Activities of lipogenic enzymes in subcutaneous adipose tissue are not increased in patients with chronic kidney failure

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ABSTRACT

Since renal replacement therapy has started to be a routine procedure in chronic kidney disease (CKD), patients no longer die of end-stage renal disease (ESRD). Today, patients with CKD live longer and the most important causes of morbidity and mortality in this group are cardiovascular events. Lipid abnormalities, such as hypertriglyceridemia (HT), are an important factor of high cardiovascular risk in this group. It is known that HT is partially caused by inhibition of lipolysis, but it is also postulated that increased lipogenesis is another cause of HT. Previous studies performed in our center has provided evidence that lipogenesis is increased in the animal model of ESRD.

OBJECTIVES The aim of this study was to investigate the activities of lipogenic enzymes in the subcutaneous white adipose tissue in patients with CKD.

PATIENTS AND METHODS The study was performed on 36 patients (17 women and 19 men). Patients with ESRD were divided into 2 groups: patients on conservative treatment in the prehemodialysis period (pre-HD group, n = 18) and patients on maintenance hemodialysis (HD group, n = 18). The control group consisted of 22 patients without ESRD. The activities of lipogenic enzymes in the subcutaneous white adipose tissue (fatty acid synthase, adenosine triphosphate citrate lyase, malic enzyme, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase) were assessed by spectrophotometry.

RESULTS There were no statistically significant differences in the activities of lipogenic enzymes in a fat tissue sample between patients with ESRD and the control group.

CONCLUSIONS The results did not confirm increased lipogenesis in patients with ESRD.

INTRODUCTION Various metabolic abnormalities of lipids, carbohydrates, and proteins are observed in patients with chronic kidney disease (CKD). Most of these abnormalities are typical for stage 5 CKD but some are observed in the earlier stages. They participate in the development of atherosclerosis and are responsible for high mortality in patients with CKD. The pathogenesis of metabolic disorders is not completely understood and is probably secondary to the retention of uremic toxins, abnormal electrolyte homeostasis, acidosis, or some hormonal disturbances. Compared with the general population, mortality rates among patients with CKD are significantly higher mainly due to cardiovascular disease (CVD). Morbidity and mortality rates associated with CVD are higher in this group compared with age- and sex-matched groups and the progression of disease is faster. About 40% of patients with stage 5 CKD experienced CVD and, consequently, age-adjusted cardiovascular mortality in these patients is 10- to 30-fold higher than in the general population. CVD accounts also for increased hospitalization rates and high cost of healthcare in this group.

Atherosclerosis is observed in the majority of patients with CKD on conservative and on renal replacement therapy (RRT). Advanced atherosclerosis is not only the main cause of mortality
but also a contraindication for renal transplantation. Numerous factors contribute to atherosclerosis in stage 5 CKD. Some of these factors are the same as observed in the general population (e.g., arterial hypertension, glucose intolerance) while others are specific for CKD (e.g., metabolic acidosis, hyperphosphatemia, or hyperparathyroidism). Hypertriglyceridemia (HT) is the most common type of dyslipidemia in CKD and is observed in up to 60%–70% of patients with stage 5 CKD in the predialysis period, patients on maintenance hemodialysis (HD), and patients after renal transplantation. \(^\text{5,10,11}\) HT is not corrected during RRT \(^\text{5,10,11}\); therefore, it seems to be one of the key risk factors for atherosclerosis and probably accounts for high cardiovascular morbidity. HT is also known to be a risk factor for renal failure. \(^\text{5,11}\)

HT results from a defective removal of triglycerides (TG) caused by low plasma lipolytic activity, which has been proved in previous studies on animals and humans. \(^\text{14-16}\) The issue of whether HT could also be caused by increased de-novo synthesis of TG (lipogenesis) is still controversial. \(^\text{17}\) Studies on lipogenesis yielded contradictory results. \(^\text{18,19}\) We observed an increase in the activity of lipogenic enzymes in our previous experimental studies, \(^\text{5,19,20}\) but the results have not been confirmed in humans.

Lipogenesis in patients with stage 5 CKD can be affected by uremic toxins, inadequate food supply, hormonal disorders, or a number of drugs. Lipogenesis is active mainly in the liver and adipose tissues. In the present study, human adipose tissue was used due to the limited availability of liver specimens.

