

Omental Adipocyte Hypertrophy relates to Coenzyme Q10 Redox State and Lipid Peroxidation in Obese Women

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ABSTRACT

Occurrence of oxidative stress in white adipose tissues contributes to its dysfunction and the development of obesity-related metabolic complications. Coenzyme Q10 (CoQ10) is the single lipophilic antioxidant synthesized in humans and is essential for electron transport during mitochondrial respiration. To understand the role CoQ10 in adipose tissue physiology and dysfunction, the abundance of the oxidized (CoQ10_{ox}) and reduced (CoQ10_{red}) isoforms of the coenzyme Q10 were quantified in subcutaneous and omental adipose tissues of women covering the full range of body mass index (BMI), values from 21.5 to 53.2 kg/m². Lean women displayed regional variations of CoQ10 redox state between the omental and subcutaneous depot, despite similar total content. Obese women had reduced CoQ10_{red} concentrations in the omental depot, leading to increased CoQ10 redox state and higher levels of lipid hydroperoxides. Women with low omental CoQ10 content had a greater visceral and subcutaneous adiposity, increased omental adipocyte diameter. Higher circulating IL-6 and CRP levels and were more insulin resistant. The associations between abdominal obesity-related cardiometabolic risk factors and CoQ10 content in the omental depot were abolished after adjustment for omental adipocyte diameter. This study shows that hypertrophic remodeling of visceral fat closely relates to depletion of CoQ10, lipid peroxidation and inflammation.

KEYWORDS

Adipose tissue, Obesity, Adipocytes, Antioxidant, Mitochondria, Ubiquinol, Ubiquinone, Body fat distribution, Cardiometabolic-risk factors

ABBREVIATIONS

4-HNE : 4-hydroxy-2-nonenal, **ATP** : Adenosine triphosphate, **CCCP**: Carbonyl cyanide *m*-chlorophenyl hydrazine, **COQ2**: Coenzyme Q2 4-Hydroxybenzoate Polyprenyltransferase, **CoQ10** : Total coenzyme Q10, **CoQ10_{red}**: Reduced coenzyme Q10; **CoQ10_{ox}**: Oxidized coenzyme Q10, **CRP**: C-reactive protein, **DEXA**: Dual-energy x-ray absorptiometry, **GPx**: glutathion peroxidase, **IL-6**: Interleukin-6, **LPO**: Lipid hydroperoxide, **NADPH**: Nicotinamide adenine dinucleotide phosphate, **Nox**: NADPH oxidase, **ROS**: reactive oxygen species, **SOD**: superoxide dismutase,

INTRODUCTION

The metabolic consequences of excess fat accumulation, especially in intra-abdominal adipose tissue compartments, are closely related to white adipose tissue dysfunction. This phenomenon is characterized by adipocyte hypertrophy, inflammatory cytokine secretion and altered postprandial lipid fluxes (1). A growing body of evidence suggests that oxidative stress might be involved early in this pathological state. In white adipocytes, reactive oxygen species (ROS) production is mainly driven by NADPH oxidase (Nox) activity instead of xanthine oxidase or mitochondrial respiration (2). Moreover, a reduction in expression levels and activity of antioxidant enzymes, like superoxide dismutase (SOD), glutathion peroxidase (GPx) and catalase, was observed in obesity (2). This phenomenon suggests that greater formation of toxic byproducts, derived from lipid peroxidation and the protein carbonylation process, occurs during the synthesis of hydrogen peroxide or 4-hydroxy-2-nonenal (4-HNE). These byproducts impair adiponectin secretion (3) and increase cytokine production by mature adipocytes (2). Exposure to 4-HNE also promotes lipolysis (4) and induces insulin resistance in skeletal muscle (5, 6).

CoQ10 is a molecule at the crossroad of mitochondrial metabolism and reactive oxygen species detoxification. It is the most prevalent form of coenzyme Q in humans. It is ubiquitous and present under three different redox states: fully oxidized (ubiquinol- CoQ10_{ox}), fully reduced (ubiquinol - CoQ10_{red}) and the semiquinone radical (7). CoQ10 is mostly found in cellular membranes because of the lipophilic properties of the isoprenoid tail. Its biosynthetic pathway mainly occurs in mitochondria, where the condensation of the isoprenyl side chain with the benzoquinone nucleus is catalyzed by the COQ2 enzyme (8). This is the cellular compartment with the highest concentration, where it shuttles electrons from complexes I and II to the complex III during oxidative phosphorylation (7, 9). Moreover, CoQ10 has been shown to modulate the activity of uncoupling protein (9). However, CoQ10 was also found in the Golgi apparatus, the endoplasmic reticulum and plasma membrane (10). The extra mitochondrial CoQ10 pool is involved in plasma membrane electron transport catalyzed by NAD(P)H-dependent enzymes (11). Moreover the reduced isoform displays direct or indirect antioxidant properties by scavenging lipid peroxy radical or reducing α -tocopherol (12).

A previous publication has shown that a depletion of CoQ10 content was found in SC adipose tissue of obese subjects and mice (13). Overexpression of COQ2 in 3T3-F442A preadipocytes decreased the CoQ redox state and promoted ROS synthesis by the mitochondria (13). Interestingly, the adipogenic potential of these cells was almost completely blunted, suggesting an important role of CoQ content and redox state during adipogenesis and adipose tissue expansion. However, the variation in CoQ10 content and redox state in subcutaneous and omental adipose tissues has not been investigated in humans. Moreover, the potential contribution of these parameters to white adipose tissue dysfunction and obesity-related metabolic complications remains unclear.

