background—Lipoatrophy modestly improves when the thymidine analogue nucleoside reverse transcriptase inhibitor (tNRTI) is removed. *In vitro*, uridine (NucleomaxX ®; Pharma Nord, Vojens, Denmark) reversed tNRTI mitochondrial toxicity.

Methods—All patients had lipoatrophy on a tNRTI-containing regimen with HIV RNA<400 copies/ml. A randomized 48-week study switched patients from tNRTI to tenofovir (TDF) or added uridine (continuing tNRTI). End points were changes in limb fat (DEXA), subcutaneous abdominal fat mitochondrial DNA (mtDNA) and mitochondrial RNA (mtRNA), inflammation markers (soluble tumour necrosis factor receptors, high-sensitivity C reactive protein [hsCRP], interleukin-6 [IL-6], soluble vascular cell adhesion molecule 1), bone mineral density (BMD) of the hip and spine, HIV-1 RNA, CD4+ T-cells and fasting metabolic parameters.

Results—Fifty patients were enrolled (*n*=24 TDF switch; *n*=26 uridine); median age 48 years; 54% white; 86% male; limb fat 4,494 g. Baseline characteristics were similar between groups. In the NucleomaxX® arm, mtRNA increased (all *P*<0.001), hsCRP and IL-6 increased (both *P*=0.02), whereas fat mtDNA decreased without changes in limb fat. In the TDF-switch arm, fat mtDNA and inflammation markers did not change; however, significant increases in mtRNAs (*P*<0.001), limb fat (409 g; IQR −59–1,155) and CD4+ T-cell count (*P*=0.03), and decreases in total and hip BMD (median −3.3%; IQR −5.1−0; *P*=0.005) were observed. Between-group changes were significant for fat mtDNA, hsCRP, IL-6, limb fat and hip BMD. No correlation was found between changes in limb fat and those of fat mtRNA, inflammation markers or protease inhibitor duration.

Conclusions—In HIV lipoatrophy, NucleomaxX® improved mtRNA, but worsened inflammation markers and fat mtDNA without changes in limb fat. Switching from a tNRTI to TDF for 48 weeks increased limb fat and fat mtRNA. Large decreases in total and hip BMD were seen after TDF switch.

Introduction

Lipoatrophy of the face, arms, buttocks and/or legs is a distressing and stigmatizing long-term effect of antiretroviral therapy (ART) that is currently used to treat HIV patients globally. The pathogenesis of lipoatrophy is not fully understood, but several lines of evidence point towards an important role of nucleoside reverse transcriptase inhibitors (NRTIs) in general and the mitochondrial toxicity of thymidine NRTIs (tNRTIs) in particular [1–3]

Some NRTIs, in particular tNRTIs (zidovudine and stavudine), impair DNA polymerase-γ or thymidine-kinase 2 and therefore interfere with the replication of mitochondrial DNA (mtDNA), leading to depletion in mtDNA, increased adipocyte apoptosis and ultrastructural abnormalities of adipocyte mitochondria [1–3]. Lipoatrophy mildly improves after discontinuation of tNRTI therapy, but this improvement is only partial [4–6]. *In vitro* and animal studies as well as one small clinical trial have shown that uridine supplementation in the form of NucleomaxX® (Pharma Nord, Vojens, Denmark) might prevent and even reverse mitochondrial toxicity of tNRTIs [7–9], and thus is a promising strategy for lipoatrophy. Indeed, in cultured adipocytes and hepatocytes, uridine supplementation prevented and treated stavudine-related mtDNA depletion, mitochondrial dysfunction and consequent adipocyte apoptosis [7,8]. However, we have recently found that in the multicentred clinical trial ACTG 5229, NucleomaxX® was not well tolerated and did not improve limb fat in patients with lipoatrophy [10]. This clinical study did not assess changes in mitochondrial indices in an effort to understand the clinical findings. Also, to date, no human study has assessed the mitochondrial and adipocyte effect of adding NucleomaxX® versus switching from tNRTI to tenofovir (TDF) in HIV-infected patients with established lipoatrophy. In our study, as a source of uridine, we used NucleomaxX®, a dietary supplement that has been shown to increase the serum concentration of uridine in humans from an average of about 5 µM to more than 150 µM owing to its high content (17%) of uridine and triacetyluridine [11].