**Patients and Methods**

**Patients** The study was performed on 36 patients with stage 5 CKD treated in the Department of Nephrology, Transplantology and Internal Diseases, Medical University of Gdańsk, Poland. Patients with CKD were classified into 2 groups: patients on conservative treatment in the pre-HD period (pre-HD group, \(n = 18\)) and patients on maintenance HD (HD group, \(n = 18\)). There were 17 men (10 in the pre-HD and 7 in the HD group) and 19 women (8 in the pre-HD and 11 in the HD group). Patients from the pre-HD group were prepared for RRT and started HD from 1 week to 3 months after the adipose tissue had been collected and biochemical tests had been done. Patients from the HD group were on HD at least 2 months (up to 8 years). There were no patients on peritoneal dialysis. Chronic primary glomerulonephritis was the most common cause of CKD and was observed in 12 patients (7 in the pre-HD and 5 in the HD group), 8 patients had interstitial nephritis (3 in the pre-HD and 5 in the HD group); 4 patients had polycystic kidney disease diagnosed as primary nephropathy (all in the HD group). In the remaining patients, the cause of CKD could not be established.

The control group consisted of 22 previously healthy patients who underwent trauma surgery at the Department of Surgery, Medical University of Gdańsk. None of the patients had proteinuria or kidney failure.

None of the patients either in the control or CKD groups had diabetes mellitus, glucose intolerance, or a history of familial hypercholesterolemia. None of the patients received statins or steroid treatment.

Lipogenic enzyme activity was measured in the subcutaneous white adipose tissue collected during vascular access operation (in patients with CKD) or during the upper limb surgery (in the control group). The vascular access in patients with CKD was performed on the forearm or arm with native vessels. In the control group, tissue samples were also obtained from the forearm or arm. The samples were taken from all patients staying at the surgery unit who met the inclusion criteria and signed consent during the study period.

**Enzyme activity assay** One gram of the white adipose tissue was placed in 8 ml ice-cold 20 mmol/l Tris-Cl buffer (pH 7.8) containing 0.2% TritonX-100. The tissue was minced finely with scissors, homogenized manually with a Teflon-pestle homogenizer, and centrifuged at 30,000 g for 20 minutes. The resulting supernatant was decanted, and the pellet was resuspend in 5 ml isolation medium, rehomogenized, and centrifuged as above. The resulting supernatant was combined with the one obtained after the first centrifugation and used for the enzyme assay. The fatty acid synthase, adenosine triphosphate citrate lyase, malic enzyme and hexose monophosphate dehydrogenases (glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) were assayed as described previously. \(^\text{21}\)

Blood samples for biochemical tests (creatinine, cholesterol, TG, total protein, and albumin levels) were collected from fasting patients in the morning before the surgery. The parameters were measured using routine methods in our diagnostic laboratory. \(^\text{22}\)

In dialysis patients, \(Kt/V\) was used to quantify HD treatment adequacy. \(Kt/V\) was calculated in these patients every month as a routine procedure. In this study, we analyzed the last \(Kt/V\) calculated before the surgery.

The statistical analysis was performed using STATISTICA 8.0. Data are expressed as mean values ± standard deviation). The Shapiro-Wilk test was used to determine normal distribution. Intergroup differences were assessed by the Mann-Whitney, Wald-Wolfowitz, and Kolmogorov-Smirnov tests. A \(P\) value less than 0.05 was considered statistically significant.

**Results** There was no significant difference in the mean age between the CKD and control groups. Hypertension was diagnosed in 77% of the patients in the HD group, 88% of those in the pre-HD group, and 63% of the controls.
The results of enzyme activity measurement is presented in Table 2. Two findings are crucial. First, the activity of the studied enzymes was low. Low activity is typically observed in healthy population but in our study it was observed also in patients with CKD. Second, there were no significant differences between patients with CKD and the control group, in fact there was no increase in the activity of lipogenic enzymes. There was also another interesting finding, namely, the activity of all enzymes was higher in the HD group compared with the pre-HD group. The difference was statistically significant for 6-phosphogluconate dehydrogenase ($P < 0.05$).

Patients on HD had significantly lower weight than patients on conservative treatment ($P = 0.028$) and controls ($P = 0.009$). The overall characteristics and biochemical data of all 3 groups are presented in Table 1.

