Liver fat, visceral adiposity, and sleep disturbances contribute to the development of insulin resistance and glucose intolerance in nondiabetic dialysis patients


Departments of 1Nephrology, 2Radiology, 3Biochemistry, 4Biomathematics, School of Medicine, University of Thessaly, Larissa; 5Department of Sport Science, University of Thessaly, Trikala; 6Institute for Human Performance and Rehabilitation CE.RE.TE.TH, Trikala, Greece; and 7Institute of Biomedical Research and Technology, CE.RE.TE.TH, Larissa, Greece

Submitted 31 December 2007; accepted in final form 23 September 2008

Insulin resistance (IR) is generally described as an impaired ability of plasma insulin to adequately promote tissue glucose disposal. It is frequently, but not invariably, accompanied by hyperinsulinemia and an impaired glucose tolerance. In chronic renal failure, IR (37) and glucose intolerance (19) are very common in nondiabetic hemodialysis patients, secondary to renal disease. IR is present in almost all patients with end-stage renal disease; however, only 30–50% will finally manifest an impaired glucose tolerance (37). In addition, IR significantly contributes to the increased cardiovascular morbidity and mortality in these patients (60). Both disorders are considered to be caused by uremia (12), a notion strongly supported by the fact that renal replacement therapy and renal transplantation significantly improve uremia and ameliorate IR and glucose intolerance (31). Nevertheless, the pathogenic mechanisms underlying these abnormalities of glucose metabolism in renal disease and uremia have not been fully elucidated.

Main target organs for insulin action and, therefore, sites responsible for IR, are the liver, skeletal muscles, and adipose tissue (58). Reduced responsiveness to insulin in these tissues would lead to IR and later on to glucose intolerance (47). In individuals with normal renal function, various factors have been implicated in the development of IR including an excess of intrahepatic fat (22), skeletal muscle’s fat infiltration (17, 18), an increased central obesity (16) and visceral adiposity (4), the degree of malnutrition (62), poor physical activity levels (20), and, recently, sleep disorders (45, 49). All of the above factors or symptoms also have been shown to be important determinants of the quality of life (QoL). A strong body of evidence suggests that IR results from the accumulation of intracellular lipid metabolites in target organs as, for example, reported in studies of both humans (30) and knockout animals (42).

In the past, we have shown that hemodialysis patients suffer from muscle atrophy (54) and weakness (25, 26), exhibit increased levels of intramuscular fat content (26, 57), and have increased functional capacity and low daily physical activity levels (24). Moreover, hemodialysis patients also suffer from sleep disorders (46, 55) and show increased levels of visceral adiposity. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Springer
adiposity and central obesity (43, 56). It seems, therefore, that factors involved in the development of IR and glucose intolerance in nonrenal failure populations, are present and might even be compounded under uremic conditions. The relationship between all of these contributing factors and their role in the development of IR and glucose intolerance in hemodialysis patients has not yet been clearly ascertained. Notably, there are no data available assessing intrahepatic fat content in relation to IR and glucose intolerance in hemodialysis patients.

The aims of the present study were 1) to measure intrahepatic, intramuscular, visceral, and total body fat content to examine the possible role of the adipose tissue in the development of IR and glucose intolerance in a group of nondiabetic hemodialysis patients; 2) to evaluate sleep disorders, nutritional status, muscle size, and physical activity levels to investigate how parameters related to the QoL may predispose to IR; 3) to assess the differences in the above parameters between the hemodialysis patients with normal and the hemodialysis patients with disturbed glucose tolerance; and 4) to develop a model for predicting glucose intolerance in stable nondiabetic hemodialysis patients.

METHODS

Subjects. Hemodialysis patients were recruited from the dialysis unit at the University General Hospital of Larissa, Greece. Entry criteria included being on chronic hemodialysis for 6 mo or more with dialysis delivery (KT/V) > 1.1. Patients were excluded if they had diabetes mellitus as well as reasons for being in a catabolic state, such as hyperthyroidism, active vasculitis, malignancies, HIV and opportunistic infections, or inflammations that required intravenous antibiotics within 3 mo prior to enrollment, since those conditions are known to affect muscle size, body composition, and functionality. The causes of renal failure in the participants were glomerulonephritis (10), polycystic kidneys (9), renovascular disease (4), hypertension (5), and unknown reasons (6). None of our patients were alcoholic or consuming more than 5 units of alcohol per week. All patients gave informed consent prior to study participation. The study was approved by the Ethics Committee on Human Research at the University Hospital of Larissa, Greece.

Study design. Patients participated in an all-night in-hospital polysomnographic session for the assessment of sleep quality and quantity, followed the next morning by a computed tomography (CT) scan, oral glucose tolerance test (OGTT), body composition assessment [dual energy X-ray absorptiometry (DEXA)], and a series of functional tests, before their dialysis session. Routine monthly laboratory results were also recorded. A single-pool Kt/V was calculated from pre- and postdialysis blood urea nitrogen measurements using the Daugirdas II equation (10).