Methods

Study design and population

This was a phase II, randomized, comparative study of uridine supplementation in the form of NucleomaxX® versus tNRTI switch to TDF for the treatment of HIV-associated lipoatrophy. All patients were enrolled at the John T Carey Special Immunology Unit, Cleveland, OH, USA, and provided a written informed consent approved by the local Institutional Review Board. Also, an investigational new drug application for the use of NucleomaxX® in HIV lipoatrophy was obtained. Eligibility criteria included HIV-infected men and women of ≥18 years of age with established lipoatrophy. For this study, lipoatrophy was defined as patient self-reported and investigator-confirmed fat wasting in at least two of the following areas: face, arms, legs or buttocks. Patients were required to have a plasma HIV-1 RNA level ≤400 copies/ml and be receiving a stable thymidine analogue-containing antiretroviral regimen for ≥12 consecutive weeks prior to study entry, with no plan at study entry to significantly alter ART for the duration of the study. Exclusion criteria included pregnancy, breastfeeding, liver failure, severe lactose intolerance, active endocrine disorders, diabetes requiring hypoglycaemic agents as well as the use of didanosine, hormonal or immunomodulating therapies. Exclusionary laboratory results were creatinine clearance <50 ml/min, aspartate aminotransferase and/or alanine aminotransferase >5× upper limit of normal, lipase >2.5× upper limit of normal, haemoglobin <9.0 g/dl, platelet count <50,000/mm^3 or absolute neutrophil count <750/mm^3.

Intervention

The patients were randomized by the investigational pharmacist 1:1 in an open-labelled fashion to either continue all ART unchanged, including tNRTI, and add a NucleomaxX® 36 g sachet three times daily every other day or switch tNRTI to TDF and continue all other ART unchanged. NucleomaxX® was bought in sachets of 36 g each from Pharma Trade Healthcare AB (Spånga, Sweden) and was provided to study participants. Study participants were instructed to drink the entire contents of one sachet after thoroughly mixing it with milk, juice or water. Because the dosing was on an every other day schedule, instructions were given that if all three sachets were not taken in one day (first 24-h period), the unused sachet(s) could be taken the next day (second 24-h period), so that patients would receive a total of three sachets (108 g total) in a 48-h period. There were no food restrictions. Antiretroviral drugs were not provided by the study. The patients were told to maintain their current ART, and not modify their exercise or dietary habits during the course of the study period.

Outcome and follow-up

Study evaluations included physical examination, fasting blood assessments, whole-body DEXA scanning, and lumbar spine and hip DEXA at study entry and week 48. Additional safety visits were carried out at weeks 4, 8, 12, 18 and 36 for the purpose of clinical and laboratory monitoring for toxicities, intolerance and adherence. The laboratory assessments were done in a fasting state of at least 8 h and included peripheral blood mononuclear cell (PBMC) mtDNA levels, fasting lipids and insulin, fasting blood lactate measured per AIDS Clinical Trials Group standards, HIV-1 RNA, CD4+ T-cell counts, and plasma inflammation markers (soluble tumour necrosis factor receptors I and II [sTNFRI and sTNFRII], high-sensitivity C-reactive protein [hsCRP], interleukin-6 [IL-6], and vascular cell adhesion molecule 1 [VCAM-1]). In addition, patients underwent an excisional subcutaneous fat biopsy from the lower abdomen. These biopsies were done under local anaesthesia by an experienced plastic surgeon. Laboratory personnel were blinded to patients' characteristics. Permanent cessation of the drug was mandatory for grade 4 adverse events, development of diabetes or pregnancy.

Mitochondrial DNA and RNA measurements

Analysis of fat or PBMC mtDNA copies/cell was conducted, in a blinded fashion, by absolute quantitative real-time PCR as previously described [12]. Samples were run in duplicate and results were then analysed with Roche Version 4.0 LightCycler software (Roche, Indianapolis, IN, USA). Absolute mtDNA copies/cell for each sample was calculated by dividing mean mtDNA values by mean genomic DNA values, and then multiplying by two. Total RNA was isolated from subcutaneous adipose tissue biopsies stored at −70°C in RNA Later (Sigma-Aldrich, St Louis, MO, USA) and quantification of ND1, ND6 and CytB copies was calculated by the ratio of each mitochondrial transcript mean concentration to the mean ribosomal L13 concentration (as nuclear control) for each sample as previously described [12].

