Changes in fat mass after initiation of maintenance dialysis is influenced by the uncoupling protein 2 exon 8 insertion/deletion polymorphism

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Abstract

Background. A high body mass index (BMI) has been reported to confer a survival advantage in end-stage renal disease (ESRD) patients. On the other hand, body fat accumulation, especially visceral adipose tissue, is an important risk factor for cardiovascular disease, as well as a clinically important source of adipokines. Uncoupling protein 2 (UCP2) uncouples respiration from ATP synthesis, thus regulating energy expenditure and fat oxidation. In this longitudinal cohort study, we investigated the impact of the UCP2 insertion/deletion (ins/del) polymorphism on body composition changes in ESRD patients starting dialysis.

Methods. A total of 222 incident Caucasian ESRD patients (mean age 53 ± 12 years; 60% males) were investigated close to the start of dialysis with peritoneal dialysis (PD; n = 126) or haemodialysis (HD; n = 96), and again after about 1 year (n = 159). Genotyping of the UCP2 ins/del polymorphism was performed in the patients and in 207 healthy controls. Dual-energy X-ray absorptiometry was conducted at baseline and after 1 year to monitor body composition.

Results. While HD patients and PD patients with the ins/del genotype did not display any changes in body composition, the 48 PD patients with the del/del genotype that completed follow-up had a significant increase; ΔBMI (0.7 ± 1.8 kg/m²), Δbody fat mass (3.5 ± 3.8 kg) and Δtruncal fat mass (1.7 ± 1.2 kg). In a multiple linear regression analysis, the del/del genotype was an independent predictor of the increase in truncal fat mass in PD patients (F-ratio = 7.99, P < 0.05) together with age and diabetes mellitus.

Conclusions. PD patients, but not HD patients, with the UCP2 del/del genotype showed a significant increase in total and truncal fat mass during the first year of dialysis therapy, suggesting a possible role for UCP2 in dissipating the excess energy of a high-glucose environment.

Keywords: body composition; dialysis; fat mass; polymorphism; UCP2

Introduction

Many patients on peritoneal dialysis (PD) show increased amounts of total body fat and intra-abdominal fat after initiation of dialysis [1–3]. While haemodialysis (HD) patients with higher body mass index (BMI) are reported to enjoy a survival advantage [4], data regarding the protective effect of overweight in PD patients have been conflicting [5–7]. However, BMI is not a precise marker of nutritional status and does not accurately reflect body composition. Therefore, it is not clear if the improved survival at higher BMI in HD patients is a result of greater amounts of adipose tissue or of increased lean body mass [8]. Accumulation of body fat, especially visceral adipose tissue, maybe be an important cardiovascular risk factor, as adipose tissue is a source of adipokines, such as IL-6, TNF-α and leptin [9], that have been shown to have adverse effects on vascular health.

Uncoupling protein 2 (UCP2), which uncouples respiration from ATP synthesis by providing an alternative route for the protons to enter into the mitochondrial matrix and also functions as a transporter of fatty acids into the mitochondrial matrix may play an important role in regulating the energy balance by participating in both resting energy expenditure and fatty acid oxidation [10]. The UCP2 insertion/deletion (ins/del) polymorphism is a 45 bp insertion in exon 8 in the 3’ untranslated region of the
UCP2 gene that may affect UCP2 mRNA stability, post-transcriptional modification, or translation, all of which could result in altered levels or activity of the mature protein [11]. The ins/del genotype, when compared with del/del and ins/ins genotypes, has been shown to be associated with an increased basal metabolic rate, increased 24 h energy expenditure and decreased BMI in Pima Indians [11]. Furthermore, we have previously shown in a small group of PD patients that with a similar energy intake and physical activity, the del/del genotype is associated with a higher body weight at baseline and a more marked gain in fat mass during 12 months of PD than is the ins/del genotype [12].

In the present study, we aimed to increase the validity of this finding by investigating the effect of the UCP2 ins/del polymorphism on body composition changes in a larger cohort of incident patients starting either HD or PD.

