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ARTICLE

Strong association between mitochondrial DNA copy number and lipogenesis in human white adipose tissue

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Abstract
Aims/hypothesis Recent studies suggest a link between insulin resistance and mitochondrial function in white fat cells. The aim of this study was to evaluate adipocyte mitochondrial DNA (mtDNA) copy number in relation to adipocyte and clinical variables that are related to insulin sensitivity.
Methods We studied a group of 148 healthy volunteers with a large inter-individual variation in BMI. Relative amounts of mtDNA and nuclear DNA were determined by quantitative RT-PCR. The mtDNA:nuclear DNA ratio reflects the tissue concentration of mtDNA per cell.
Results The mtDNA copy number was enriched in adipocytes of adipose tissue and decreased slightly by ageing (p=0.015) and increasing BMI (p=0.004); however, it was not influenced by sex, energy-restricted diets or marked long-term weight reduction. Adipose mtDNA copy number was not independently related to resting energy expenditure, overall insulin sensitivity or adipocyte lipolysis. However, it showed a strong positive correlation with basal (p=0.0012) and insulin-stimulated lipogenesis (p<0.0001) in fat cells, independently of age and BMI, and a weak positive correlation with levels of mRNA from several genes involved in mitochondrial oxidative capacity (r=0.2–0.3).
Conclusions/interpretation The mtDNA copy number in human white fat cells is fairly stable within healthy individuals. It is not influenced by sex or weight loss and is not important for overall insulin sensitivity or energy expenditure at rest. However, it is strongly related to adipocyte lipogenesis and weakly to mitochondrial oxidative capacity, suggesting that adipocyte mitochondria are, above all, local regulators.

Keywords Adipocytes · Energy expenditure · Insulin resistance · Lipogenesis · Lipolysis · Mitochondria · White adipose tissue

Abbreviations

ATP6	ATP synthase 6
COX1	cytochrome *c* oxidase 1
CPT1A	carnitine palmitoyltransferase 1A
CPT1B	carnitine palmitoyltransferase 1B
HOMA2 %S	homeostasis model assessment of insulin sensitivity
LPL	lipoprotein lipase
MCAD	medium-chain specific acyl-coenzyme A dehydrogenase
mtDNA	mitochondrial DNA
NDUFB4	NADH dehydrogenase 1 β subcomplex 4
ND1	NADH dehydrogenase subunit 1
pD_2	$-\log_{10}$ half maximum effective hormone concentration
PGC1A	peroxisome proliferator-activated receptor γ coactivator 1α
RQ	respiratory quotient
TZD	thiazolidinedione
$\dot{V}CO_2$	carbon dioxide produced
$\dot{V}O_2$	oxygen uptake
WAT	white adipose tissue

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Introduction

The mitochondrion and its energy expenditure have recently attracted attention as candidate factors involved in obesity, insulin resistance and type 2 diabetes. Mitochondria are key players in energy turnover, and are where the tricarboxylic acid cycle and beta oxidation of fatty acids take place. The latter process produces NADH and $FADH_2$ as proton donors for oxidative phosphorylation where movement of protons is used to synthesise ATP from ADP and phosphate. Skeletal muscle and brown adipose tissue, the latter of which is not abundant in adult humans, have a large number of mitochondria. Environmental stimuli regulate mitochondria. The transcriptional co-activator peroxisome proliferator-activated receptor γ coactivator 1α (PGC1A), induced by cold, has been demonstrated to coordinately regulate mitochondrial biogenesis, quantified as mitochondrial density and mitochondrial DNA (mtDNA) copy number, as well as oxidative capacity in muscle and adipocytes [1, 2]. One implication of this is that coregulation of mtDNA copy number and mitochondrial function would be expected in vivo.

Earlier human studies focused on mitochondria in skeletal muscle. Microarray studies have implicated mitochondrial dysfunction in skeletal muscle cells in the development of insulin resistance and type 2 diabetes [3, 4]. Elderly individuals and offspring of type 2 diabetic patients have been shown to have impaired mitochondrial function in muscle [5, 6].