Inflammation markers

The following plasma biomarkers were measured: inflammatory cytokines (sTNFRI and sTNFII, IL-6, and hs-CRP) and the endothelial activation marker soluble VCAM-1. The markers were measured in duplicate and averaged using commercially available enzyme-labelled immunosorbent sandwich assays (Searchlight; Thermo Fisher Scientific, Wobern, MA, USA). The median intraassay coefficients of variation for sTNFRI, sTNFRII, IL-6, hsCRP and soluble VCAM-1 were 8.8%, 8.6%, 11.7%, 6.9% and 8.7%, respectively. The median interassay coefficients of variation for each assay were 10.8%, 6.0%, 10.4%, 4.5%

and 13.4%, respectively. The median inter-assay and intra-assay coefficients of variation for mtDNA and mitochondrial RNA (mtRNA) were <5%.

Statistical analysis

The primary outcome measure was the change in fat mtDNA from baseline to week 48 by as-treated analysis. A sample size of 22 patients per arm (total of 44) was required to ensure >80% power to significantly ($P<0.05$) detect a difference of 30% in fat mtDNA from baseline to week 48 between the two treatment arms, allowing for a dropout rate of ~15%. Secondary outcome measures included changes in mtRNA, limb fat, spine and hip bone mineral density (BMD), lipids, glucose metabolism and inflammation markers. The changes from baseline to week 48 were compared between groups using a stratified Wilcoxon rank-sum test, whereas within-group changes were tested using a Wilcoxon signed-rank test. The level of significance was set at 0.05 for all analyses. All analyses were carried out using SAS v.8.2 (The SAS Institute, Carey, NC, USA).

Results

Patient characteristics

A total of 50 patients were enrolled from 9 May 2005 to 27 November 2007 from the John T Carey Special Immunology Unit, Cleveland, OH, USA, and randomized to receive NucleomaxX® (n=26) or switch tNRTI to TDF (n=24). Table 1 shows the baseline characteristics of the study groups. Overall 43 (86%) were male, 27 (54%) were White, and 20 (40%) were African American. The overall median age at enrolment was 48 years. The baseline median fat mtDNA was 644.5 copies/cell, limb fat was 4,494 g, body mass index was 26.1 kg/m^2 and CD4$^+$ T-cell count was 564 cells/mm^3; 40 (80%) were on an zidovudine-containing regimen, and 10 (20%) were on a stavudine-containing regimen. Overall, 47 (94%) had HIV-1 RNA<50 copies/ml. Demographics, clinical and HIV disease parameters were similar between the groups. In particular, there were no statistically significant differences in demographics, fat mtDNA levels, PBMC mtDNA levels, body mass index, limb fat, lipids, lactate, glucose parameters, BMD or inflammation markers at study entry.

Patient disposition

Of the 50 patients who received study treatment, 45 (90%) completed 48 weeks of study. The reasons for discontinuation were grade 3 diarrhoea (one patient on NucleomaxX®), intolerance to NucleomaxX® taste (two patients), schedule conflict (one patient on NucleomaxX®) and fear of repeat biopsies (one patient in the switch arm). During the study period, no patient discontinued the tNRTI in the NucleomaxX® arm.

Changes in fat mtDNA levels: primary end point

The primary end point, change in fat mtDNA levels from baseline to week 48, is depicted in Table 2. The median (IQR) change in fat mtDNA (copies/cell) from baseline to week 48 in the NucleomaxX® arm was a significant decrease of −169 (−778− 64) compared with a non-significant increase of 321 (−298–669) in the switch arm (P=0.03 and P=0.37, respectively, for within-arm changes and P=0.02 for between arms). PBMC mtDNA did not change significantly within each of the arms or between the arms. The changes in fat mtDNA did not correlate with baseline mtDNA levels and body mass index or with duration of tNRTI or protease inhibitor before study entry.