IR. Glucose tolerance was assessed using a 75-g, 2-h OGTT (2) by measuring the area under the glucose curve (AUC\text{Glucose}) using a trapezoidal integration. Insulin sensitivity indices were calculated using the insulin sensitivity index by Matsuda et al. (IS\text{Matsuda}) (39), the oral glucose insulin sensitivity (OGIS) index (38), the quantitative insulin-sensitivity check index (QUICKI) (29) as well as the homeostasis assessment model of IR (HOMA-IR) (14) (Table 1). Glycosylated hemoglobin (HbA1c) was also measured using in hospital routine clinical laboratory procedures.

Body composition. Whole body, regional fat, and lean body mass were measured by a DEXA system (Lunar model DPX Madison, WI). Post hoc regional analysis was performed as previously described (36). The waist-to-hip ratio (WHR) was calculated as waist circumference at midway between the iliac crest and the lowest margins of the ribs over the hip circumference at the maximum circumference of buttocks. An average of three readings of each measurement was taken for the calculation of the WHR. Visceral and subcutaneous adipose tissue of the abdominal areas at the level of L4-L5 lumbar area, was assessed by analyzing images collected by a CT system (model Tomoscan SR5000; Philips) (35).

Fat infiltration. Intrahepatic fat accumulation was assessed by the same CT system at the level of T12-L1 vertebrae using the ratio of mean liver-to-spleen attenuation (11). Muscle cross-sectional area and intramuscular fat (extramyocellular lipids) accumulation were assessed by images collected using the same CT system by collecting six images 2-cm apart at the larger girth of the right thigh for each patient as previously described (32).

Image analysis of the CT slices was performed using a customized software program written in Interactive Data Language (Research Systems, Boulder, CO). This software, based on variations in signal intensity, allowed for the quantification of fat (visceral and subcutaneous adipose tissue), muscle, liver, and miscellaneous components (connective tissue, fascia, intramuscular or intrahepatic fat) expressing the data as area (cm²) and percentage of tissue composition (%fat, %muscle, or %liver, and %miscellaneous) (26, 56).

Functional capacity. Functional capacity was assessed by a battery of tests including the North Staffordshire Royal Infirmary (NSRI) walk test, the time to perform five sit-to-stand cycles, the number of sit-to-stand cycles achieved in 60 s, and the time to walk a distance of 6.06 meters (20 ft) at normal (slow walk) and fast pace. These tests are described in detail elsewhere (56).

Nutritional status. The nutritional status of the patients was assessed using the seven-point subjective global assessment scale. The validity and reliability of this method of nutritional assessment has been previously reported in dialysis patients (15).

Polysomnography. Polysomnograms (Somnoscreen; Somnomedics, Randersacker, Germany) were collected overnight at the Sleep Disorders Laboratory of University General Hospital of Larissa, as previously reported (9). Sleep stages and arousals were determined using standard criteria (59a). Obstructive apnea was defined as the presence of chest/abdominal wall motion in the absence of airflow for at least 10 s in duration. Hypopnea was defined as: 1) a reduction in airflow signal amplitude of at least 50% compared with baseline, 2) the presence of chest/abdominal wall motion, and 3) oxygen desaturation of hemoglobin by 4% or with an arousal. The respiratory disturbance index was equal to the sum of the number of hypopneas and obstructive and mixed apneas (apneas with both central and obstructive components) per hour of sleep. The arousal index was defined as the total number of arousals in sleep, divided by the total sleep time. The oxygen desaturation index was defined as the average of desaturation events per hours of recording. Desaturation events were defined as O₂ saturation < 90% for > 15 s. Sleep stages and arousals were determined using standard criteria (59a).

Questionnaires. The questionnaire materials were completed with the interview method by experienced personnel (G. K. Sakkas, C. D. Giannaki, C. Karatzafieri). QoL was assessed by using the Sort Form Questionnaire 36 (SF36 QoL) adjusted and validated in dialysis patients (40). The Zung Self-Rating Depression Scale was used to assess levels of depression. This questionnaire is very sensitive to the early signs of depression and has been previously used in hemodialysis patients (41).

Biochemical assessment. Biochemical examination was performed at the ClinLab of the University Hospital under routine procedures (including glucose, insulin, C-reactive protein, parathyroid hormone, vitamin D, blood urea nitrogen). Monthly laboratory results were recorded for all hemodialysis patients participating in our study.