Patients and methods

Study populations

A total of 222 Caucasian end-stage renal disease (ESRD) patients (134 males, 60%) with a mean age of 54 ± 13 years were evaluated shortly before the initiation of renal replacement therapy (RRT). The patients were all a part of an ongoing prospective study [13], where incident patients between 18 and 70 years of age starting RRT and without overt inflammation, infection or liver disease are included. We selected all patients in this cohort where DNA was available, then analysed these patients post hoc and followed them retrospectively for a period of 12 months or until death or transplantation (range 2–12 months). The causes of ESRD were chronic glomerulonephritis in 72 patients (32%), diabetic nephropathy in 68 patients (31%), polycystic kidney disease in 22 patients (10%) and other or unknown causes in 60 patients (27%). As genetic controls, we also included 207 unrelated Caucasians living in the Stockholm area (62% males; mean age 40 ± 1 years). The study protocol was approved by the Ethics Committee of the Karolinska Institutet at Karolinska University Hospital Huddinge, and informed consent was obtained from all patients and control subjects.

Laboratory methods

Biochemical analyses used are original measurements from the ongoing prospective study. These were taken after an overnight fast, using venous blood samples for genotyping of the UCP2 ins/del polymorphism and analysis of blood lipids (cholesterol, triglycerides and HDL cholesterol), blood glucose, insulin, markers of inflammation (IL-6 and hsCRP) and adipocytokines (leptin and adiponectin). Most analyses were done using routine methods at the Department of Clinical Chemistry, Karolinska University Hospital Huddinge. Plasma IL-6 levels were measured by a high-sensitivity photometric enzyme-linked immunosorbent assay (ELISA) (Boehringer Mannheim, Mannheim, Germany). Serum leptin levels were analysed with a commercially available RIA kit (Linco Research, Inc., St. Charles, MO). Adiponectin levels were measured by a high-sensitivity photometric ELISA (Boehringer Mannheim, Mannheim, Germany). Inflammatory status was defined as a hsCRP ≥ 10 mg/l. Insulin resistance was estimated using the homeostatic model assessment (HOMAIR): HOMA IR = fasting blood glucose (mmol/l) × fasting insulin (μIU/ml)/22.5. Glomerular filtration rate (GFR) was estimated as the mean of creatinine and urea clearance from a 24 h collection of urine and corrected for body surface area.

Genotyping

All patients and genetic controls were genotyped for the ins/del variation in the UCP2 gene. This polymorphism consists of a 45 bp ins or del in the 3′-untranslated region of exon 8. The ins variant contains a duplication of a fragment shown in upper case as follows: 5′-CCCTCTTTCCCAAGCTCTTT CCTTTTCCGCTTACCTACCTCTT-3′. The polymorphic region was amplified with polymerase chain reaction (PCR) using forward primer 5′-CAG TGA GGG AAG TGG GAG G-3′ and reverse primer 5′-GGG CAGGAA CGAAGATTCC-3′, yielding products of 457 and 502 bp for the deletion and insertion, respectively. The PCR products were separated on 1% ethidium bromide stained agarose gels and photographed under ultraviolet transillumination.

Dual-energy X-ray absorptiometry (DEXA)

Body composition was measured by DEXA at baseline and after about 1 year follow-up. It was performed with a DPX-L machine (Lunar Corp., Madison, WI, USA), and the data were evaluated using Lunar Software version 3.4.

Nutritional status assessment and definition of cardiovascular disease (CVD)

Subjective global nutritional assessment (SGA) was used to evaluate the overall protein-energy nutritional status. SGA included six subjective assessments, three that were based on the patient’s history of weight loss, incidence of anorexia and incidence of vomiting, and three that were based on the subjective grading of muscle wasting, the presence of oedema and the loss of subcutaneous fat. Based on the three consecutive assessments, each patient was given a score that reflects the nutritional status as follows: 1 = normal nutritional status, 2 = mild malnutrition, 3 = moderate malnutrition and 4 = severe malnutrition. For the purpose of this study, malnutrition was defined as a SGA > 1. BMI was defined as the weight in kilograms divided by the square of the height in metres. Patients who had a history of or clinical signs of cerebrovascular, cardiovascular and/or peripheral vascular disease at the time of inclusion were defined as having clinical CVD.