Changes in mtRNA levels: secondary end points

As shown in Table 2, in either study arm, changes from baseline to week 48 were significant for ND1/L13, ND6/L13 and for CYT b/L13 ($P \leq 0.001$ for changes in all markers in either arm). However, changes in all three mtRNAs were higher in the switch arm when compared with the NucleomaxX® arms, although only CYT b/L13 was significantly different between the groups ($P=0.01$).

Changes in limb fat and trunk fat by DEXA: secondary end points

Changes in limb fat were significantly different between arms ($P=0.04$). Although median (IQR) limb fat did not change from baseline to week 48 in the NucleomaxX® arm (84 g [−34–314]; $P=0.65$), it increased significantly in the switch arm (409 g [−59–1,155]; $P=0.007$). Similarly median (IQR) trunk fat change from baseline to week 48 did not change in the NucleomaxX® arm (181 g [−524–596]; $P=0.97$) but increased significantly in the switch arm (654 g [−36–1,359]; $P=0.02$); $P=0.02$ for the changes between arms.

No correlation was found between changes in limb fat and baseline or changes in fat mtRNA, PBMC mtDNA, protease inhibitor duration or non-NRTI duration. In addition, limb fat changes were similar in stavudine-treated versus zidovudine-treated patients at entry and were independent of the degree of lipoatrophy at baseline as assessed by limb fat. In the switch arm, changes in limb fat negatively correlated with baseline fat mtDNA ($r=−0.55$; $P=0.006$) and with cumulative tNRTI duration at the time of study entry ($r=−0.66$; $P<0.001$), positively correlated with baseline CD4$^+$ T-cell count ($r=0.44$; $P=0.03$) and trended to correlate with changes in fat mtDNA ($r=0.41$; $P=0.05$).

Changes in bone mineral density by DEXA: secondary end points

The percentage change in lumbar spine BMD did not change within either study arm ($P \geq 0.42$ for both) or between arms ($P=0.28$). However, median percentage changes in hip BMD significantly decreased from baseline to week 48 in the switch arm (−3.3% [IQR −5.1−0.0]; $P=0.01$ versus 0.5% [IQR −0.3%, 3.6%] in the NucleomaxX® arm; $P=0.16$). The difference in changes between arms was also significant ($P=0.002$).

Changes in inflammation markers: secondary end points

As detailed in Table 2, most inflammation markers significantly increased from baseline to week 48 in the NucleomaxX® arm, but not in the switch arm. In the NucleomaxX® arm, the changes from baseline to 48 weeks were statistically significant for IL-6, hsCRP, sTNFRII and VCAM-1. In addition, the between-arm changes were significant for IL-6, hsCRP and VCAM-1 ($P=0.02$, $P=0.02$ and $P=0.04$, respectively). The changes in the inflammation markers did not correlate with changes in limb fat or mitochondrial indices.

Other metabolic end points

No statistically significant within or between-arm differences were seen in body mass index. No differences in changes from baseline to week 48 were seen in fasting glucose, insulin, homeostatic model assessment of insulin resistance, lactate, total cholesterol, low-density lipoprotein cholesterol or high-density lipoprotein cholesterol between the NucleomaxX® and switch arms. Fasting triglyceride levels did not change in the NucleomaxX® arm, but there was a trend towards a decrease in the median [IQR] triglyceride levels in the switch arm (−38 mg/dl [−125−32]; $P=0.05$ within switch arm and $P=0.07$ between arms).

Adverse events and effect on HIV disease activity

There was a total of 13 (26%) patients who had one adverse event each related to the biopsy procedure; seven were grade 1 and six were grade 2. All these complications resolved

without permanent sequelae. None of these led to the premature discontinuation from the study. The most common biopsy-related complication (8/13 [62%]) consisted of a haematoma at the site of the biopsy. In one patient, the haematoma required aspiration; and in another the haematoma became infected, requiring oral antibiotics. In addition, three other patients had seromas at the site of biopsies; two of these required aspiration. Lastly, one patient developed minimal self-limited irritation at the site of the biopsy and another developed incisional neuritis that resolved after a course of non-steroidal anti-inflammatory agent.