Statistical analysis. The Spearman Rank Correlation Test was used to assess the relationship between the IS\text{Matsuda} and the examined variables. Moreover, to compare the two groups (normal glucose tolerant vs. impaired glucose tolerant) for differences in the fat accumulation (visceral, intrahepatic, intramuscular, total body), body composition, sleep quality and quantity, as well as functional capacity and QoL, unpaired t-tests for continuous normally distributed vari-
Table 1. Patient characteristics presented as pool data and divided in NGT and IGT groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pool Data</th>
<th>Spearman Rank Correlation to ISI Matsuda</th>
<th>NGT</th>
<th>IGT</th>
<th>P Values*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr *</td>
<td></td>
<td></td>
<td>19</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>18–74</td>
<td></td>
<td>7/12</td>
<td>4/11</td>
<td>0.05</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.1±4.1</td>
<td>r = −0.527, P = 0.01</td>
<td>25.8±3.9</td>
<td>26.4±3.9</td>
<td>0.06</td>
</tr>
<tr>
<td>KTV*</td>
<td>1.3±0.5</td>
<td>r = 0.135, P = 0.45</td>
<td>1.1±0.3</td>
<td>1.4±0.5</td>
<td>0.05</td>
</tr>
<tr>
<td>Years in Dialysis</td>
<td>2.5±1.5</td>
<td>r = −0.006, P = 0.70</td>
<td>2.2±1.2</td>
<td>2.9±1.8</td>
<td>0.20</td>
</tr>
<tr>
<td>SGA, A/B/C</td>
<td>29/50</td>
<td>r = −0.175, P = 0.48</td>
<td>13/60</td>
<td>14/10</td>
<td>0.05*</td>
</tr>
<tr>
<td>Glucose disposal assessment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting plasma glucose, mg/dl</td>
<td>91.9±12.7</td>
<td>r = −0.684, P = 0.01</td>
<td>86.0±8.3</td>
<td>98.5±13.8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Plasma glucose at 120 min, mg/dl</td>
<td>140.9±48.0</td>
<td>r = −0.445, P = 0.01</td>
<td>107.9±20.0</td>
<td>178.3±32.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Fasting plasma insulin, μU/ml</td>
<td>9.3±9.5</td>
<td>r = −0.815, P = 0.01</td>
<td>8.9±12.2</td>
<td>9.7±5.6</td>
<td>0.88</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.1±0.5</td>
<td>r = 0.12, P = 0.51</td>
<td>4.9±0.4</td>
<td>5.4±0.5</td>
<td>0.01</td>
</tr>
<tr>
<td>AUC_Glucose (0–120 min) mg·m²·min⁻¹</td>
<td>146.3±31.7</td>
<td>r = −0.565, P = 0.01</td>
<td>125.8±17.7</td>
<td>172.3±26.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>G0/I0 ratio</td>
<td>14.8±9.3</td>
<td>r = 0.770, P = 0.01</td>
<td>15.7±10.3</td>
<td>13.8±8.2</td>
<td>0.53</td>
</tr>
<tr>
<td>ISI Matsuda Index</td>
<td>9.2±7.0</td>
<td>r = 0.428, P = 0.01</td>
<td>10.7±7.0</td>
<td>7.7±8.4</td>
<td>0.25</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.2±2.3</td>
<td>r = −0.856, P = 0.01</td>
<td>2.1±2.8</td>
<td>2.4±1.5</td>
<td>0.60</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.35±0.0</td>
<td>r = 0.864, P = 0.01</td>
<td>0.36±0.0</td>
<td>0.35±0.0</td>
<td>0.14</td>
</tr>
<tr>
<td>OGIS, ml·min·m⁻²</td>
<td>431±64</td>
<td>r = 0.896, P = 0.01</td>
<td>456±59</td>
<td>400±58</td>
<td>0.01</td>
</tr>
<tr>
<td>Hypertension prevalence</td>
<td>7 (20%)</td>
<td></td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>4 (12%)</td>
<td></td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ankle brachial index</td>
<td>1.2±0.2</td>
<td>r = −0.287, P = 0.26</td>
<td>1.2±0.2</td>
<td>1.2±0.2</td>
<td>0.54</td>
</tr>
<tr>
<td>Body Composition (DEXA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LB/M weight, kg/m</td>
<td>27.8±4.5</td>
<td>r = −0.135, P = 0.68</td>
<td>28.6±5.4</td>
<td>25.9±4.8</td>
<td>0.60</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>26.1±12.0</td>
<td>r = −0.212, P = 0.41</td>
<td>29.7±9.6</td>
<td>22.7±11.1</td>
<td>0.14</td>
</tr>
<tr>
<td>Trunk fat/height, kg/m</td>
<td>5.6±3.0</td>
<td>r = −0.492, P = 0.05</td>
<td>5.8±2.2</td>
<td>6.6±3.2</td>
<td>0.53</td>
</tr>
<tr>
<td>WHR</td>
<td>1.0±0.1</td>
<td>r = −0.580, P = 0.01</td>
<td>1.0±0.1</td>
<td>1.0±0.0</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Data are mean ± SD. NGT, the normal glucose tolerance group; IGT, the impaired glucose tolerance group; BMI, body mass index; KTV, dialysis efficiency; ISI, insulin sensitivity index; SGA, subjective global assessment; HbA1c, glycosylated hemoglobin; AUC, area under the OGTT curve; G0/I0, fasting glucose-to-fasting insulin ratio; HOMA-IR, homeostasis model assessment for insulin resistance; QUICKI, quantitative insulin-sensitivity check index; OGIS, oral glucose insulin sensitivity; OGTT, oral glucose tolerance test; LBM, lean body mass; Trunk fat, fat in kg calculated from DEXA analysis; WHR, waist-to-hip ratio. *Age and KTV were included as a covariate in the analysis. The Spearman Rank correlation test was used to assess the relation between the ISI Matsuda index and the examined variables. An unpaired t-test was used for assessing the differences between the two groups.