In the present study, we have explored physiological variations in CoQ10 content and redox state in omental and subcutaneous adipose tissue of lean, overweight and obese women. We hypothesized that CoQ10_{red} content is lower in omental fat of obese individuals and is associated with lipid peroxidation. The loss of lipophilic antioxidant capacity of this molecule could be an important mechanism. We also postulate that this phenomenon could link visceral adipose tissue hypertrophy to systemic low grade inflammation and insulin resistance.

MATERIALS AND METHODS

Participants

Women undergoing elective hysterectomy (n = 29) without (n = 17) or with unilateral (n = 4) or bilateral salpingo-oophorectomy (n = 8) were recruited at the gynecologic unit of the Laval University Medical Center. Surgery was performed for the following reasons: menorrhagia/menometrorrhagia (n = 9), myoma/fibroids (n = 15), incapacitating dysmenorrhea (n = 1), pelvic pain (n = 1), benign cyst (n = 5), endometriosis (n = 2), pelvic adhesions (n = 1), hypermenorrhea (n = 1), excessive anemia-causing uterine bleeding (n = 1), endometrial hyperplasia (n = 2), or polyp (n = 2). All scientific protocols were approved by the Research Ethics committee of Laval University Medical Center. Subjects provided written informed consent before their inclusion in the study.

Adipose tissue sampling

Subcutaneous adipose tissue samples were collected at site of the surgical incision (lower abdomen) while omental samples were removed from the distal part of the greater omentum. Fresh samples were immediately carried to the laboratory and used for adipocyte isolation. A portion of each sample was flash frozen in liquid nitrogen and stored at -80°C.

Adipocyte isolation and cell sizing

Tissue samples were digested with collagenase type I in Krebs-Ringer-Henseleit buffer for 45 minutes at 37°C according to a modified version of the Rodbell method (14-16). Cell suspensions were filtered through nylon mesh and washed 3 times with Krebs-Ringer-Henseleit buffer. To determine adipocyte diameter, pictures of 250 cells were taken with a phase contrast microscope and analyzed with Scion Image software. The collagenase digestion was not performed for three samples because of the limited amount of tissue.

Measurement of body composition and adipose tissue distribution

The determination of body fat and lean body mass was performed by dual-energy x-ray absorptiometry (DEXA) using a Hologic QDR-2000 densitometer and the enhanced array whole-body software V5.73A (Hologic, Bedford, MA). Regional adipose tissue distribution was measured by computed tomography as previously

described (14, 17) using a GE Light Speed 1.1 CT scanner (General Electric Medical Systems, Milwaukee, WI). Briefly, the cross-sectional scan was performed at the L4-L5 vertebrae level in supine position with arms over the head. The exact scanning position was determined using a scout image. Adipose tissue areas of interest including visceral and subcutaneous adipose tissue areas, the latter included superficial and deep subcutaneous adipose tissues, were highlighted and computed with attenuation ranging from -190 to -30 Hounsfield units with ImageJ 1.33u software (National Institutes of Health, Bethesda, MD). Two obese volunteers exceeded the CT scanner field of view and only the visceral adipose tissue area was determined. One of these women also exceeded the upper limit of weight allowed for the DEXA table and did not undergo this test.

Quantification of lipid hydroperoxide levels in white adipose tissues

Lipid hydroperoxides (LPO) were quantified using a lipid hydroperoxide assay kit purchased from Cayman Chemical (Ann Harbors, Michigan, USA), performed according to supplier recommendations with minor modifications. Briefly, 50 mg of OM and SC adipose tissues were homogenized in methanol. Lipid extraction was achieved by adding 500 µl of water and 1 ml of chloroform. The mixture was centrifuged at 1500 x g and the organic phase was collected. An aliquot was used to perform the colorimetric reaction and the absorbance was read at 500 nm with a 96-well plate reader.

Quantification of coenzyme Q10 isoforms

The quantification of both isoforms was performed by reverse-phase HPLC with electrochemical detection as previously described (24). The HPLC-EC system is composed of a Gilson 307 pump, a Rheodine injector, an analytical column and an ESA Coulochem 2 Electrochemical Detector (Model 5200 A) with a Pentium II computer/controller with EuroChrom 2000 Integration Package. The analytical cell (ESA Model 5010- porous graphite) consisted of a series of two coulometric electrodes and was connected in series to the analytical column: the first electrode (E2) was for reduction of CoQs and the second electrode (E3) was for detection of these reduced coenzymes Q. The identification of the different forms were set by the chromatographic separation with an analytical column, a reverse-phase Hypersil BDS C18 column (4 mm x 25 cm, 5 µm bead). The mobile phase for the isocratic elution of internal standard from ChromSystem and CoQ10s containing sodium acetate,

acetic acid and 2-propanol, methanol and hexane, suited for EC detector. The standards of oxidized coenzyme Q10 (CoQ10) were obtained from Sigma. Identification and quantification of oxidized and reduced forms of CoQs were performed using in-house external standards. The concentration of the working solution was confirmed by measuring absorbance at a wavelength of 275 nm and by reference to known coefficients ($E_{1\text{cm}}^{1\%}$ 185 for CoQ9 and 165 for CoQ10). Then the solution of oxidized standard conditioned was reduced in loop to -1000 mV in the system pump-guard cell. The determination of the concentration of each form was compared with the QA program organized by the National Institute of Standard Technology (NIST) on lyophilized serums. Calibration curves were performed with mixed of 3 different working diluted solutions of CoQ10s stock solutions. Before injection, each standard solution was prepared as biological sample in propanol. A highly linear relationship was observed between the area of the peak (mV/min) and the molecular concentration ratios of each compound over a wide concentration from 10-15 nM to 3000 nM ($r = 0.99$). Acceptable repeatability was obtained for CoQs with a coefficient of variation below 5%, respectively 3.5 and 3.7% for CoQ10red and CoQ10ox. The limits of detection per injected quantity were 21 pmol for CoQred and 15 pmol for CoQox.