Recent studies have highlighted the importance of mitochondrial mass in white adipose tissue (WAT) as well. Fat cells from *ob*/*ob* mice display reduced mitochondrial mass and altered mitochondrial structure, which are normalised by treatment with rosiglitazone, an insulin-sensitising thiazolidinedione (TZD) [7]. The treatment is accompanied by increased adipocyte fatty acid oxidation. Mitochondria of human white fat cells might also be of importance for whole-body energy expenditure and insulin resistance. Although only around 0.5% of the fatty acids that are produced in human adipocytes from lipolysis are oxidised, this process has been shown to be highly regulated [8]. Heat production in adipocytes from obese individuals increases after weight reduction, indicating a role for mitochondria in human white fat cells for energy turnover and obesity [9]. Treatment of human fat cells with forskolin (a cAMP-inducing agent) increases mtDNA copy number and fatty acid oxidation in the cells [10]. The expression of genes controlling oxidative phosphorylation in mitochondria is downregulated in visceral adipose tissue of type 2 diabetic patients [11]. TZDs induce mitochondrial biogenesis in human WAT in vivo in type 2 diabetic patients [12, 13]. In addition, *n*-3 polyunsaturated fatty acids, known to prevent obesity and insulin resistance, upregulate mitochondrial biogenesis in white fat [14]. Mitochondrial biogenesis in these studies is accompanied by an increase in adipose tissue gene expression of several genes involved in fatty acid oxidation and improvement of overall insulin-stimulated glucose uptake [12–14].

Taken together, the findings mentioned above strengthen the idea that the mitochondria in WAT play an important role in energy metabolism and in the development of obesity, insulin resistance and, ultimately, type 2 diabetes. However, a number of questions have not yet been answered regarding the role of mitochondria in human white fat cells. Is there an influence of age, sex, obesity or body weight changes? Is it related to overall insulin sensitivity or to whole-body energy expenditure? Is it above all a local (i.e. adipocyte) regulator? Although the amount of mitochondria seems important for fatty acid oxidation by human white fat cells [12, 13], it is not clear how it relates to other metabolic features of these cells. It has been shown that adipocyte lipogenesis and lipolysis require the functionality of mitochondria [15].

This study was undertaken primarily to study the in vivo regulation of mtDNA copy number in human WAT. We also investigated the relationship between mtDNA copy number and adipocyte metabolism, as well as whole-body insulin sensitivity and resting energy expenditure. We quantified mtDNA copy number in abdominal subcutaneous WAT in a large cohort (n=148) of healthy male and female volunteers with considerable inter-individual variation in age and BMI.

Methods

Volunteers The study was approved by the ethics committee at Karolinska University Hospital, and informed consent was obtained from all participants. The study population was recruited by local advertisement and obesity was defined as BMI ≥30 kg/m^2. Clinical data for the whole group are presented in Table 1.

We investigated 116 women (72 obese, aged 38±8 years [means±SD], BMI 37±5 kg/m^2, 44 non-obese, aged 39±12 years, BMI 24±3 kg/m^2) and 32 men (23 obese, aged 41±

Table 1 Clinical characteristics of the study population

Measure	Mean±SD
Sex distribution (men/women)	32/116
Age (years)	39±9
BMI (kg/m^2)	33±7
Plasma insulin (pmol/l)	77±59
Plasma glucose (mmol/l)	5.2±0.7
HOMA2 %S	111±98
i.v. insulin tolerance (% fall in glucose/min)	4.1±1.3
RQ at rest	0.83±0.05
Energy expenditure at rest (J kg^{-1} day^{-1})	7,286,468±1,526,792

6 years, BMI 36±5 kg/m^2, nine non-obese, aged 33±10 years, BMI 27±2 kg/m^2); all were healthy and free of continuous medication. Sixty-eight of the obese participants were investigated before and after a 10-week energy-restricted diet. They were randomised to either a low-fat or a high-fat content diet. The dietary intervention was as previously described [16]. No effect of diet composition on results was observed. The average weight loss induced by the diet was 7.0±3.1 kg. Nine obese women were investigated before intense anti-obesity therapy with a gastric banding operation or behavioural modification and when they had reached a non-obese weight-stable state after 2–5 years. The nine obese participants undergoing intense anti-obesity therapy have been previously described [17]. Seven obese women were investigated before gastric banding, after 2 years when body weight was reduced but not stable and after 3–4 years when body weight was further reduced and stable.