‘Glucose disposal assessment’ and ‘Body Composition (DEXA)’ were measured, and the χ²-test for categorical variables, were used. Two-tailed P values < 0.05 were considered statistically significant. Age and dialysis efficiency indices (KTV) were added as a covariate for all the comparisons between the two groups.

To predict the probability of glucose intolerance in stable nondiabetic hemodialysis patients, a logit model was fitted. The quality of the biological markers was evaluated independently using a receiver operator characteristics (ROC) curve analysis. The ROC curve analysis was applied for the identification of the IR. The quality of biological markers was assessed based on the AUC metric. For each ROC curve, a cutoff point was determined as the value of the parameter that maximizes the sum of specificity and sensitivity, equally weighting their significance.

All statistical analyses were performed using commercially available statistical analysis software (SPSS 15.0). Reported data are means ± SD and percentage (%) values.

RESULTS

Patient characteristics, dialysis compliance, glucose disposal indices, and body composition parameters, are shown in Table 1. Briefly, 45 hemodialysis patients went through the screening process for this study. Eleven hemodialysis patients were diabetic and, therefore, were not eligible to participate in the study. Thirty-four hemodialysis patients were found eligible to participate, and they underwent a 2-h, 75-g OGTT examination to determine the glucose tolerance as well as various indices of IR [ISI_Matsuda, OGIS, QUICKI, HOMA-IR, fasting glucose-to-fasting insulin ratio (G0/I0)]. Patients were divided into two groups according to their OGTT status: the normal glucose tolerance (NGT) group with the 2-h postload glucose levels < 140 mg/dl (7.8 mmol/l, n = 19, 7 females, ages 47 ± 18, postload glucose 108 ± 20 mg/dl) and the impaired glucose tolerance (IGT) group with the 2-h postload glucose values > 140 mg/dl but < 200 mg/dl (11.1 mmol/l, n = 15, 4 females, ages 58 ± 11, postload glucose 178 ± 33 mg/dl), as shown in Table 1. Data are also presented as pool data and are correlated with the ISI_Matsuda index as shown in Tables 1–3.

The groups were found to differ statistically in age and KT/V, and, therefore, those values were used as covariable in all the comparisons between the two groups. By definition the groups differed in many of the IR and glucose intolerance-related parameters (Table 1). Body mass index correlated with WHR (r = 0.412, P = 0.02) and %total body fat (DEXA) (r = 0.711, P = 0.001). KTV correlated moderately with the Respiratory Disturbance Index (r = −0.403, P = 0.02), the AUC_Glucose with OGTT (r = 0.438, P = 0.01), and weakly with intrahepatic lipids (r = 0.392, P = 0.02). VAT correlated strongly with ISI_Matsuda (r = −0.686, P = 0.01). Our patients received weekly doses of EPO 50 U/kg body wt as standard care (NGT Hct 38.7 ± 3.2% vs. IGT Hct 37.8 ± 2.9%, P = 0.95). C-reactive protein levels were within the normal range for both groups (NGT, 0.37 ± 0.9 vs. IGT, 0.55 ± 0.5 mg/dl, P = 0.94). Groups did not differ, and none of our patients were found to be vitamin D deficient (NGT, 40.8 ± 85 ng/ml vs. IGT, 51.2 ± 114 ng/ml, P = 0.77) or having uncontrolled hyperparathyroidism (NGT, 373 ± 191 vs. IGT, 276 ± 178 pg/ml, P = 0.18). All patients were receiving 1-(OH)
cholecalciferol as standard care up to three times weekly (≈3 μg/wk).

None of the above parameters were correlated significantly with the ISI_matsuda index.

**Body composition.** Parameters of body composition and post hoc regional analysis of DEXA images did not differ statistically between the NGT and IGT groups. Trunk fat assessed by DEXA and WHR correlated significantly with ISI_matsuda index (Table 1).

Visceral adiposity assessed by CT was found to be increased in IGT patients compared with NGT patients, while the size of thigh muscles did not differ between the two groups (Table 2).

**Fat infiltration.** Although content of the intrahepatic lipids was 10-fold increased in the IGT group compared with the NGT group, the thigh intramuscular lipid content (known also as extramyocellular lipids) did not differ between the two groups. The ISI_matsuda index did not correlate significantly with extramyocellular lipids, while there was a week correlation with intrahepatic lipids content (Table 2).

**Functional capacity and QoL.** In the pool data, the ISI_matsuda correlated significantly with the NSRI functional test time performance (Table 2). Moreover, the ISI_matsuda correlated significantly with the depression score and the QoL Total and Vitality scores (Table 2). Functional capacity, assessed by a battery of five tests did not differ between the two patient groups. The QoL score was reduced by 18% in the IGT patients compared with their NGT counterparts (Table 2).

**Polysomnography.** The majority of the parameters examined during the sleep assessment did not differ between the two groups (Table 2). However, the %oxygen saturation during sleep was (P = 0.04) lower in the IGT group compared with the NGT group. The ISI_matsuda correlated significantly with %oxygen saturation in the pool data set (Table 2).