The protocol-defined toxicities were diarrhoea and hyperglycaemia (expected toxicities of NucleomaxX®). Two patients (both on NucleomaxX®) developed diarrhoea; in one patient, grade 3 diarrhoea developed after 1 week of NucleomaxX® and led to premature discontinuation of the study. Another patient had self-limited grade 1 diarrhoea that resolved with continuation of NucleomaxX®. No cases of diabetes occurred during the study. Four patients (two on NucleomaxX®) had hyperglycaemia (grade 1–2) during the study. The repeat glucose levels during the study were back to normal levels except for one patient (in the NucleomaxX® arm) who continued to have grade 1 or 2 hyperglycaemia until the end of the study. In addition, 8/50 (16%) patients developed hypophosphataemia (grade 2 in five patients and grade 3 in three patients). Four of these were on TDF (all grade 2). Of the patients with data available, 22/24 (92%) patients in the NucleomaxX® arm and 23/23 (100%) patients in the switch arm had HIV-1 RNA≤50 copies/ml at week 48 (P=0.70). One of the NucleomaxX® patients had HIV-1 RNA of125 copies/ml and another 726 copies/ml at week 48. Both patients had a history of missing several doses of ART in the period preceding the week 48 blood draw. The median (IQR) change in CD4+ T-cell count from baseline to week 48 was 83 cells/mm^3 (−79–102) and 70 cells/mm^3 (−31–175) for the NucleomaxX® and switch arms, respectively (within-arm P=0.14 and P=0.03, respectively; P=0.63 between arms).

Discussion

In this comparative study of two parallel interventions for HIV lipoatrophy, uridine supplementation in the form of NucleomaxX® improved mtRNA levels, but worsened fat mtDNA and did not change limb fat. Uri-dine is incorporated during transcription and this might explain the increase in mtRNA levels [13]. The worsening in fat mtDNA is probably because of the continued use of tNRTI in this study arm and the inability of NucleomaxX® to counteract and reverse mitochondrial effects on adipose tissue. The lack of reversal of mtDNA depletion is in contrast to prior *in vitro* studies where uridine supplementation of hepatocytes and adipocytes abrogated mitochondrial toxicities of tNRTIs [7,14,15]. This could be owing to the uridine being used during the glycolysis pathway of galactose instead of transcription [16]. Uridine is one of the nucleosides of RNA and also plays a role during glycolysis pathway of galactose where uridine diphosphate is transferred to the galactose and glucose-1-phosphate is produced so that glycolysis can proceed. Another possibility for the lack of improvement in limb fat on NucleomaxX® is the fact that several inflammation markers significantly worsened during the study period on NucleomaxX®. This is a novel observation that has never been previously investigated in cell culture or *in vivo*. Although we did not have a control group where no intervention has been performed, the fact that these same inflammation markers did not significantly change in the comparator arm (the switch arm) makes it likely to be a real observation that requires further investigation. The aetiology of the increase in inflammation markers after NucleomaxX® in our study is unclear and is in contrast to animal studies showing that exogenous uri-dine reduces oedema formation, leukocyte infiltration and tumour necrosis factor-α concentrations in a lung inflammation model [17].

By contrast, switching from a tNRTI to TDF for 48 weeks led to significant increases in limb fat and in fat mtRNA levels, without a change in fat mtDNA levels. Similar subcutaneous fat increases have been observed in some studies [18–21], but not others [15,22]. Prior cumulative tNRTI therapy of at least 3 years was found to jeopardize the limb fat response to the switch [20]. It is notable that the favourable changes in limb fat seen in the switch arm of our study happened despite the fact that our study patients in the switch arm had a median duration of prior tNRTI of 79 months, with 75% of them having received >3 years of tNRTI at the time of enrolment into our study. We have also previously shown that limb fat correlates with mtRNA levels, and not with mtDNA levels, in patients with lipoatrophy [12]. The TDF increase in fat mitochondrial transcripts is a novel observation that could be compensatory for the increase in limb fat cells; the mechanism should be studied further. Thus, we showed a significant change in limb fat despite a median duration of prior tNRTI therapy of 79 months (IQR 48, 86) in the switch arm. However, even in our study, changes in limb fat after tNRTI-to-TDF switch negatively correlated with cumulative tNRTI duration at the time of study entry. Thus, it is imperative to promptly switch patients off tNRTI therapy to ensure better and faster recovery of limb fat.