The participants were investigated at 0800 hours after an overnight fast. Biopsies of the subcutaneous abdominal adipose tissue (0.5–2 g) were obtained by needle aspiration under local anaesthesia. Venous blood samples were taken for measurements of insulin and glucose, and HOMA2 %S was calculated from these values, reflecting insulin sensitivity [18]. Height, weight and waist and hip circumferences were measured. To quantify body fat, bioimpedance was measured with Bodystat equipment (Bodystat, Douglas, UK). Indirect calorimetry was performed for 25 min using an open-system ventilated hood (Deltatrac II; Datex-Ohmeda, Helsinki, Finland). This system measures caloric needs and estimates substrate oxidation by continuously measuring oxygen uptake ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). After indirect calorimetry, an i.v. insulin tolerance test was performed in 64 participants as previously described [19].

Adipocyte experiments Isolated fat cells were prepared according to the collagenase procedure described by Rodbell [20] and van Harmelen et al. [21]. In some experiments we saved the non-adipose cells harvested from the stromal fraction of adipose tissue that was obtained after collagenase treatment. Mean fat cell weight and volume were determined and lipolysis and lipogenesis experiments conducted as described [17]. Briefly, in the lipolysis experiments diluted cell suspensions (2% *v*/*v*) were incubated in duplicate for 2 h with air as the gas phase at 37°C in Krebs–Ringer phosphate buffer (pH 7.4) supplemented with glucose (8.6 mmol/l), ascorbic acid (0.1 mg/ml) and BSA (20 mg/ml) without (basal) or with increasing concentrations (10^{-16}–10^{-4} mol/l) of either noradrenaline or insulin, and glycerol release (lipolysis index) was determined. In the lipolysis experiments with insulin, the standard medium was also supplemented with 8-bromo-cAMP (10^{-3} mol/l) and adenosine deaminase (1 mU/l).

In the lipogenesis experiments, the standard medium was supplemented with a low concentration of unlabeled and tritiated glucose (10^{-6} mol/l) and insulin (0, 10^{-16}–10^{-6} mol/l) and radioactive incorporation into total lipids was determined. Results were expressed as amount of glycerol release or amount of glucose incorporated into lipids (nmol glucose 2 h^{-1} [10^7 fat cells]$^{-1}$). Half maximum effective hormone concentration (EC_{50}) was determined and turned into a pD_2 value ($-\log_{10}$ EC_{50}). Maximum effect was calculated as lipolysis or lipogenesis at maximum effective concentration.

DNA and RNA isolation Adipose tissue was brought to the laboratory in saline and pieces were immediately frozen in liquid nitrogen. Adipose tissue pieces (300 mg), 200 µl isolated adipocytes and isolated stromal cells were kept at −70°C. NucleoSpin RNA II (740955.50; Macherey-Nagel, Düren, Germany) in combination with a NucleoSpin RNA/DNA Buffer Set (740944; Macherey-Nagel) were used to separately isolate RNA and DNA from the same adipose tissue lysates. The same procedure was used for isolated adipocytes and stroma cells. The concentrations of the DNA and RNA were measured spectrophotometrically.

Quantification of mtDNA copy number Relative amounts of nuclear DNA and mtDNA were determined by quantitative RT-PCR as described [12]. The ratio of mtDNA to nuclear DNA reflects the tissue concentration of mtDNA per cell. Briefly, a 120 nucleotide-long mtDNA fragment within the *ND1* gene (also known as *MT-ND1*) for NADH dehydrogenase subunit 1 was used for quantification of mtDNA [22]. The PCR product has previously been cloned into a plasmid [12]. Plasmid standards of known copy number were used to generate a log–linear standard curve, from which the *ND1* copy numbers of studied samples could be determined by quantitative RT-PCR. A 120 bp region of the nuclear gene for lipoprotein lipase (*LPL*), was used to normalise results. Plasmid standard curves containing the *LPL* fragment were used to determine copy numbers of studied samples. The ratio of *ND1* to *LPL* copy number reflected the tissue concentration of mitochondria per cell [12].

Primers and Taqman probes (Applied Biosystems, Foster City, CA, USA) were for *ND1* probe: 5′-CCATCAC CCTCTACATCACCGCCC-3′, forward primer 5′-CCCTAAAACCCGCCACATCT-3′, reverse primer 5′-GAGCGATGGTGAGAGCTAAGGT-3′, and for *LPL* probe: 5′-ACATTCACCAGAGGGTC-3′, forward primer 5′-CGAGTCGTCTTTCTCCTGATGAT-3′, reverse primer 5′-TTCTGGATTCCAATGCTTCGA-3′. The amplification assays were as reported but performed on an iCycler (BioRad Laboratories, Hercules, CA, USA) [12].