**Probability model and ROC analysis.** The probability model for the prediction of the glucose intolerance of the hemodialysis patients indentified four parameters to fit the model significantly: %oxygen saturation during sleep, visceral adipose tissue over total adipose tissue, QoL and %intrahepatic lipids (Fig. 1; Table 3). The area under the ROC curve of the intrahepatic fat content and the AUCGlucose 0–120 value were significantly larger than the area under the diagonal reference line (Fig. 2). The cutoff points for the diagnosis of IR were > 3.97% for intrahepatic fat (sensitivity 100, specificity 35.7) and > 181.37 mg·dl⁻¹·min⁻¹ for AUCGlucose 0–120 (sensitivity 100, specificity 66.7) as shown in Table 4.

**DISCUSSION.** Our results demonstrated that the excess of intrahepatic fat deposition in hemodialysis patients is an important contributor to the development of IR and glucose intolerance. Forty-four percent of the non-diabetic hemodialysis patients examined in this study were found to have abnormal glucose tolerance. Four parameters, the increased fat deposition in the liver, the in-

### Table 2. Polysomnographic evidence, CT analysis, functional capacity, and quality of life parameters presented as pool data (both groups) and divided as NGT and IGT groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Pool Data</th>
<th>Spearman Rank Correlation With ISI Matsuda</th>
<th>NGT</th>
<th>IGT</th>
<th>P Values*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polysomnography</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sleep duration, min</td>
<td>303.4±78.2</td>
<td>r = 0.274, P = 0.15</td>
<td>315.2±86.8</td>
<td>289.1±66.5</td>
<td>0.40</td>
</tr>
<tr>
<td>Sleep efficiency, %</td>
<td>69.1±18.8</td>
<td>r = 0.203, P = 0.28</td>
<td>72.1±20.5</td>
<td>65.9±16.7</td>
<td>0.19</td>
</tr>
<tr>
<td>RDI</td>
<td>11.5±18.3</td>
<td>r = -0.362, P = 0.04</td>
<td>15.2±23.1</td>
<td>7.1±8.3</td>
<td>0.13</td>
</tr>
<tr>
<td>Arousal index, events/h</td>
<td>31.7±22.7</td>
<td>r = -0.051, P = 0.77</td>
<td>30.1±23.8</td>
<td>33.6±21.9</td>
<td>0.85</td>
</tr>
<tr>
<td>%O2 saturation during sleep</td>
<td>94.1±2.1</td>
<td>r = 0.551, P = 0.01</td>
<td>94.8±1.9</td>
<td>93.2±2.1</td>
<td>0.04</td>
</tr>
<tr>
<td>ODI, events/h</td>
<td>19.9±27.2</td>
<td>r = -0.437, P = 0.02</td>
<td>21.9±33.7</td>
<td>17.6±18.3</td>
<td>0.58</td>
</tr>
<tr>
<td>CT Analysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal CSA, cm²</td>
<td>629.2±152.8</td>
<td>r = -0.666, P = 0.01</td>
<td>620.7±170.5</td>
<td>640.4±150.4</td>
<td>0.67</td>
</tr>
<tr>
<td>VAT/TAT</td>
<td>0.4±0.1</td>
<td>r = -0.429, P = 0.02</td>
<td>0.3±0.1</td>
<td>0.4±0.1</td>
<td>0.01</td>
</tr>
<tr>
<td>VAT/SAT</td>
<td>0.6±0.3</td>
<td>r = -0.373, P = 0.04</td>
<td>0.5±0.2</td>
<td>0.8±0.4</td>
<td>0.01</td>
</tr>
<tr>
<td>Thigh Muscle Fat Deposition (%)</td>
<td>17.3±6.8</td>
<td>r = 0.031, P = 0.88</td>
<td>16.9±6.3</td>
<td>17.6±7.7</td>
<td>0.98</td>
</tr>
<tr>
<td>Thigh muscle CSA, cm²</td>
<td>99.6±24.4</td>
<td>r = 0.071, P = 0.68</td>
<td>99.8±25.8</td>
<td>99.2±23.7</td>
<td>0.95</td>
</tr>
<tr>
<td>Intrahepatic fat deposition, %</td>
<td>3.2±5.8</td>
<td>r = -0.350, P = 0.06</td>
<td>0.6±0.9</td>
<td>6.7±7.8</td>
<td>0.01</td>
</tr>
<tr>
<td>Functional Tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal walk, s</td>
<td>6.2±1.6</td>
<td>r = -0.167, P = 0.35</td>
<td>6.4±1.7</td>
<td>6.0±1.6</td>
<td>0.52</td>
</tr>
<tr>
<td>Fast walk, s</td>
<td>4.2±1.4</td>
<td>r = -0.194, P = 0.28</td>
<td>3.9±0.9</td>
<td>4.4±1.3</td>
<td>0.23</td>
</tr>
<tr>
<td>Sit to stand 5 reps, s</td>
<td>10.1±3.7</td>
<td>r = -0.436, P = 0.01</td>
<td>9.7±3.1</td>
<td>10.6±3.4</td>
<td>0.37</td>
</tr>
<tr>
<td>Sit to stand 60-s, reps</td>
<td>29.4±9.2</td>
<td>r = 0.292, P = 0.12</td>
<td>29.2±10.1</td>
<td>29.7±8.2</td>
<td>0.95</td>
</tr>
<tr>
<td>NSRI walk test, s</td>
<td>81.7±34.1</td>
<td>r = -0.522, P = 0.01</td>
<td>79.9±36.6</td>
<td>84.1±31.5</td>
<td>0.73</td>
</tr>
<tr>
<td>Questionnaires</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zung depression scale</td>
<td>36.9±7.1</td>
<td>r = -0.500, P = 0.01</td>
<td>35.2±7.4</td>
<td>38.7±6.5</td>
<td>0.21</td>
</tr>
<tr>
<td>SF36 total score</td>
<td>71.6±17.8</td>
<td>r = 0.317, P = 0.08</td>
<td>78.1±12.9</td>
<td>63.7±20.2</td>
<td>0.01</td>
</tr>
<tr>
<td>SF36 physical function</td>
<td>69.8±30.9</td>
<td>r = 0.430, P = 0.01</td>
<td>68.1±34.5</td>
<td>72.0±26.9</td>
<td>0.72</td>
</tr>
<tr>
<td>SF36 vitality</td>
<td>71.9±18.2</td>
<td>r = 0.432, P = 0.01</td>
<td>79.7±13.8</td>
<td>62.6±18.8</td>
<td>0.01</td>
</tr>
<tr>
<td>SF36 mental health</td>
<td>71.9±19.1</td>
<td>r = 0.237, P = 0.19</td>
<td>78.7±13.9</td>
<td>63.8±21.7</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Data are mean ± SD. Polysomnographic evidence, CT analysis, functional capacity and quality of life parameters presented as pool data (both groups) and divided as NGT and IGT groups. RDI, Respiratory disturbance index (the sum of the number of hypopneas, obstructive and mixed apneas (apneas with both central and obstructive components), per hour of sleep); Arousal index, defined as the total number of arousals in sleep, divided by the total sleep time; ODI, oxygen desaturation index (the total number of desaturations events per hour of recording); CSA, cross-sectional area; VAT, visceral adipose tissue; SAT, total adipose tissue; SAT, subcutaneous adipose tissue; NSRI, North Staffordshire Royal Infirmary; SF36, sort form 36 quality of life questioner An unpaired t-test was used for assessing the differences between the two groups. Spearman Rank correlation test was used to assess the relation between ISI matsuda index and the examined variables.
creased visceral adiposity, the low oxygen blood saturation during sleep, and the low overall QoL appeared to be involved in the development of glucose intolerance in hemodialysis patients. The level of intrahepatic fat content appeared to be a sensitive indicator for predicting IR in nondiabetic stable hemodialysis patients.