To performed CoQ10 extraction, frozen tissues (100 mg) were added to 0.9 ml of 2-propanol and homogenized with an Ultraturax blender. 100 μ l of this homogenate were mixed with 500 μ l of 2-propanol during 30 s and then centrifuged (10 000 trs/min for 3min). 50 μ l of the supernatant were directly injected in the system. This extraction procedure with only propanol was chosen because it was simple to perform and avoid oxidation of reduced coenzyme Q forms as it was demonstrated and validated by using various molecules well known to modify electrons flow at different levels of the respiratory chain. As expected, CoQ redox state was significantly decreased in the presence of antimycin and significantly increased in the presence of rotenone and CCCP. The details of the validation were reported by *Galnier and al.* (24). The total coenzyme Q10 pool was described as the sum of CoQ10_{ox} and CoQ10_{red} concentrations. Results were expressed as nmol/g of tissue. The redox state was calculated as: $[\text{CoQ10}_{\text{ox}}] / [\text{CoQ10}_{\text{Tot}}] \times 100$.

Estimation of daily dietary CoQ10 consumption

Dietary intake was assessed using a food frequency questionnaire validated by Goulet et al. (23) that was interviewer-administered by the same investigator within 2 weeks preceding or following the surgery, and was

structured to represent food habits throughout the previous month. The questionnaire was based on foods available in the Quebec City area and reflected Canadian food habits. The participants were questioned about the quantity and frequency intake of each item among different food groups: vegetables; fruits; legumes; nuts and seeds; cereals and grain products; milk and dairy products; meat/processed meat; poultry; fish; eggs; sweets; oil and fats; fast foods; and drinks. The content in CoQ10 by portion of each food groups was estimated using the data compiled by *Pravst and al.* (21) and is reported in supplementary Figure 1. The total daily consumption of CoQ10 was calculated as the sum each food source.

Statistical analyses

All tables shows mean \pm standard error of the mean. Each graph shows dots as a single observation and horizontal lines as the mean of the distribution. Differences among body weight categories and adipose tissue depots were analyzed by two-way ANOVA, followed by Tukey's post-hoc test. The stratification of CoQ10 content in the omental and subcutaneous depot was performed using a cutoff value of 14.0 and 15.0 nmol/g of tissue, respectively. These values were selected from a previous publication (13). Differences between women with low and high concentrations of CoQ10 were analyzed by Student's t-test or Mann-Whitney test. Multiple univariate Pearson's linear correlations were performed to find associations between adiposity measurements and the concentrations of the CoQ10 isoforms for both depots. Log10 and Box-Cox transformations were used for correlations with non-normally distributed residuals. Statistically significant correlations (in omental depot) between body fat distributions parameters and CoQ10 isoforms were adjusted for omental fat cell diameter to investigate the confounding effect of visceral fat hypertrophy. Statistical analyses were performed with the JMP 11.0 (SAS Institute, Cary, NC) and Graphpad Prism 6 (GraphPad, a Jolla, CA) software.

RESULTS

Volunteer characteristics

Twenty-nine Caucasian women aged between 40.4 and 55.5 years (mean of 48.1 ± 4.1) were recruited. The anthropometric and metabolic characteristics are shown in **Table 1**. They were characterized by a wide range of BMI values (from 21.5 to 51.3 kg/m²) with an average around the class I obesity threshold (30.0 ± 6.8 kg/m²). Of the 29 volunteers, 7 were lean (BMI < 25.0 kg/m²), 12 were overweight (BMI: 25.1 kg/m² to 30.0 kg/m²) and 10 were obese (BMI > 30.0 kg/m²). Twelve of the twenty-nine subjects were insulin resistant based on a HOMA-IR value greater than 2.5, but none of them had type 2 diabetes. This sample was specifically designed to identify cardiometabolic risk factors and adipose tissue dysfunction, features that relate to obesity and fat distribution in women.

Dietary CoQ10 consumption and smoking status

Using a food frequency questionnaire, we found that women included in our study consumed an average of 4.6 mg of CoQ10 per day with a range from 2.0 to 8.0 mg/day. BMI of the volunteers did not influence their daily consumption of CoQ10 from diet (**Supp. Fig 1A**). We also found that vegetal sources (oils, nuts, vegetables and fruits) were the main sources of CoQ10 in this French Canadian population (**Supp. Fig 1C**). In this study, CoQ10 consumption did not influence the total levels and redox state of this molecule in the omental (**Supp. Fig 2 A-C**) or the subcutaneous (**Supp. Fig 2 B-D**) adipose tissue compartment. Only 4 volunteers were smokers and were distributed among BMI subgroups. We did not observe any specific pattern of CoQ10 content or redox state in adipose tissues of these individuals (data not shown).