Quantification of gene expression RNA samples were treated with RNase-free DNase (Qiagen, Hilden, Germany).

RNA quality was verified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Kista, Sweden). One microgram of RNA was reverse transcribed using an Omniscript RT kit (Qiagen) and random hexamer primers. Specific mRNAs were quantified using TaqMan assays and Universal Master Mix (4318157; Applied Biosystems). We quantified genes for medium-chain specific acyl-coenzyme A dehydrogenase (*MCAD* [also known as *ACADM*], Hs00163494_m1), carnitine palmitoyltransferase 1A (*CPT1A*, Hs00912670_m1), carnitine palmitoyltransferase 1B (*CPTIB*, Hs00189258_m1), ATP synthase 6 (*ATP6* [also known as *MT-ATP6*], Hs02596862_g1), cytochrome *c* oxidase 1 (*COX1* [also known as *MT-CO1*], Hs02596864_g1), NADH dehydrogenase 1 β subcomplex 4 (*NDUFB4*, Hs00853558_gl) and PGC1A (*PGC1A* [also known as *PPARGC1A*], Hs00173304_ml). The control gene, encoding 18S rRNA, was quantified using an SYBR Green-based assay with primers CACATGGCCTCCAAGGAGTAAG and CCAG CAGTGAGGGTCTCTCT. A direct comparative method was used for data analysis (Applied Biosystems).

Statistical analysis Values for HOMA2 %S, mtDNA copy number per cell and mRNA expression levels were $\log_{10}$-transformed before statistical analysis to become normally distributed. Values are shown as means±SD. Simple and multiple regression analysis and Student's paired or unpaired *t* test were used for statistical comparison. Correlations were evaluated by the *z* test.

Results

In the whole studied cohort (n=116 women and 32 men) investigated before the energy-restricted diet there were, on average, about 1,000 mtDNA copies per cell in adipose tissue. In a subgroup of 24 investigated individuals, the mtDNA copy number was found to be markedly enriched in adipocytes with around 15 times higher values in isolated adipocytes vs adipose tissue pieces (Fig. 1a). mtDNA copy number was measured in cells from the stromal vascular fraction of adipose tissue in five participants. It was ranged between four and ten copies per cell and was significantly lower than the number in isolated fat cells or adipose tissue (p<0.0001). There was no difference in age or BMI between men and women (data not shown), nor was there a difference in mtDNA copy number between the sexes (Fig. 1b).

In the whole studied cohort investigated before the energy-restricted diet, mtDNA copy number per cell correlated inversely and significantly with age (Fig. 2a) and BMI (Fig. 2b). These relationships were weak (r=0.20–0.24) but independent of each other (partial r=0.20–0.24). There was no correlation between HOMA2 %S or i.v. insulin tolerance and mtDNA copy number per cell when age and BMI were included in multiple regression analysis (partial r=0.06–0.10). mtDNA copy number was not associated with respiratory quotient (RQ, calculated as $\dot{V}CO_2/\dot{V}O_2$) (r=0.06) or resting energy expenditure when calculated per total body fat (r=0.15) or per kg fat-free mass (r=0.02) (graphs not shown).

In analysis of adipocyte metabolism in the same cohort there was no association between any measure of lipolysis (basal, maximal noradrenaline-induced, noradrenaline sensitivity, maximal anti-lipolytic effect of insulin, insulin sensi-

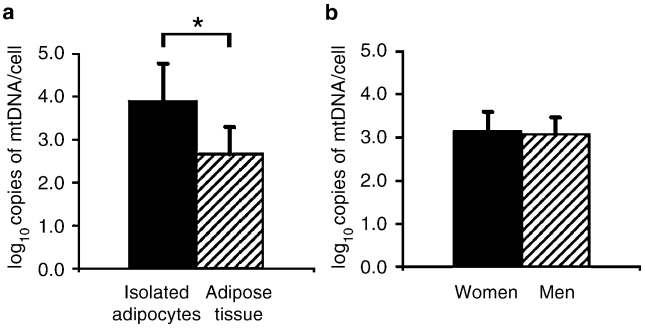

Fig. 1 mtDNA copy number ($\log_{10}$ copies of mtDNA/cell) in human subcutaneous abdominal adipose tissue. **a** Isolated fat cells vs adipose tissue pieces of the same participants (n=24). **b** Comparison of mtDNA copy number in women (n=116) and men (n=32). Data are presented as means±SD. *p<0.0001 from Student's paired or unpaired *t* test