In this study, we have selected nine parameters that, according to the literature, significantly contribute to the development of IR and glucose intolerance in nonrenal failure populations, and we tested them in a model as predictors for glucose intolerance in our hemodialysis population. Intrahepatic fat content, visceral adiposity, oxygen blood saturation during sleep, and QoL fit statistically in the probability model (Fig. 1). Moreover, the intrahepatic fat content (apart from the obvious effect on the AUCGlucose) came out as a very sensitive index in establishing a cutoff threshold in the subsequent ROC analysis (Table 4).

The suggested prediction model can be useful at two levels: first, it could help to identify hemodialysis patients at high risk for developing type 2 diabetes mellitus (Fig. 1), and, second, it could help to improve the design of the potential rehabilitation approaches for reversing or reducing the glucose intolerance in hemodialysis patients.

The 3.97% of intrahepatic fat content cutoff threshold found in this study, could be helpful for the identification of IR state in hemodialysis patients. According to this threshold, 30% of our hemodialysis patients are insulin resistant, and the majority of them are also glucose intolerant (in agreement with the AUCGlucose delineation of IR). Interestingly, the intrahepatic fat threshold for IR development determined in this study is very close to the intrahepatic fat threshold established for the diagnosis of nonalcoholic fatty liver disease (cutoff > 5.0% intrahepatic fat) in obese patients with normal renal function (8, 50). This is the first study in hemodialysis patients examining their intrahepatic fat content. Notably, 18% of the patients examined were found to have intrahepatic lipid content above the 5% threshold for the diagnosis of nonalcoholic fatty liver disease. Unfortunately, our patients were not further examined by an independent specialist to establish a nonalcoholic fatty liver disease diagnosis.

Although, the sensitivity for all the parameters examined by the ROC analysis was 100%, the intrahepatic lipids threshold showed lower specificity (35.7%) compared with AUC for the glucose values (66.7%). Both analyses, however, showed very similar area under the ROC curve (0.97 and 0.98 for intrahepatic fat and OGTT, respectively) making them equally significant for the assessment of IR.