Regional variation of CoQ10 in obesity

We were able to quantitatively assess the levels of CoQ10_{ox} and CoQ10_{red} in 24 omental and 22 subcutaneous samples. We excluded data from 5 omental (overweight: n=3, obese: n=2) and 7 subcutaneous (lean: n=2, overweight: n=4, obese: n=1) samples because of the complete oxidation of the CoQ10_{red} isoform during the extraction process. We considered the CoQ10_{ox} levels as the total CoQ10 pool for these samples. However, only

women with available data of CoQ10_{ox}, CoQ10_{red} and total CoQ10 within the same depot were included in this set of analysis. We first analyzed the regional variation of CoQ10 content in lean, overweight or obese patients. We did not observe difference in total CoQ10 content between the omental and subcutaneous adipose tissue of women within the same BMI category (**Fig 1A**). However, we observed a depletion of the CoQ10 pool in omental adipose tissue of overweight and obese women, as well as in subcutaneous adipose tissue of obese women (BMI effect: $p = 0.001$) (**Fig 1A**). In lean individuals, we measured higher concentrations of the CoQ10_{red} isoform in the omental depot compared to subcutaneous tissue ($p = 0.003$) (**Fig 1B**). However, we found higher levels of CoQ10_{ox} isoform in subcutaneous adipose tissue of these women ($p = 0.01$) (**Fig 1C**). As expected, they displayed greater CoQ10 redox state in the subcutaneous depot (Depot effect: $p = 0.0003$) (**Fig 2A**), suggesting that omental and subcutaneous adipose tissues have distinct CoQ10 redox statuses in healthy volunteers. The content in CoQ10_{red} was decreased specifically in omental adipose tissue of obese women ($p = 0.007$) (**Fig 1B**). A reduction in CoQ10_{ox} concentrations was also observed in subcutaneous adipose tissue of overweight and obese volunteers (**Fig 1C**). Because of these modifications, regional differences in CoQ10 redox state were no longer present in obesity (**Fig 2A**).

CoQ10 redox state and oxidative stress in abdominal adipose tissues

For samples with valid CoQ10 redox state data, 16 omental and 18 subcutaneous fat samples were available for lipid hydroperoxides (LPO) levels quantification as a marker of oxidative stress. The omental depot had higher levels of LPO than the subcutaneous compartment (depot effect: $p < 0.0001$) (**Fig 2B**). We also observed a significant increase in the omental LPO content of obese women compared to lean and overweight volunteers ($p = 0.01$ and 0.02 respectively) (**Fig 2B**). A positive association was found between the CoQ10 redox state and LPO content in omental adipose tissue ($r = 0.67$, $p = 0.005$) (**Fig 2C**), supporting a role of the CoQ10 redox state in the regulation of redox status and oxidative stress in visceral fat. However, the association between the total CoQ10 and LPO levels was not statically significant in this sample of patients ($r = -0.41$, $p = 0.11$). LPO concentration in subcutaneous adipose tissue was similar for all groups (**Fig 2B**) and was not linked with the CoQ10 redox state (data not shown).

Influence of adipose tissue distribution and adipocyte diameter on CoQ10 content

Table 2 provides the Pearson and Spearman Rho values describing the correlations between adipose tissue areas measured by axial tomography and the abundance of CoQ10 isoforms in subcutaneous and omental adipose tissues. We found some significant negative associations between visceral ($r = -0.63, P = 0.0009$), subcutaneous ($r = -0.49, P = 0.02$) and total adipose tissue areas ($r = -0.58, P = 0.005$) with CoQ10_{red} levels in omental adipose tissue. Moreover, the total CoQ10 pool negatively correlated with visceral ($r = -0.50, P = 0.006$), superficial subcutaneous ($r = -0.40, P = 0.04$) and total ($r = -0.43, P = 0.02$) adipose tissue areas. The presence of the CoQ10_{ox} isoform in omentum was not associated with visceral or subcutaneous adiposity. The CoQ10 redox state of the visceral depot was also weakly associated with visceral adipose tissue area ($r = 0.37, P = 0.08$) and deep subcutaneous adipose tissue area ($r = 0.39, P = 0.07$). To test the hypothesis that visceral adipocyte hypertrophy is a major contributor to CoQ10 depletion in obesity, we adjusted the previous correlations for omental fat cell diameter. The association between omental CoQ10_{red} content and adipose tissue areas were no longer significant. Adjustment also abolished the relationship between the total CoQ10 pool and visceral adipose tissue area, although the correlation with subcutaneous adipose tissue areas remained statistically significant ($r = -0.40, P = 0.05$).

Metabolic effects of low adipose tissue CoQ10 concentration

In a previous study, the concentration of 13.26 nmol of CoQ10 per gram of subcutaneous adipose tissue was identified as a phenotypic threshold value below which 95 % of volunteers are obese (13). To quantify the metabolic effect of low and high CoQ10 content, we used a cutoff value of 14.0 and 15.0 nmol/g of subcutaneous and omental adipose tissues. This stratification generated two groups with either low or high total CoQ10 concentrations within the same tissue (**Fig 3A-C**). The women with low levels of CoQ10 in omental adipose tissue also displayed lower CoQ10_{red} concentrations ($p < 0.01$) (**Fig 3A**). The amount of CoQ10_{ox} (**Fig 3A**) and the redox state (**Fig 3B**) were not statistically different. Low CoQ10 content in the subcutaneous compartment was related to reduced CoQ10_{red} ($p = 0.02$) and CoQ10_{ox} ($p = 0.05$) (**Fig 3C**). The redox state (**Fig 3D**) was similar in these subgroups.