Consistent with the prior preclinical and clinical data [7,9,10], our study did not detect a significant interaction between NucleomaxX® and immunological parameters. Similarly switching tNRTI to TDF preserved virological suppression without significant adverse events, with the exception of the large drop seen in hip BMD. Although the mechanism of bone loss in HIV remains unclear, prospective studies of patients initiating their first ART regimen showed that TDF-containing regimens led to a significantly larger decline in spine and hip BMD than other NRTIs [23,24]. Tenofovir might affect bone through proximal tubule toxicity resulting in phosphate wasting and increased bone turnover [25]. In addition, in a prior switch study, there was more bone loss in suppressed patients switched to TDF than those switched to abacavir [26], although the degree of bone loss after the switch was less pronounced than in our study. This loss of BMD after the switch should not be perceived as clinically insignificant or the natural bone loss that occurred after 1 year in our aging population, because even at the most vulnerable skeletal time in women – during the first 2 years of menopause – the mean annual rates of bone loss is between 1.2 and 1.6% [27]. However, it is important to note that in both treatment initiation studies and in switch studies, the drop in BMD after introduction of TDF is mostly seen during the first 24–48 weeks after TDF, following which BMD stabilizes.

We recognize the limitations of this study, mainly the relatively small sample size. Another important limitation is the lack of a control arm where therapy with tNRTI was continued unchanged without the addition of any intervention. However, such a study in lipoatrophic patients would not be feasible or ethical. Our findings should be interpreted with consideration that a large number of comparisons and correlations were made without adjustment for multiple comparisons. Lastly, our measure of adherence to NucleomaxX® might be arguably suboptimal, but the sachet/pill count has been validated for use in other studies. In addition, measurements of plasma uridine levels have not been done because their utility to assess adherence is questionable. After ingestion of NucleomaxX®, the uridine plasma levels peak at about 1.3 h, after which they decline rapidly (half-life <2 h) [11] because the erythrocytes serve as a carrier to distribute the uridine quickly throughout the body. Measurement of the intracellular levels is technically challenging and we were unable to find a laboratory with the required experience to check these intracellular levels.

In summary, although NucleomaxX® was safe and well tolerated by most patients, we failed to show any improvement in mtDNA and limb fat on NucleomaxX® in patients with lipoatrophy who continued their tNRTI therapy. Switching tNRTI to TDF led to significant improvement in limb fat and fat mtRNA levels; however the swift drop in hip BMD after the

switch is concerning. The unexpected large increases in inflammation markers after NucleomaxX® might partially explain the failure of NucleomaxX® to reverse lipoatrophy and deserves further investigation.

Acknowledgments

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Table 1

Baseline characteristics by study arm

Characteristic	NucleomaxX® (*n*=26)	TDF switch (*n*=24)
Age, years	48 (41–51)	48 (43–51)
Male sex, %	86	86
Race, %		
White	57	50
African American	43	36
Hispanic	0	9
Asian	0	1
Body mass index, kg/m^2	26.1 (19.0–28.0)	26.1 (22.0–28.0)
Limb fat	4.4 (2.9–6.3)	5.5 (3.4–7.0)
Phosphate, mg/dl	3.30 (3.15–3.60)	3.20 (2.80–3.60)
CD4$^+$ T−cell count, cells/mm^3	578 (439–781)	532 (334–858)
Known duration of HIV, months	146 (78–184)	108 (68–183)
Receiving stavudine at entry, %	27	17
Receiving zidovudine at entry, %	73	83
Receiving PI at entry, %	38	50
Duration of thymidine	41	79
NRTI, months	(24–86)	(48–86)

Data presented as median (IQR) unless otherwise indicated. Baseline characteristics were well balanced between study arms. NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; TDF, tenofovir.