In this study, intrahepatic fat, visceral adiposity, sleep, and QoL appeared to be good predictors of the development of glucose intolerance. It is conceivable, therefore, to hypothesize that by targeting these important contributors, we might be able to improve insulin sensitivity and glucose intolerance in hemodialysis patients. Studies in patients with no kidney disease have shown that weight reduction by diet and/or exercise

![Fig. 1. Probability models for glucose intolerance in hemodialysis patients based on the %oxygen saturation during sleep, VAT/TAT, SF36 QoL questionnaire, and %intrahepatic fat accumulation. VAT/TAT, visceral adipose tissue over total adipose tissue; SF36, sort-form questionnaire 36; QoL, quality of life.](image)

Table 3. Spearman’s correlation coefficients between the four parameters used in the probability models

<table>
<thead>
<tr>
<th>Variables</th>
<th>VAT/TAT</th>
<th>SF36 QoL</th>
<th>Intrahepatic Fat</th>
<th>% O₂ Saturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>VAT/TAT</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF36 QoL</td>
<td>−0.05, P = 0.77</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intrahepatic fat</td>
<td>−0.06, P = 0.71</td>
<td>−0.16, P = 0.07</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>% O₂ saturation</td>
<td>−0.33, P = 0.08</td>
<td>−0.11, P = 0.55</td>
<td>−0.22, P = 0.23</td>
<td>1.000</td>
</tr>
</tbody>
</table>

QoL, quality of life.
ISI-Matsuda and OGIS indices were sensitive enough to detect
Bearing in mind the small sample size of our study, it seems
while being within the accepted normal range (4.5–6.3%).
was found to be statistically different between the two groups,
due to the shortened red blood cell survival in uremia (27), it
index for long-term glycemic control in hemodialysis patients,
although the HbA1c is not considered to be the most accurate
differences between the NGT and IGT groups. In addition,
OGIS, HOMA-IR, QUICKI, G0/I0). Our data showed that only
training resulted in reduced visceral adiposity and intrahepatic
fat content, significantly improving insulin sensitivity and
glucose intolerance (51, 61). Very often, exercise training has
been used as a means of improving glucose tolerance in
prediabetes patients, since exercise is known to have an insul-
in-like effect (34). It remains to be seen whether interventions,
such as exercise training and diet alterations, could reduce
visceral and intrahepatic fat content in hemodialysis patients
and thus improve IR and prevent or defer the development of
glucose intolerance.

It is well known that hemodialysis patients suffer from IR
that seems to be directly related to their uremic state (33).
Various indices of IR have been used for assessing the insulin
action in hemodialysis patients and for this reason we have
included in our study the five most accepted indices (ISI-Matsuda,
OGIS, HOMA-IR, QUICKI, G0/I0). Our data showed that only
ISI-Matsuda and OGIS indices were sensitive enough to detect
 differences between the NGT and IGT groups. In addition,
the HbA1c is not considered to be the most accurate
index for long-term glycemic control in hemodialysis patients,
due to the shortened red blood cell survival in uremia (27), it
was found to be statistically different between the two groups,
while being within the accepted normal range (4.5–6.3%).
Bearing in mind the small sample size of our study, it seems
that even when fasting glucose and insulin, as well as glyco-
sylated hemoglobin, are within the normal range, implying
good glycemic control, a significant number of hemodialysis
patients remain undiagnosed for IR, having thus a significant
risk for developing cardiovascular events (60) and/or type 2
diabetes in the coming years (21).

Strong correlations were found between ISI*Matsuda (and
OGIS) and indices of central adiposity, sleep quality, func-
tional capacity, and QoL, implying that disturbances of insulin
action are closely related to those parameters in this patient
population. However, although diet, muscle mass, and intra-
muscular fat content, are recognized as important contributors
to IR development in nonrenal populations, in our study, none
of those correlated significantly with any of the insulin sensi-
tivity indices nor did they differ significantly between the two
groups. In accordance with the notion that physical activity
promotes euglycemia, in our study, the NSRI test score was
correlated significantly with ISI*Matsuda (6, 48).

We have divided our patient population into two groups
according to their OGTT postload glucose levels. Although, by
definition, none of our patients suffered from diabetic nephrop-
athy or diabetes, there were significant differences between the
two groups, closely related to the pathophysiology of IR (58).
More specifically, visceral adiposity was 31% higher in the
IGT group compared with the NGT group, similar to what
others have found in nonrenal failure glucose-intolerant pa-
tients (5, 16). Moreover, the IGT group exhibited increased
intrahepatic fat accumulation by 10-fold, even exceeding the
levels for the diagnosis of nonalcoholic fatty liver disease. This
is in agreement with the general notion that IR and distur-
bances in liver metabolism are concomitant (30). In addition,
the IGT group had significantly lower oxygen saturation during
sleep, implying an increased hypoxic stress during sleep (1,
53). Sleep disorders have been linked to IR in nonrenal failure
patients (23); however, this link has not been studied in
hemodialysis patients. In the present study, the respiratory
disturbance index did not correlate with ISI*Matsuda; neither did
it differ significantly between the two groups. This result is
somehow unexpected since hemodialysis patients with sleep
apnea syndrome have reduced functional capacity and altered
muscle composition (55), both predisposing to impaired glu-
sose tolerance. It is possible that the longer the patients suffer
from sleep disorders, the more insulin resistant they become;
however, no such data are yet available for this population of
hemodialysis patients.