Table 3 compares the anthropometric and metabolic characteristics of women with low vs. high CoQ10 content in both tissues. As expected, volunteers with a lower amount of CoQ10 in the omental depot were more obese

and had higher fat mass and fat-free mass. We also found higher visceral and subcutaneous adipose tissue areas, without differences in the VAT/SAT areas ratio. Even if they were more obese, these volunteers did not display any alteration in plasma lipid levels. However, an increase in mean omental adipocyte diameter, higher IL-6 and CRP plasma levels and insulin resistance were observed. On the other hand, the group of women with lower CoQ10 content in subcutaneous adipose tissue was not characterized by higher adiposity or changes in body fat distribution. We did not observe any variation of the metabolic parameters except for the fasting glucose level, which was slightly higher in women with higher CoQ10 content. We did not find any statistically significant variation of insulin levels or HOMA-IR index in these subgroups.

DISCUSSION

Most of the studies that investigated the link between CoQ10 and cardiometabolic health have focused on plasma levels of this molecule. The circulating pool of CoQ10 is mostly found in high- and low-density lipoproteins, where it fulfills antioxidant roles (12). The redox state is often used as a marker of oxidative stress, and has been shown to be higher in patients with type 2 diabetes (20). In this study, we examined the regional variations of CoQ10 content and redox state in two adipose tissue depots. We showed for the first time that CoQ10 redox state was fundamentally different among the omental and subcutaneous adipose tissues of lean individuals, despite similar total content. Indeed, we found that the omental depot displayed lower redox state, which is mainly driven by the high content in CoQ10_{red}. In lean and healthy women, regional variations of these parameters were not linked to adipocyte hypertrophy or inflammation, suggesting physiological variation of CoQ10 redox status between the intra-abdominal and subcutaneous adipose compartments. We also ruled-out the contribution of CoQ10 from food as a major contributor of the adipose CoQ10 pool. Indeed, we established the daily consumption of CoQ10 between 2 to 8 mg/day our sample of the French Canadian population. These results are similar to a previous study that reported a consumption of 3 to 5 mg of CoQ10 per day in a sample of Danish (22). However, no association were found between the dietary intake and the adipose tissue content in CoQ10, suggesting that the intracellular levels were mainly derived from endogenous production.

We also observed that overweight volunteers had a reduced total CoQ10 pool in omental adipose tissue, without modification of the redox state or lipid peroxidation. However, obese women displayed lower concentrations of total CoQ10 with reduced levels of the antioxidant isoform (CoQ10_{red}) and greater LPO content. This indirectly suggests that the depletion in CoQ10 alone is not sufficient to trigger a sustained oxidative stress response. Nevertheless, the CoQ10_{red} levels and the lower redox state observed in obese women are closely related to LPO production in omental adipose tissue. We can hypothesize that depletion of the total CoQ10 pool measured in overweight volunteers may predispose omental adipocytes to oxidative damage in response to metabolic stress occurring mostly in obesity. Based on this assumption, a cohort of overweight patients with severe metabolic dysfunctions might also display impaired CoQ10 redox status and oxidative stress in the omental depot.

The loss of omental content in CoQ10 and CoQ10_{red} was strongly associated with both visceral and subcutaneous fat accumulation. Our results also suggest that hypertrophic remodeling of the visceral depot plays a critical role in this phenomenon. Moreover, we did not observe any difference in plasma lipids among women with low or high levels of CoQ10 in the omentum, suggesting that modification of total CoQ10 content is not related to dyslipidemia. However those participants with low CoQ10 content displayed higher IL-6 and CRP plasma levels, suggesting early adipose tissue dysfunction. This group of volunteers also displayed insulin resistance and hyperinsulinemia without dysglycemia. Taken together, our results suggest that CoQ10 depletion and oxidative stress might be involved in visceral adipose tissue dysfunction leading to insulin resistance and increased risk of developing type 2 diabetes.

Surprisingly, we did not find any difference in CoQ10 concentrations in the subcutaneous adipose tissues of overweight vs. obese individuals. A previous study found a reduction of CoQ10 levels in subcutaneous samples from obese (BMI from 31 to 40 kg/m²) and morbid obese (BMI over 40 kg/m²) men and women, compared to lean individuals (13). In that study, overweight patients displayed CoQ10 content that was similar to that of lean volunteers. The conclusion drawn from the latter investigation differs from this work, but could be explained by the nature of the population studied. Most of the women included in the present study had a BMI lower than 40 kg/m². The relation between mean adipocyte size and adiposity level is mostly linear in the subcutaneous depot, until it reaches a plateau in very obese women (reviewed in (18)). This suggests that most patients included in our investigation still had an important potential for hypertrophic remodeling of the subcutaneous depot despite their excess of body fat mass. If we assume that adipocyte hypertrophy has the same effect on CoQ10 in fat from all anatomical locations, it is possible that adipocyte dysfunction associated with CoQ10 depletion only occurs in females with very high BMI values. We observed a specific and significant reduction of the CoQ10_{ox} content in overweight and obese volunteers, without any variation of CoQ10_{red} levels or redox state. However, this decrease in CoQ10_{ox} levels was not related to lipid peroxidation. Moreover, the metabolic profile of participants with low vs. high CoQ10 content in subcutaneous adipose tissue is similar between groups. Despite variation in the absolute CoQ10_{red} and CoQ10_{ox} levels, we did not observe modifications in plasma lipid levels, insulin resistance and cytokine secretion. This suggests that the lipophilic antioxidant system in subcutaneous adipose tissue is not

a major factor influencing metabolic health of women with moderate obesity. Other antioxidant enzymes like catalase, manganese-dependent superoxide dismutase or glutathione peroxidase might be involved in the regulation of the intracellular redox status. Indeed, obese Zucker rats had reduced activity of these enzymes in the inguinal fat pad (2).