Statistical analysis

Patients were classified according to initial dialysis modality. Change of body composition and survival analysis were assessed in an intention-to-treat analysis during follow-up.
Variables were given as mean ± SD unless stated otherwise. Allele frequencies were compared between populations using Chi-square test. The non-parametric Wilcoxon rank sum test was used to evaluate differences between two groups. Comparisons between two groups of nominal variables were made with Fisher’s exact test. Paired t-test was used to analyse the changes in the same group of patients. Spearman’s correlation was used to analyse the correlation between two variables. Independent associations with log transformed changes in fat mass were evaluated using a linear regression model incorporating all factors associated with changes in fat mass in univariate analysis. All analyses were carried out with SAS statistical software version 8.2 (SAS Inc., Cary, N.C., USA). A two-tailed P-value of <0.05 was considered significant.

Results

Baseline characteristics

Among the 222 ESRD patients enrolled, 96 (43%) patients started HD and 126 (57%) started PD. Baseline characteristics of these patients, grouped according to genotype, are summarized in Table 1. Briefly, there were no significant differences in any studied parameter between those patients who started on HD and those who started on PD.

Association of baseline truncal fat mass with clinical and biochemical markers

As expected, total and truncal fat mass was positively associated with leptin ($\rho = 0.65$ and 0.62; $P < 0.001$, respectively), hsCRP ($\rho = 0.17$ and 0.20; $P < 0.05$, respectively), IL-6 ($\rho = 0.13$ and 0.17; $P < 0.05$, respectively), serum triglycerides ($\rho = 0.15$ and 0.16; $P < 0.05$, respectively) and HOMA index (in non-DM, $n = 158$; $\rho = 0.33$ and 0.33; $P < 0.001$, respectively) and negatively associated with adiponectin ($\rho = -0.27$ and $-0.32$; $P < 0.01$, respectively). Truncal, but not total, fat mass also correlated negatively with HDL-cholesterol ($\rho = -0.15$; $P < 0.05$).

Genotype distribution

The genotype distribution was similar in patients and healthy genetic controls, as well as between PD and HD patients, and the allele frequencies of this polymorphism satisfied the Hardy-Weinberg equilibrium law in all three groups. The frequencies of the del/del, ins/del and ins/ins genotypes were 56.8, 36.7 and 6.5%, respectively in ESRD patients and 54.6, 36.2 and 9.2% in controls. Basic characteristics including age, gender and proportion of DM, CVD, inflammation and residual renal function were similar between the ins/ins, ins/del and del/del genotypes in HD and PD patients (Table 1).

Association between changes of body composition and the UCP2 ins/del genotype in HD and PD patients

Patients were evaluated again after about 12 months of study. At that time, 159 patients (72%) remained in the study, while 28 (13%) had received a kidney transplant and 29 (14%) had died (six patients were lost due to other causes). Table 2 shows that patients who did not complete follow-up were more often diabetics and had more inflammation than those who completed the study. Of the 159 patients who completed the study, only the 48 PD patients with del/del genotype significantly altered their body composition due to an increase in truncal fat mass ($1.7 ± 1.2$ kg; $P < 0.001$) (Figure 1) and an associated increase in BMI ($0.7 ± 1.8$ kg/m$^2$; $P < 0.05$). All of the HD-patients, regardless of genotype, as well as PD-patients with the ins/del and ins/ins genotypes did not change their
body fat stores significantly during the study period (Table 3).

During the study, lean body mass decreased significantly in both HD and PD patients and the extent of decrease was not associated with UCP2 genotype. However, the extent of changes of lean body mass correlated negatively with that of truncal fat mass \( (n = 158, \rho = -0.28, \ P < 0.001) \) and body fat mass \( (n = 158, \rho = -0.22, \ P < 0.01) \).

**Predictors of changes of truncal fat mass in PD**

A linear regression model, incorporating all factors significantly associated with changes of truncal fat mass, showed that besides age and DM, the UCP2 genotype was an independent predictor of the change of truncal fat mass during the first year of PD therapy (Table 4).

**Discussion**

The present study investigated the impact of a UCP2 polymorphism and dialysis modality on body composition changes during the first year of RRT. Our study confirms our previous observation [12] in a smaller group of PD patients that the del/del genotype is significantly and independently associated with increased fat mass accumulation during the first year.
of dialysis therapy. This may suggest that genetic factors play an important role in the accumulation of fat mass in PD patients. The present study also extends our previous findings by showing that fat mass did not change significantly in HD patients. We speculate that continuous intraperitoneal glucose absorption provides an energy-rich environment in PD patients, thus stimulating glycogen storage and glucose oxidation while suppressing fat oxidation, and that this exposes PD patients to the increased propensity for accumulating fat mass.