Overall, we found in this small group of patients on hemo-
dialysis that they share common contributors in the develop-
mnt of IR and glucose intolerance with patients with normal
kidney function. Whether the pathogenic mechanism of IR in
these cases involves only uremia or other additional factors,
i.e., inflammation, similar to other chronic diseases, remains
to be investigated.

Table 4. AUC values produced from the ROC curve analysis

<table>
<thead>
<tr>
<th>Variables</th>
<th>NGT vs. IGT</th>
<th>Cutoff Threshold</th>
<th>Sensitivity, %</th>
<th>Specificity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intrahepatic fat, %</td>
<td>0.97 (0.935–1)</td>
<td>3.97</td>
<td>100</td>
<td>35.7</td>
</tr>
<tr>
<td>GlucoseAUC, mg·dl⁻¹·min⁻¹</td>
<td>0.99 (0.957–1)</td>
<td>181.37</td>
<td>100</td>
<td>66.7</td>
</tr>
</tbody>
</table>

Data are presented as AUC (95% CI). ROC, receiver operator characteristics.
Contrary to other studies, which linked IR to a sedentary lifestyle and to poor physical activity levels in nonrenal failure populations (7, 20), in our study no differences in functional capacity were found between the two groups. This possibly could be masked under the already low fitness levels that hemodialysis patients experience (24).

Although the hemodialysis patients’ QoL is very poor compared with other populations (44), in our study, the hemodialysis patients in the IGT group reported even poorer QoL scores compared with the NGT group. In our study, vitality and mental health stood out as the components that mainly influenced the total score of the SF36 questionnaire (Table 2). As QoL components can both affect and be affected by disease symptoms, we are not able to say whether QoL could affect insulin action or the other way around. Our observations though are in agreement with other studies in nonrenal failure patients (52), which show reduced QoL in patients with IR and metabolic syndrome. Still it should be noted that our hemodialysis patients come from a more or less uniform background as they all receive state disability support, they receive free medical treatment, they live with their families, they are coming from the same agricultural area, and they share similar cultural and religious beliefs.

Low-grade systemic inflammation is considered a potentially important candidate in the pathogenesis of IR in end-stage renal disease patients (28, 63). In our study, there were no differences in C-reactive protein levels between the two groups nor was there a significant correlation with any of the insulin sensitivity indices in the pool data. It is possible that a higher body mass index and/or a higher percentage of total body fat could be required for this factor to gain strength in contributing to the prediction of IR in hemodialysis patients.

It has been reported that IR is closely related to muscle wasting (33, 59) and malnutrition (13) in nondiabetic hemodialysis patients. In our study, no such a relationship was found between ISImatsuda and total lean body mass (by DEXA) or thigh muscle cross-sectional area (by CT) or nutritional status, with the exception of WHR, which correlated significantly with ISIman in the pool data. As WHR reflects total central adiposity, it seems that it can still be useful during outpatients screening for IR in hemodialysis patients.

In our study, two important weaknesses have to be acknowledged. At first, a sample size of 34 hemodialysis patients is not sufficient enough to support strong statements for the prediction of IR and glucose intolerance. Second, since the gold standard technique for the assessment of IR is the hyperinsulinemic euglycemic clamps, the OGTT-derived values of insulin sensitivity should be interpreted with caution. It is possible that the calculated indices of IR could be affected in an unknown manner by other factors related to either the dialysis procedure or the nature of the renal diseases.

**Perspective and Significance**

We have shown for the first time that the intrahepatic fat content could be an important contributor for IR and glucose intolerance in hemodialysis patients. One out of three nondiabetic hemodialysis patients had abnormal glucose tolerance while 18% had high liver fat content. Health parameters that affect QoL, such as sleep status and central adiposity, but also the QoL score per se should also be considered as important players for glucose intolerance. Patients with end-stage renal disease and a “normal” body mass index may at the same time have fatty liver and increased visceral fat depots. Future research on interventions for better diet, increased exercise, and overall improved physical activity, in an effort to preserve a fat-free body composition may be the next steps for improving treatment in this patient group. Consideration of all these data into a model to predict glucose intolerance could help minimize the number of hemodialysis patients that remain undiagnosed with IR and improve prognosis. Furthermore, development of cheaper methods than the CT scan or MRI and spectroscopy to determine liver fat content could have a considerable impact on health costs.

**ACKNOWLEDGMENTS**

We thank Professor Morrie Schambelan, Dr. Kathleen Mulligan, and Dr. Kirsten Johansen from the University of California, San Francisco, for their comments during the preparation of this manuscript. We also thank the nursing staff at the hemodialysis unit of the University Hospital of Larissa for their cooperation and all hemodialysis patients for participating in this elaborating study.

**GRANTS**

This study was supported by the 03ED375 research project, implemented within the framework of the “Reinforcement Programme of Human Research Manpower” and cofinanced by National and Community Funds (25% from the Greek Ministry of Development-General Secretariat of Research and Technology and 75% from the EU-European Social Fund).

**REFERENCES**