Table 2

Changes in the metabolic end points by study arm

	NucleomaxX® (n=26)			TDF switch (n=24)			Between-group P-value
	Baseline	Change to week 48[a]	P-value	Baseline	Change to week 48[a]	P-value	
Body mass index, kg/m²	26 (19–28)	0 (–0.4–0.5)	0.71	26 (22–28)	0 (–0.6–0.7)	0.70	0.53
Limb fat, kg	4.4 (2.9–6.3)	0.1 (–0.03–0.3)	0.65	5.5 (3.4–7.0)	0.4 (–0.06–1.2)	0.007	0.04
Trunk fat, kg	7.9 (5.6–11.6)	0.2 (–0.5–0.6)	0.97	7.7 (5.8–10.1)	0.7 (–0.04–1.4)	0.02	0.02
Fat mtDNA, copies/cell	662 (395–1,410)	–169 (–778–64)	0.03	641 (396–1,343)	321 (–298–669)	0.37	0.02
PBMC mtDNA, copies/cell	301 (253–424)	–24 (–158–91)	0.44	290 (225–381)	–52 (–105–108)	0.63	0.81
ND1/L13	2.7 (2.0–8.9)	3.7 (–0.2–8.0)	0.001	6.3 (3.1–11.4)	6.3 (3.7–10.9)	<0.001	0.07
ND6/L13	0.8 (0.5–2.0)	2.2 (0.2–5.0)	<0.001	1.5 (0.8–4.0)	3.9 (1.6–6.4)	<0.001	0.17
CYTB/L13	2.2 (1.3–4.0)	2.8 (0.8–4.5)	<0.001	4.7 (2.0–7.1)	7.1 (3.9–10.1)	<0.001	0.01
Lumbar spine BMD, g/cm²	1.1 (0.9–1.2)	0.39% (–0.96–1.68)	0.52	1.05 (1.0–1.1)	0.0% (–2.9–1.76)	0.50	0.28
Hip BMD, g/cm²	0.81 (0.73–0.97)	0.45% (–0.27–3.56)	0.14	0.81 (0.74–0.94)	–3.3% (–5.1—0.0)	0.01	0.002
IL–6, pg/ml	2.6 (2.0–3.8)	26.0 (10.6–53.7)	<0.001	4.2 (2.0–14.2)	2.4 (–1.7–55.0)	0.21	0.02
hsCRP, mg/l	2.0 (0.8–4.5)	15.9 (4.5–29.9)	<0.001	3.6 (1.4–8.4)	–1.1 (–4.3–7.1)	0.69	0.02
sTNFRI, pg/ml	590 (536–831)	48 (–137–274)	0.27	615 (509–894)	66 (–195–169)	0.95	0.63
sTNFRII, pg/ml	214 (139–411)	724 (371–1,091)	<0.001	344 (133–723)	189 (–223–1,355)	0.10	0.18
sVCAM1, ng/ml	450 (305–611)	1,986 (912–2,805)	<0.001	731 (420–1,769)	521 (–164–1,991)	0.11	0.04
LDL cholesterol, mg/dl	123 (96–148)	–2 (–16–11)	0.43	111 (90–127)	–5 (–19–20)	0.98	0.78
Triglycerides, mg/dl	163 (109–241)	17 (–34–42)	0.65	187 (132–363)	–38 (–125–32)	0.05	0.07
Glucose, mg/dl	86 (83–91)	4 (–3–6)	0.40	85 (78.5–94)	0 (–6–12)	0.32	0.90
Insulin, mIU/l	10 (7–16)	1 (–4–3)	0.60	10 (4–12)	–0.5 (–3.5–3)	0.64	0.54

Data presented as median (IQR).

[a] Change from baseline to week 48 represent the absolute change in values, except for lumbar spine and hip bone mineral density (BMD), where percentage change from baseline is shown. hsCRP, high-sensitivity C-reactive protein; IL–6, interleukin-6; LDL, low-density lipoprotein; mtDNA, mitochondrial DNA; PBMC, peripheral blood mononuclear cell; sTNFRI, soluble tumour necrosis factor receptor I; sTNFRII, soluble tumour necrosis factor receptor II; sVCAM1, soluble vascular cell adhesion molecule 1.