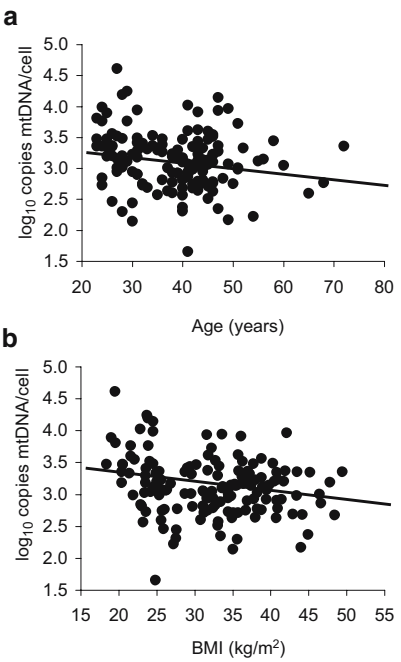

Fig. 2 Simple linear regression between mtDNA copy number ($\log_{10}$ copies of mtDNA/cell) in women (n=116) and men (n=32) vs age (**a**) (r=0.20, p=0.015) or BMI (**b**) (r=0.24, p=0.004)

tivity) and mtDNA copy number per cell (r=0.002–0.14) (graphs not shown). In contrast, there was a strong positive association between mtDNA and basal lipogenesis, as well as responsiveness of lipogenesis to insulin independently of age and BMI (partial r=0.23–0.31 in joint analysis of all participants put together). Figure 3a–f displays the results of simple linear regression between basal lipogenesis and responsiveness to insulin vs mtDNA copy number for non-obese and obese individuals separately to illustrate that these associations were not secondary to difference in obesity status between participants. In the obese group there was a trend towards a positive association between basal lipolysis and mtDNA copy number, although the result was non-significant. All other subgroup analyses were significant. About 14% of the variance in lipogenesis could be explained by the variance in mtDNA (i.e. adjusted r^2 for the whole cohort). In contrast, there was no association between mtDNA copy number and sensitivity of lipogenesis to insulin (r=0.003; graph not shown).

A 10-week low- or high-fat energy-restricted diet in the obese cohort (n=68), resulting in a 2.6±1.0 and 2.2±1.1 kg/m^2 decrease in BMI, respectively (Fig. 4a), did not influence mtDNA copy number (Fig. 4b). It is probable that fat cell number was not affected by this energy-restricted diet. Nine obese women were investigated before intense anti-obesity therapy and after they had reached a steady non-obese state 2–5 years after intervention, when they had decreased in BMI by 15±6 kg/m^2 (Fig. 4c). In spite of this marked reduction in BMI to normal values, there was no difference in adipose tissue mtDNA copy number before and after weight loss (Fig. 4d). Seven obese women were investigated before gastric banding, 2 years thereafter and 3–4 years following surgery when body weight was stable. BMI at these three different occasions were 44.3±3.2, 33.8±2.8 and 30.0±3.8 kg/m^2 (significantly different from each other). mtDNA copy number, however, was the same at all three occasions (values not shown).

The expression of several genes regulating mitochondrial oxidative capacity was measured in the 136 of the 148 participants for which high-quality RNA was available, and set in relation to mitochondrial number using a correlation matrix (Table 2). *PGC1A*, an important regulator of