Creatinine and $\text{Kt/V}$ Patients with stage 5 CKD had higher creatinine levels compared with the control group. Patients in the HD groups had good dialysis adequacy. The mean $\text{Kt/V}$ was $1.348 \pm 0.28$ with the mean protein catabolic rate of $1.238 \pm 0.419 \text{ g/kg/d}$.

Total protein and albumin Total protein levels were lower in the pre-HD group compared with the control and HD groups ($P = 0.0006$ and $P = 0.03$, respectively). Albumin levels were higher in the control group compared with the pre-HD group and HD group ($P = 0.0005$ and $P = 0.00001$, respectively). Hypoalbuminemia was detected in 61.5% and hypoproteinemia in 25% of the patients in the pre-HD group.

Lipids There were no significant differences in cholesterol concentrations between the control and CKD groups. In pre-HD and HD groups, TG concentrations were higher than in the control group ($P = 0.01$ and $P = 0.0005$, respectively). There were no differences in TG levels between the pre-HD and HD groups although HT was more common in the HD group.

**Discussion** The aim of this study was to investigate the differences in the activity of lipogenic enzymes between patients with CKD and healthy individuals. Half of the patients with CKD had already been on HD and half were scheduled...
for dialysis. Of all study patients with CKD, 48% of had TG levels above 2.0 mmol/l and 56% had hypercholesterolemia (total cholesterol level >5.2 mmol/l). According to the available data, overt dyslipidemia is frequently detected in late stage of CKD. The most common abnormality is HT, which was first described by Bagdade et al. in 1968. HT is observed in up to 60%–70% of patients with glomerular filtration rate below 15 ml/min. Hypercholesterolemia is less common and is detected in 20%–30% of patients with CKD. Patients with HT have increased TG levels in very-low-density lipoprotein, intermediate-density lipoprotein, and low-density lipoprotein fractions of lipoproteins. TG levels are decreased in the high-density lipoprotein fraction, but the proportion of TG to other lipids (e.g., cholesterol) is relatively increased. Some other lipid disturbances can be observed before overt dyslipidemia is detected, namely, reduced apolipoprotein A-I and A-II, increased lipoprotein(a), apolipoprotein CIII, and significantly lower apoA-I/apoC-III ratio. These abnormalities form a strong atherogenic pattern, which can explain why patients without overt dyslipidemia present atherosclerotic lesions at a relatively early stage of renal failure.

There is no doubt that dyslipidemia occurs in uremic patients. A reduced removal of TG due to decreased activities of lipoprotein and hepatic lipases has been proved in several studies. There is still controversy regarding the role of lipogenesis in the development of HT in CKD. The available studies have yielded contradictory results. In 1978, Bagdade et al. showed that the activity of acetyl-CoA carboxylase was decreased; on the other hand, a study performed in 1982 showed that the activity of lipogenic enzymes was nonsignificantly increased. Our previous studies on animals suggested that lipogenesis could be increased in stage 5 CKD. In the present study, we measured the activity of lipogenic enzymes in patients with stage 5 CKD and in the control group. In the pre-HD group, 61.5% of the patients had hypoalbuminemia and 25% had hypoproteinemia suggesting malnutrition. The higher levels of total protein and albumin in patients on HD indicated that they were better nourished than patients on conservative treatment.

There were no differences between patients with CKD and controls and our results did not confirm increased lipogenesis in stage 5 CKD. This finding suggests that a decreased elimination of TG is most probably the only cause of HT in stage 5 CKD. Nevertheless, our study have several limitations that have to be acknowledged.

First, the only human tissue that can be easily collected except blood is the adipose tissue. Although lipogenesis is present in the adipose tissue, its activity is relatively lower than that in the liver. It is also known that the activity of lipogenic enzymes is lower in humans compared with rats. The adipose tissue is a relatively large organ and probably can play an important role in lipogenesis but it is not an ideal tissue for experimental studies.

Second, we collected only the subcutaneous tissue from the upper limb during vascular access surgery. It has not been confirmed that this tissue has the same importance and activities as the visceral adipose tissue. Yet studies on the subcutaneous adipose tissue have some advantages, e.g., lipogenesis in this tissue has the same activity irrespective of sex, while in the visceral adipose tissue it is more active in men. We excluded patients on peritoneal dialysis, and it is possible that there are some changes in the activity of lipogenic enzymes in this group of patients.