The CoQ10 redox state might have different physiological significance depending on the cellular localization on the CoQ10 pool in adipocytes. During mitochondrial respiration, the CoQ10_{ox} is reduced by complex I or II and carries electrons to complex III. At high mitochondrial membrane potential, a low CoQ10 redox state promotes ROS production. Indeed, higher levels of CoQ10_{red} in mitochondrial membranes promotes the formation of reactive semiquinone radicals at the Q-binding site of complex I. (Reviewed in (7)). The CoQ10 redox state also favors ROS production at the Q_o site of the complex III under proper circumstances. On the other hand, ROS derived from complex III are released in the intermembrane space (7). They can diffuse directly in the cytosol and may act as “signaling ROS” to trigger adaptive responses to oxidative stress. Primary cultures of skin fibroblasts isolated from patients harboring *COQ2* and *PDSS2* mutations displayed defects in ATP synthesis and oxidative damage (19). Otherwise, the CoQ10_{red} in other cellular membranes displayed antioxidant effects. The cross-sectional and retrospective nature of this study leads to several technical limitations. We were not able to specifically measure the CoQ10 content and redox state in separate cell compartments. Moreover the depletion of the CoQ10 pool might affect mitochondrial metabolism and ROS production only under specific metabolic conditions. We could not exclude that defects in mitochondrial function could occur in the postprandial state instead of fasting. Moreover the population studied only included a relatively small sample of Caucasian women from 40 to 55 years of age. These results cannot be extrapolated to cohorts of men, pediatric or adolescent obese individuals and other ethnic groups. Further studies should be conducted to clarify the importance of this phenomenon in the general population.

CONCLUSION

Hypertrophic remodeling of the omental fat depot is related to a smaller CoQ10 pool, which is mostly explained by loss of the antioxidant isoform (CoQ10_{red} - Ubiquinol). This phenomenon is associated with the presence of oxidative stress markers in this depot, systemic low-grade inflammation and insulin resistance. However, it was

not directly linked to dyslipidemia in this sample of healthy women, suggesting that modifications of the CoQ10 redox state and content might be an early mechanism leading to visceral fat dysfunction.

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FIGURE LEGENDS

Figure 1: Content in total (A), reduced (B) and oxidized (C) forms of coenzyme Q10 per gram of omental and subcutaneous fat. The horizontal bar is the mean of the distribution. #: $p \leq 0.10$ &: $p \leq 0.05$, && : $p \leq 0.01$ versus lean subjects, † : $p \leq 0.05$, ††: $p \leq 0.01$, OM versus SC adipose tissues. Dark grey and light grey dots respectively represent omental and subcutaneous adipose tissue samples. OM (n = 24), SC (n = 22).

Figure 2: Redox state of coenzyme Q10 (A) and lipid hydroperoxide levels (B) in the omental and subcutaneous compartment. Spearman correlation between lipid hydroperoxides levels, coenzyme Q10 redox state (C) and coenzyme Q10 content (D) in the omental depot. The horizontal bar is the mean of the distribution. &: $p \leq 0.05$ body weights versus obese, †: $p \leq 0.05$, ††: $p \leq 0.01$, †††: $p \leq 0.001$, OM versus SC adipose tissues. Dark grey and light grey dots respectively represent omental and subcutaneous adipose tissues samples. Redox state; OM: (n = 24), SC (n = 22). LPO; OM (n = 16), SC (n = 18).

Figure 3: Content in reduced, oxidized and total forms of coenzyme Q10 per gram of omental (A) and subcutaneous (C) fat in volunteers stratified by low or high total coenzyme Q10 content. Coenzyme Q10 redox state of the omental (B) and subcutaneous (D) depot was also determined. Data are means \pm standard error of the mean. * $p \leq 0.05$, ** $p \leq 0.01$; CoQ10_{red} content versus low CoQ10 groups, † $p \leq 0.05$; CoQ10_{ox} content low CoQ10 groups. White and grey bars respectively represent the oxidized and reduced isoform of coenzyme Q10. Low CoQ10 content group: OM (n = 9) and SC (n = 6). High CoQ10 content group: OM (n = 15), SC (n = 16)

TABLES

Table 1: Anthropometric characteristics (n=29)