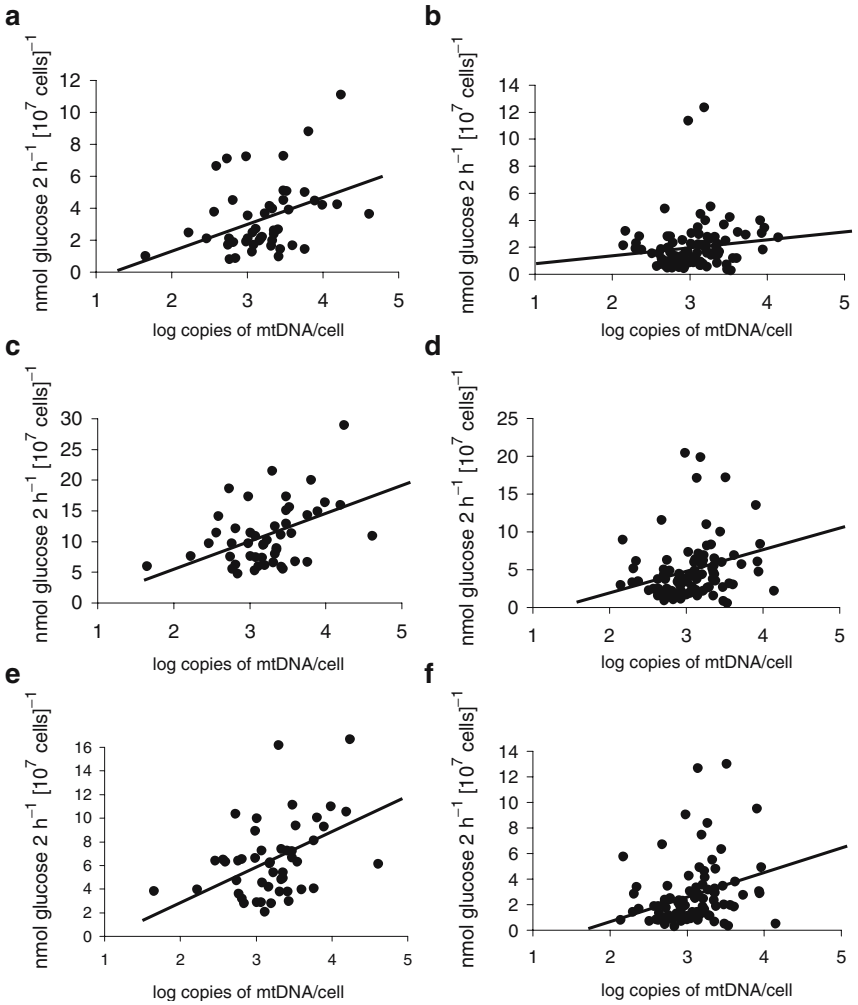

Fig. 3 Simple linear regression between mtDNA copy number ($\log_{10}$ copies of mtDNA per cell) and lipogenesis in isolated fat cells in the basal (non-stimulated) state for non-obese (**a**) (r=0.35, p=0.016) and obese volunteers (**b**) (r=0.10, p=0.32); at a maximum effective insulin concentration for non-obese (**c**) (r=0.43, p=0.0026) and obese (**d**) (r=0.22, p=0.034); and as maximum effective insulin concentration minus basal for non-obese (**e**) (r=0.43, p=0.0036) and obese (**f**) (r=0.27, p=0.0079). Lipogenesis is expressed as nmol glucose incorporated into lipids 2 h^{-1} [10^7 cells]$^{-1}$

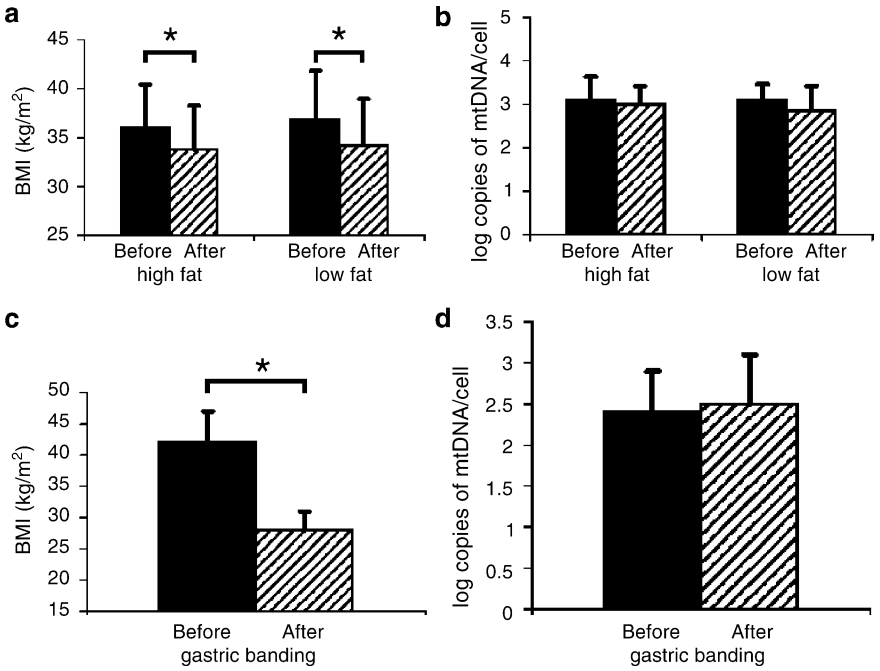

Fig. 4 Effect of weight reduction on BMI (**a**, **c**) and mtDNA copy number ($\log_{10}$ copies of mtDNA per cell) (**b**, **d**) in 68 obese participants undergoing high- or low-fat energy-restricted diet intervention for 10 weeks (**a**, **b**) and in nine obese participants before and after intense anti-obesity therapy (**c**, **d**). Data are presented as means±SD. *$p<0.0001$ from Student's paired t test