Third, our patients had advanced disease and metabolic abnormalities could depend not only on uremic toxins but also on some secondary changes, such as inadequate food intake due to lack of appetite, metabolic acidosis, drugs, etc. The rate of lipogenesis is regulated by glucose supply and could be affected by diet, which in many cases is insufficient. In our study, patients with stage 5 CKD had low levels of total protein and albumin, which is typical for malnutrition. Of note, these abnormalities were more advanced in pre-HD patients. Despite this fact, lipogenesis was not decreased. We can only speculate that there are 2 opposite factors affecting the rate of lipogenesis—low glucose supply and uremic toxins.

This hypothesis can be partially supported by comparing the activity of enzymes between pre-HD and HD patients. As mentioned before, the activity of several enzymes was lower in the pre-HD group. The activity of 2 enzymes (i.e., malic enzyme and 6-phosphogluconate dehydrogenase) showed even a significant difference between the 2 groups. This suggests that there is a dialyzable toxin compound accumulating in stage 5 CKD patients, which may inhibit the activity of lipogenic enzymes.

Considering the above issues, it is worth to perform further studies, preferably with the use of the visceral adipose tissue, to elucidate the role of lipogenesis in the development of HT in patients with stage 5 CKD.

In summary, in the present study, the activity of lipogenic enzymes in the adipose tissue of patients with stage 5 CKD was not increased. These results are in contrast with the previous studies on rats. This discrepancy could be explained, for example, by the difference in metabolism and food intake between rats and humans. Nevertheless, the rate of lipogenesis in humans is so low that this process probably has no effect on HT in CKD, especially nowadays, when patients with stage 5 CKD rarely experience severe metabolic complications of uremia.

We hope that further studies will help develop new therapies to lower the mortality rates in patients with CKD.
REFERENCES


Aktywności enzymów związanych z lipogenezą nie są podwyższone u pacjentów z przewlekłą niewydolnością nerek

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lipogeneza,
miażdżyca, przewlekła choroba nerek, terapia nerkozastępcza

STRESZCZENIE

WPROWADZENIE Od czasu, gdy leczenie nerkozastępcze stało się rutynową terapią w leczeniu przewlekłej choroby nerek (PChN), pacjenci nie umierają z powodu schyłkowej niewydolności nerek (SNN). Chorzy z SNN żyją obecnie coraz dłużej i głównym czynnikiem wpływającym na chorobowość i śmiertelność w tej grupie są powikłania sercowo-naczyniowe. Jednym z istotnych czynników zwiększających ryzyko sercowo-naczyniowe w tej grupie są zaburzenia lipidowe, takie jak hiperlipidemia (HT). Wiadomo, że za powstanie hiperlipidemii odpowiada częściowo zahamowanie lipolizy, ale postuluje się także udział zwiększonej lipogenezy w rozwoju HT. Badania wykonane wcześniej w naszym ośrodku potwierdziły zwiększenie lipogenezy na zwierzęcym modelu z SNN.

CELE Celem przedstawionego badania była ocena aktywności enzymów związanych z lipogenezą w podskórnej białej tkance tłuszczowej u pacjentów z PChN.

PACJENTI I METODY Badanie przeprowadzono na 36 chorych (17 kobietach i 19 mężczyznach). Chorzy z SNN byli podzielni na dwie grupy – chorych leczonych zachowawczo w okresie przed rozpoczęciem hemodializ (grupa pre-HD, n = 18) oraz przewlekle hemodializowanych (grupa HD, n = 18). Grupę kontrolną stanowiły 22 osoby bez niewydolności nerek. Aktywności enzymów związanych z lipogenezą w podskórnej tkance tłuszczowej (syntazy kwasów tłuszczowych, liazy ATP-cytrynianowej, enzymu jabłczanowego, dehydrogenazy glukozo-6-fosforanowej oraz dehydrogenazy 6-fosfoglukonianowej) oznaczono techniką spektrofotometrii.

WYNIKI Nie stwierdzono istotnych statystycznie różnic w aktywnościach enzymów lipogenezy w po- branej tkance tłuszczowej pomiędzy osobami z SNN a grupą kontrolną.

WNIOSKI Wyniki badania nie potwierdziły zwiększonej aktywności lipogenezy u pacjentów z SNN.