The observed effect of UCP2 on body composition change is likely due to its role in the regulation of energy metabolism. UCP2 dissipates the energy stored in the proton gradient across the mitochondrial inner membrane as heat and this basic proton leak is associated with increased resting metabolic rate [14].

UCP2 mRNA levels in adipose tissue were found to be positively related to resting metabolic rate, which accounts for 60–70% of 24 h energy expenditure [15]. The UCP2 45-base-pair ins/del in the non-coding region of exon 8, investigated in the present study, has previously been found to be positively associated with energy expenditure [11]. In addition, numerous data in animals and humans have suggested that the function of UCP2 in the skeletal muscle and adipose tissue may be involved in the regulation of lipids as fuel substrate [16,17]. Results regarding the regulation of UCP2 expression by glucose are conflicting [18–21]. An increase in dietary fat and serum fatty acid can lead to consistent elevations in UCP2 expression in skeletal muscle and several other organs/tissues [22,23]. Furthermore, the associations found between the polymorphism in UCP2 and significant reductions in lipid oxidation in humans are consistent with the proposed role for UCP2 in the regulation of lipids as fuel substrates [24]. Taken together, these findings suggest that UCP2 is related to energy expenditure and fat oxidation, and could, therefore, be involved in the development of obesity [10,25,26].

In this context, it is interesting that a higher BMI has been reported to confer a survival advantage in HD patients [4], while the relationship between higher BMI and survival is less evident among patients on PD [5–7]. We speculate that increased truncal fat mass
in PD patients may partially explain this difference. As fat mass accumulation reflects deposition of surplus energy, it is not surprising that the well-nourished patients had more fat mass than the malnourished patients in our study. Abundant energy stores may provide a survival advantage during intermittent catabolic events in the ESRD population. On the other hand, visceral fat mass, often approximated as truncal fat mass, is the fat tissue depot considered to be the most metabolically active and has been identified as a key factor in the development of malnutrition, insulin resistance and premature atherosclerosis by secreting adipokines and proinflammatory cytokines [27]. In agreement with many previous reports, our results show that the increase in truncal fat mass accounts for the main part of the increase in total fat mass. Truncal fat mass was positively associated with serum leptin, IL-6, hsCRP, insulin resistance and dyslipidaemia and negatively associated with adiponectin levels. An increased fat mass in PD patients may thus have adverse metabolic consequences.

Muscle wasting is a common phenomenon in ESRD patients and is associated with increased morbidity and mortality [28]. Subclinical inflammation is likely an important component of the pathophysiology of muscle wasting [29]. Recent evidence suggests a possible role for IL-6 in this process through stimulation of muscle protein breakdown and by affecting appetite and eating behaviour [30]. Adipose tissue is estimated to account for as much as 20% of systemic IL-6 concentration and is a potential source of elevated IL-6 in obese ESRD patients [31,32]. Therefore, fat mass accumulation may aggravate muscle wasting in ESRD patients. Our results are consistent with this theory, as the changes of body fat mass, especially truncal fat mass, was inversely correlated with changes in lean body mass.

Some shortcomings of the present study should be discussed. First, although DEXA has been shown to be superior to other simple non-invasive methods to determine body composition in ESRD patients, it should be noted that the estimation of lean body mass may be confounded by changes in hydration status. However, we have demonstrated previously that changes in hydration status could not explain changes in lean body mass over time [1]. Second, it should be pointed out that this is a post hoc analysis, which may limit the value of the study. Third, only a subset (72%) completed the 12 months follow-up period. As expected, these patients were significantly less inflamed and more seldom diabetics than the patients who dropped out. Finally, the limited number of patients may limit the power of the study.

In conclusion, the present study confirms that the exon 8 ins/del polymorphism of the UCP2 gene can influence fat mass accumulation after the initiation of PD in patients carrying the del/del genotype. We suggest that this may be of clinical importance as fat mass has been shown to be a metabolically active organ with links to inflammation, vascular health and insulin resistance.

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