mitochondrial biogenesis [1], *ATP6*, *COX1* and *NDUFB4* in the oxidative phosphorylation pathway, as well as *MCAD* regulating mitochondrial fatty acid beta oxidation [12] correlated significantly but weakly with mtDNA copy number per cell (r=0.17–0.30; p=0.001–0.049). *CPT1A* and *CPT1B*, regulating fatty acid oxidation, were not correlated to mtDNA copy number. There was no important effect of BMI on the relationship between mtDNA copy number and mRNA values in multiple regression except for *PGC1A*, which displayed borderline significance in this analysis (p=0.058). There was a much stronger correlation between all oxidative genes, including the two *CPT*s (r= 0.35–0.90; $p<0.0001$). When arbitrary units of mRNA levels were used instead of $\log_{10}$ values, results were not changed in any important way, although r values were slightly lower.

PGC1A mRNA in adipose tissue was downregulated in obesity ($p<0.0001$). Expression of the other measured genes regulating mitochondrial oxidative capacity was not downregulated in obesity (results not shown). There was no positive association between expression of genes regulating mitochondrial oxidative capacity and HOMA2 %S, RQ, resting energy expenditure or i.v. insulin tolerance. Basal lipogenesis was associated with expression of *COX1*, *ATP6* and *NDUFB4* (p=0.0011–0.016) and responsiveness of lipogenesis to insulin with *COX1*, *ATP6*, *MCAD*, *PGC1A* and *NDUFB4* (p=0.0001–0.013) independently of age and BMI.

Table 2 Correlation between *ND1/LPL* ratio and mRNA levels of specific genes: correlation coefficients and p values

		Correlation coefficients							
		ND1/LPL	*PGC1A*	*COX1*	*ATP6*	*NDUFB4*	*CPT1A*	*CPT1B*	*MCAD*
	ND1/LPL		0.23	0.27	0.30	0.17	−0.07	−0.04	0.21
p values	*PGC1A*	0.0075		0.65	0.67	0.59	0.35	0.55	0.62
	COX1	0.0012	<0.0001		0.90	0.80	0.62	0.57	0.65
	ATP6	0.0005	<0.0001	<0.0001		0.76	0.56	0.53	0.66
	NDUFB4	0.049	<0.0001	<0.0001	<0.0001		0.68	0.57	0.67
	CPT1A	0.42	<0.0001	<0.0001	<0.0001	<0.0001		0.50	0.42
	CPT1B	0.63	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001		0.43
	MCAD	0.014	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	

Specific mRNAs were quantified using a direct comparative method with the gene for 18S rRNA as the control gene and are expressed as arbitrary units.
All measurements were $\log_{10}$-transformed before calculating correlation coefficients.

Discussion

To assess the role of mitochondrial mass in human white fat cells, we have presently examined the relationship between WAT mtDNA copy number in the subcutaneous adipose region of a large human cohort with varied age and BMI, and clinical factors related to insulin sensitivity including energy expenditure and adipocyte metabolism.

