Insulin resistance, renal dysfunction and cardiovascular disease, studies in a high and a low risk population
Oterdoom, Leendert Harmen

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2008

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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CHAPTER 2

Validation of insulin resistance indices in a stable renal transplant population

Leendert H Oterdoom, Aiko PJ de Vries, Willem J van Son, Jaap J Homan van der Heide, Rutger J Ploeg, Ron T Gansevoort, Paul E de Jong, Rijk OB Gans and Stephan JL Bakker

*Diabetes Care. 2005; 28: 2424-2429*
Abstract

Background
To investigate the validity of established insulin resistance indices, based on fasting blood parameters, in a stable renal transplant population.

Research design and methods
Fasting insulin, Homeostasis Model Assessment (HOMA), quantitative Insulin Sensitivity Check Index (QUICKI), and McAuley’s index were assessed for correlation and agreement with whole body glucose uptake (m-value) divided by prevailing serum insulin concentrations (i-value) assessed during a hyperinsulinaemic euglycemic clamp, in 51 stable renal transplant recipients, who were at a median of 7.5 years post-transplant. Multivariate linear regression analyses were used to determine independent risk factors for insulin resistance.

Results
The $m/i$-value correlated with fasting insulin ($r=-0.56$), HOMA ($r=-0.53$), QUICKI ($r=0.52$), and McAuley’s index ($r=0.61$) (all $p$-values $<0.01$). Linear regression showed agreement between all indices and insulin resistance. However, McAuley’s index showed the strongest agreement irrespective of age, gender, renal allograft function, and obesity. In multivariate analysis, fasting insulin ($\beta=-0.59$, $p=0.002$), fasting triglycerides ($\beta=-0.33$, $p=0.04$), and body mass index ($\beta=-1.22$, $p=0.05$) were independently associated with the $m/i$-value.

Conclusions
All investigated insulin resistance indices were valid estimates of insulin resistance in the long-term stable renal transplant population. However, correlation and agreement were strongest for McAuley’s index. In addition to fasting insulin and triglyceride concentrations, of which McAuley’s index is composed, only body mass index seemed to be independently associated with insulin resistance in this population.
Introduction

The incidence and prevalence of cardiovascular disease have been estimated to be three to five times greater in the renal transplant population than in the general population.\(^1\) A recent study showed that the majority of renal transplant out-patients suffers from a constellation of cardiovascular risk factors, i.e. obesity, dyslipidemia, hypertension, and post-transplant diabetes mellitus, that is consistent with the metabolic syndrome (ms).\(^3\) According to preliminary data of the ALERT trial, ms is associated with an increased risk of cardiovascular mortality.\(^4\)

Insulin resistance is thought to be the central pathophysiological feature underlying ms.\(^5\) In order to study the role of insulin resistance in the high incidence of cardiovascular morbidity and mortality in this population, validated insulin resistance indices are needed. Insulin resistance indices have not yet been validated in comparison to the hyperinsulinaemic euglycemic clamp in the stable renal transplant population. Indices that are based on fasting blood parameters alone, have the distinct advantages over other methods of quantifying insulin resistance in that they are less cumbersome and less time-consuming for large scale epidemiological studies at out-patient clinics. However, established indices have been derived from correlates of insulin resistance in non-transplant populations. Evidence suggests that insulin resistance in the renal transplant population may be caused by other risk factors as well, such as immunosuppression and anti-hypertensive medication.\(^6\) Consequently, it remains uncertain whether these indices are applicable to the stable renal transplantation population.

The primary objective of this study was therefore, to validate established insulin resistance indices based on fasting blood parameters in a stable renal transplant population. The second objective was to investigate which risk factors, both traditional and those specifically related to the transplant population, are associated with insulin resistance.

Research Design and Methods

Study population

The Institutional Review Board approved the study protocol (METC 01/039), which was in adherence with the Declaration of Helsinki.\(^7\) Patients from the renal transplant out-patient population, who were part of a previous study cohort\(^3\), were randomly invited to participate.
Recruitment was performed in a stratified manner so that similar numbers of males and females and similar numbers of participants with a high and a low waist hip ratio would be included. Subjects were eligible for participation in the present study if they had received a renal allograft at our center at least 2 years prior to the start of the study and used cyclosporine micro-emulsion (Neoral®; in combination with prednisolone and/or azathioprine, mycophenolate mofetil, or rapamycin) as part of their immunosuppressive regimen. Inclusion required a stable allograft function, defined as a 24-hour urinary creatinine clearance of >30 ml/min, and a difference in 24-hour urinary creatinine clearance over the past year of ≤20 ml/min, to participate. Excluded from invitation were subjects with diabetes mellitus, defined as plasma glucose ≥7.0 mmol/l, and/or use of anti-diabetic medication. Sources funding this project did not play a role in either data collection or analysis or in submission and publication of the manuscript.

**Procedure**
Subjects were admitted at 8:00 am to our clinical research unit after an 8-hour overnight fasting period. Fasting blood was drawn first, after which patients were allowed to take their immunosuppressive medication. Weight, height, waist (midway between the iliac crest and the 10th rib), and hip (at the level of the trochanter major) circumference were measured secondly. Blood pressure was reported as the average of five automated measurements taken at 3-minute intervals (Dinamap; GE Medical Systems, Milwaukee, Wisconsin, USA).