Variables	Mean ± SD	Range (min-max)
Age and anthropometrics		
Age (yrs)	48.1 ± 4.1	40.4-54.5
Weight (kg)	76 ± 17	53-133
Height (cm)	159 ± 5	151-170
BMI (kg/m ²)	30.0 ± 6.8	21.5-51.3
Waist circumference (cm) ^a	97.2 ± 15.5	72.5-147.0
Total body fat mass (kg) ^a	28.8 ± 8.9	12.4-50.4
Total lean body mass (kg) ^a	43.8 ± 6.1	29.3-55.2
% fat ^a	37.6 ± 5.8	23.3-48.6
Adipose tissue areas (cm²)		
Total ^b	448 ± 151	192-773
Subcutaneous ^b	340 ± 128	129-555
Visceral	111 ± 64	44-266
Adipocyte diameter (µm)		
Subcutaneous ^a	104.0 ± 11.4	77-131
Omental ^b	90.7 ± 14.8	65-117
Cholesterol (mmol/l)^a		
Total	5.2 ± 1.1	3.2-6.9
VLDL	0.5 ± 0.2	0.4-0.6
LDL	3.3 ± 0.9	1.3-4.7
HDL	1.4 ± 0.3	0.8-2.1
Triglycerides (mmol/l)^a		
Total	1.4 ± 0.6	0.7-2.5
VLDL	0.9 ± 0.5	0.3-1.8
LDL	0.2 ± 0.1	0.1-0.6
HDL	0.2 ± 0.1	0.2-0.3
Glucose homeostasis		
Glucose (mmol/l)	5.9 ± 0.7	4.8-7.1
Insulin (µU/ml)	11.4 ± 4.4	3.3-45.3
HOMA-IR	2.4 ± 1.3	0.7-13.7

^an=28, ^bn=27

Table 2: Correlations between omental and subcutaneous adipose tissue content in CoQ10 and adipose tissue areas measured by computed tomography.

	Adipose tissue areas (cm ²)				
	Total	Visceral	Subcutaneous	Deep subc.	Superficial subc.
Omental content					
CoQ10 _{red}	-0.58**	-0.63***	-0.49*	-0.51*	-0.49*
CoQ10 _{ox}	0.25	0.14	0.26	0.33	0.21
CoQ10 _{tot}	-0.43*	-0.50**	-0.35 ^{&}	-0.34 ^{&}	-0.40*
% CoQ10 _{ox}	0.30	0.37 ^{&}	0.29	0.39 ^{&}	0.25
Omental content adjusted for omental adipocyte size					
CoQ10 _{red}	-0.18	-0.16	-0.33	-0.30	-0.30
CoQ10 _{ox}	-0.28	-0.18	-0.19	-0.23	-0.19
CoQ10 _{tot}	-0.30	-0.29	-0.40*	-0.40*	-0.37 ^{&}
% CoQ10 _{ox}	-0.05	-0.02	0.04	0.01	0.03
Subcutaneous content					
CoQ10 _{red}	-0.07	-0.19	0.01	-0.04	-0.04
CoQ10 _{ox}	-0.19	-0.12	-0.18	-0.21	-0.27
CoQ10 _{tot}	-0.15	0.03	-0.21	-0.21	-0.16
% CoQ10 _{ox}	-0.00	0.18	-0.11	-0.04	-0.05

[&] $p \leq 0.10$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$

Table 3: Anthropometric and metabolic parameters of women with low versus high CoQ10 content in omental and subcutaneous adipose tissues.

	Omental adipose tissue ^a			Subcutaneous adipose tissue ^b		
	Low CoQ10 _{tot} (n = 11)	High CoQ10 _{tot} (n = 18)	<i>p</i>	Low CoQ10 _{tot} (n = 12)	High CoQ10 _{tot} (n = 17)	<i>p</i>
Anthropometrics						
BMI (kg/m ²)	34.7 ± 2.4	27.2 ± 0.9	0.002	29.6 ± 1.8	30.4 ± 1.8	0.60
Fat mass (kg)	34.8 ± 2.6	25.5 ± 1.8	0.009	29.7 ± 2.9	28.2 ± 2.1	0.69
Fat-free mass (kg)	47.1 ± 1.6	41.9 ± 1.4	0.02	42.7 ± 1.8	44.6 ± 1.5	0.44
Percentage of fat (%)	40.9 ± 1.3	35.7 ± 1.4	0.01	39.0 ± 1.4	36.5 ± 1.6	0.26
Adipose tissue areas (cm²)						
Total	554.7 ± 45.4	394.3 ± 30.6	0.01	428.1 ± 37.4	461.4 ± 42.3	0.56
Visceral	159.4 ± 21.5	81.8 ± 8.3	0.002	102.3 ± 18.5	117.6 ± 15.6	0.60
Subcutaneous	409.2 ± 37.2	305.2 ± 29.1	0.04	321.5 ± 32.6	352.6 ± 35.3	0.52
Deep subc.	215.7 ± 21.1	155.8 ± 17.8	0.04	171.5 ± 19.2	181.5 ± 21.5	0.73
Superficial subc.	205.2 ± 20.2	156.2 ± 12.5	0.06	174.8 ± 15.1	171.1 ± 16.6	0.87
Cholesterol (mmol/L)						
Total	4.9 ± 0.3	5.3 ± 0.3	0.30	5.3 ± 0.3	5.0 ± 0.2	0.52
LDL	2.8 ± 0.3	3.1 ± 0.2	0.35	3.0 ± 0.3	2.9 ± 0.2	0.74
HDL	1.4 ± 0.1	1.6 ± 0.1	0.25	1.6 ± 0.1	1.5 ± 0.1	0.50
Triglycerides (mmol/L)	1.4 ± 0.5	1.3 ± 0.1	0.39	1.4 ± 0.2	1.3 ± 0.1	0.58
Glucose homeostasis						
Glucose (mmol/L)	6.0 ± 0.2	5.8 ± 0.2	0.45	5.6 ± 0.1	6.1 ± 0.2	0.05
Insulin (μU/ml)	15.0 ± 3.3	9.2 ± 1.0	0.04	9.7 ± 1.2	12.6 ± 2.3	0.42
HOMA-IR	4.1 ± 1.0	2.4 ± 0.3	0.04	2.5 ± 0.3	3.5 ± 0.7	0.31
Adiponectin and cytokines						
Adiponectin (pg/ml)	10.9 ± 1.4	10.9 ± 1.1	0.99	9.8 ± 1.3	11.6 ± 1.1	0.31
IL-6 (pg/ml)	1.3 ± 0.2	0.8 ± 0.1	0.03	0.9 ± 0.2	1.1 ± 0.1	0.18
CRP (mg/ml)	3.5 ± 0.9	1.3 ± 0.3	0.007	1.9 ± 0.7	2.4 ± 0.6	0.56
Adipocyte diameter (μm)						
Omental	98.5 ± 4.4	85.2 ± 3.4	0.03	90.9 ± 4.2	89.5 ± 4.2	0.81
Subcutaneous	108.8 ± 4.4	100.5 ± 1.8	0.14	101.1 ± 1.3	105.3 ± 3.6	0.28