Despite considerable inter-individual variation in mtDNA copy number per cell of WAT, it appears that the number is relatively stable within certain individuals; thus, there was only a minor decreasing effect of age and BMI. A moderate, as well as marked, weight reduction caused no change in mtDNA. This resistance to weight reduction was observed for different energy-restricted diets and when participants were investigated in a non-steady-state phase of weight reduction. It should be noted that this notion of mtDNA stability is obtained from studies of healthy participants. In participants with type 2 diabetes, WAT mtDNA copy number is decreased and normalised after TZD therapy [12]. The mtDNA copy number does not appear to be influenced by sex hormones or other sex-related factors because the number was found to be almost the same in men and women. Furthermore, there was no independent relationship between mtDNA copy number and whole-body insulin sensitivity as measured by HOMA2 %S or i.v. insulin tolerance. Finally, mtDNA copy number did not associate with RQ and metabolic rate measured at rest. Taken together, these data suggest that the amount of WAT mitochondria per se is not important for overall insulin sensitivity and resting energy expenditure in man. It should be noted that we investigated subcutaneous adipose tissue. Thus, the amount of mitochondria in other depots, such as the viscera, might be more important. This is, however, less likely because subcutaneous WAT represents approximately 80% of total body adipose tissue. In the obese, the omental depot, which is a major component of visceral adipose tissue, represents only approximately 1% of total body fat [23]. Furthermore, other aspects of mitochondrial function, such as its structure or size might be of greater importance than the mtDNA copy number itself. Unfortunately, it is not possible to measure these additional features of WAT mitochondria in the present clinical setting with only small amounts of tissue at hand.

What about a local role of WAT mitochondria? In our experiments, mtDNA copy number was substantially higher in adipocytes than whole adipose tissue. With available methods we cannot determine whether this represent a true difference in mtDNA copy number or is caused by differences in relative yield of mitochondrial vs genomic DNA from the two different tissues. It is, though, very unlikely that such methodological issues are of great importance. First, because of the fat content DNA extraction from fat cells would, if anything, give less not more yield than extraction of DNA from other cells. Second, our additional studies on stroma cells show that they have far fewer mitochondria than isolated fat cells. We found that the average number of mtDNA copies in adipose tissue was about 1,000 per cell. Mean mtDNA copy number per cell in adipose tissue has previously been reported to be from 500 to 2,000 [12, 24]. We believe the different results are primarily due to experimental variation due to different efficiencies in amplifying plasmids used for constructing standard curves, use of different DNA extracting kits and PCR machines, as well as pipetting errors. Clinical differences between study groups may also play a role in the divergent results.

Adipocyte number of mtDNA copies seems to be of little importance for lipolysis in the non-stimulated (basal) or hormone-stimulated (insulin, catecholamines) state as judged by our data with mtDNA vs glycerol release or hormone sensitivity (pD_2). On the other hand, we found a strong positive correlation between both basal and insulin-stimulated lipogenesis and mtDNA, which was independent of age and BMI. As much as 14% of the variation in lipogenesis could be explained by mtDNA copy number (i.e. adjusted r^2). The importance of mitochondria for lipogenesis was supported by a positive association between expression of genes regulating mitochondrial oxidative phosphorylation and lipogenesis. Thus mtDNA copy number and expression of genes regulating mitochondrial oxidative phosphorylation may be important for lipogenesis. However, at present, we do not know if there is a causative association. To answer this question, it is necessary to manipulate mitochondrial mass. This can be done in human fat cells using forskolin and, presumably, other cAMP-generating agents or cAMP analogues [10]. However, it is not possible to distinguish how such effects on lipogenesis are mediated by mitochondrial mass or by other cAMP-generated effects such as those on lipolysis or cAMP-mediated transcriptional effects on lipogenesis genes.

The importance of mtDNA copy number per cell for mitochondrial oxidative capacity was also evaluated indirectly by comparing the mtDNA amount with adipose mRNA levels of key genes regulating oxidative phosphorylation and fatty acid oxidation. Five out of seven genes showed a positive correlation, although weakly, with mtDNA copy number. This relationship was independent of BMI except for *PGC1A* (p=0.058 after BMI adjustment). Interestingly, *PGC1A* was the only gene measured that was influenced by obesity.

The inter-relationships between *PGC1A* and the oxidative phosphorylation and fatty acid oxidation genes were much stronger than their relationship with mitochondrial number. This indicates that metabolic adaptation in available mitochondria is more closely coregulated than mito-

chondrial biogenesis. Direct measures of mitochondrial function such as lipid oxidation or cytochrome *c* activity are not feasible to use in large clinical studies with small amounts of adipose tissue available [25].

When present and previous [10] data are considered together we propose the following role of mtDNA copy number in human WAT. It has little influence on whole-body insulin sensitivity and resting energy expenditure, but it is correlated to, and may be important for, the lipogenesis of fat cells. An impact of fatty acid capacity is less certain but there is evidence of coregulation of mtDNA copy number and genes regulating fatty and oxidation.

In summary, the WAT mtDNA copy number is fairly stable within healthy individuals and may be important for the lipogenic capacity of their adipocytes.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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