^aCut-off value of 15 nmol/g of adipose tissue, ^bCut-off value of 14 nmol/g of adipose tissue

FIGURE 1

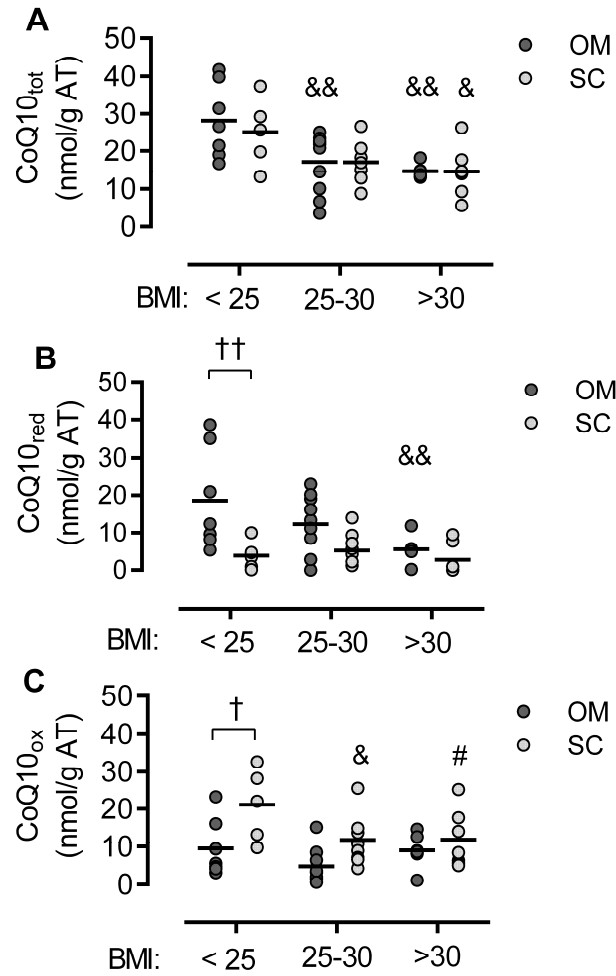


FIGURE 2

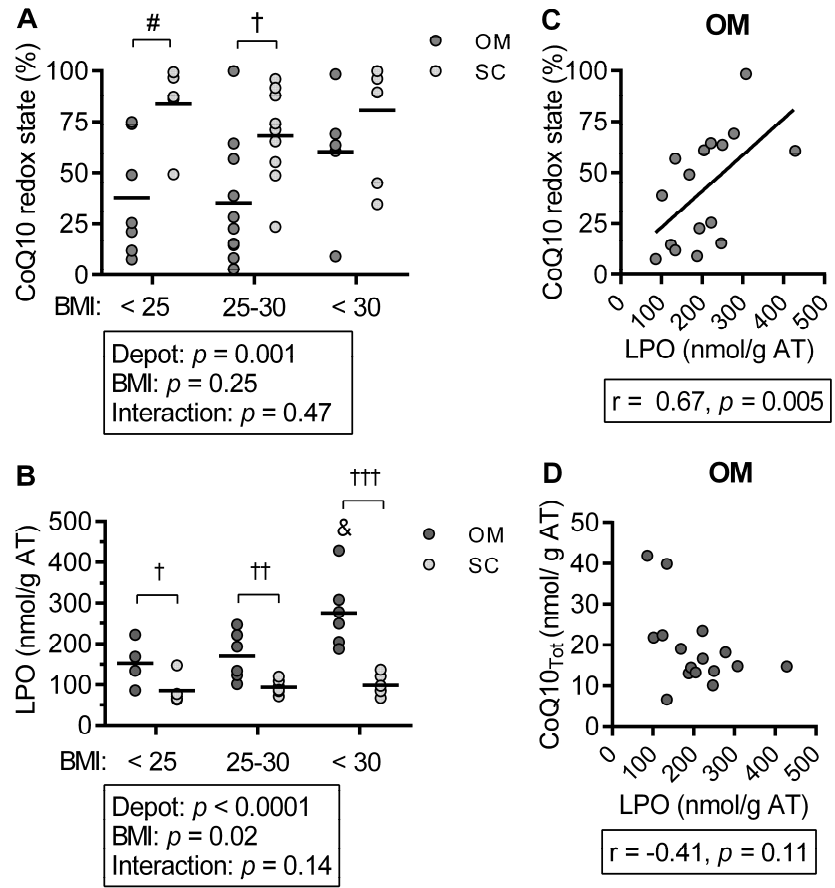


FIGURE 3