**Hyperinsulinaemic euglycemic clamp**
Insulin resistance was measured using the hyperinsulinaemic euglycemic clamp technique. The clamps were performed as described by previous investigators 8. To give a brief summary of the procedure, exogenous insulin (Velosulin, Novo Nordisk, Bagsvaerd, Denmark) was infused at a continuous rate of 50mU/kg/hour for 120 minutes. Glucose concentration of 5 mmol/L was maintained by adjusting the rate of a 20% D-glucose and 1% KCl infusion based on plasma glucose measurements performed at 5-minute intervals. Whole body glucose uptake (m-value; mg/kg/min) was determined by the total amount of glucose infused during the last 60 minutes of the clamp. Steady-state insulin concentration (i-value; pmol/l) was determined as the mean of two plasma samples at 90 minutes and 120 minutes. Insulin sensitivity was defined as the whole body glucose uptake (M-value) divided by the prevailing serum insulin concentrations.
(I-value) during the clamp (mg/kg/min per pmol/l). Insulin resistance is the reciprocal of insulin sensitivity. Clamp-assessed insulin resistance is therefore the reciprocal of the M/I-value. For convenience, the M/I-value was multiplied by 100.

**Insulin resistance indices**

The following indices were validated against the clamp: fasting insulin (in μU/mL),$^9$ Homeostasis Model Assessment (HOMA): glucose (in mmol/l) x insulin (in μU/ml) / 22.5,$^{10}$ Quantitative Insulin sensitivity Check Index (QUICKI): 1 / [(log glucose (in mg/dl) + log insulin (in μU/ml)),$^{11}$ and McAuley’s index: exp[2.63 - 0.28 ln(insulin (μU/ml)) - 0.31ln(triglycerides (mmol/l))].$^{12}$

**Laboratory measurements**

Fasting serum insulin and insulin levels during the clamp were determined using a radioactive immuno-assay (DSL-1600, Texas, USA). The intra- and inter-assay coefficients of variation at 16.9 μU/ml are 4.5% and 9.9% respectively, and at 53.4 μU/ml 6.4% and 4.7% respectively. Total cholesterol was assessed using the CHOD-PAP method and serum triglyceride level was measured using the GPO-PAP method (both on a MEGA AU 510, Merck Diagnostica, Darmstadt, Germany). High density lipoprotein (HDL) cholesterol was determined using the CHOD-PAP method on a Technikon RA-1000 (Bayer Diagnostics b.v., Mijdrecht, The Netherlands). Low density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula.$^{13}$ Total protein concentration was analyzed using the Biuret reaction (MEGA AU 510, Merck Diagnostica, Darmstadt, Germany). Creatinine clearance was calculated using 24-hour urinary creatinine excretion and serum creatinine.

**Transplant related factors**

Relevant donor, recipient, and transplant characteristics were extracted from the Groningen Renal Transplant Database. This database holds information on all renal transplantations that have been performed at our center since 1968. Parameters used for analysis were donor and recipient age and gender, dialysis modality and duration, date of transplantation, delayed graft function (i.e. days of oliguria or necessity of dialysis treatment), weight 6 months after transplantation (to calculate post-transplant weight gain), human leukocyte antigen (HLA) mismatches, cold and warm ischaemia times, cytomegalovirus (CMV) seropositivity of donor and recipient, acute rejection treatment, and immunosuppressive medication.
**Statistical analysis**

Analyses were performed using SPSS version 12.0 software (SPSS Inc. Chicago IL). The Kolmogorov-Smirnov test was used to assess the normality assumption of continuous distribution. Parametric values are presented as mean ± standard deviation, whereas non-parametric values are displayed as median [interquartile range]. A two-sided p-value of 0.05 or less was considered to indicate statistical significance. All indices and M/I values were log transformed prior to analysis.

The study sample was compared to the population from which participants were recruited, with regard to age, sex, time after transplantation, body mass index (BMI), blood pressure, renal allograft function, and proteinuria using Student’s T-test for parametric variables, and the Mann-Whitney test for non-parametric variables.

Correlation between the indices and log transformed M/I values of the clamps were analyzed by Pearson’s test for parametric variables. Agreement between the indices and the clamps was assessed by linear regression of the insulin resistance index under investigation against the M/I-values with a 95% prediction interval, as suggested by Bland and Altman when methods have different units.

To determine whether age, gender, BMI or renal allograft function influenced the association between the indices and clamp-assessed insulin resistance, correlation was re-assessed after stratification along the median of the above-mentioned variables. In case of difference in correlation, linear regression was performed to determine whether effect modification existed between the above-mentioned variables and the indices.

To determine which traditional and transplant-related risk factors were associated with insulin resistance, all putative factors that were univariately associated with log transformed M/I values at a p-value ≤ 0.1, were entered simultaneously in a backward linear regression model with log transformed M/I values as the dependent variable. The variables that were retained in the crude model were subsequently tested for interaction among covariates, goodness of fit, and higher-order (e.g. polynomial) regression by ANOVA. Residual terms were tested to determine if distribution was normal.

**Results**

Table 1 shows baseline characteristics of the 51 subjects. Mean age was 53 ± 11 years, 55% were male, median time after transplantation was 7.5 years,
<table>
<thead>
<tr>
<th><strong>Recipient demographics</strong></th>
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<tbody>
<tr>
<td>Age, y</td>
<td>53 ± 11</td>
<td>Male gender, n (%)</td>
<td>28 (55)</td>
</tr>
<tr>
<td>Time since transplantation, y</td>
<td>7.5 [5.2 - 12.0]</td>
<td>Cadaveric donor, n (%)</td>
<td>46 (90)</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th><strong>Body composition</strong></th>
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<tbody>
<tr>
<td>BMI, kg/m²</td>
<td>26.0 [23.8 - 28.6]</td>
<td>Waist circumference, cm</td>
<td>101 ± 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Waist to hip ratio</td>
<td>1.03 [0.92-1.09]</td>
</tr>
</tbody>
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<tr>
<th><strong>Renal allograft function</strong></th>
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<tbody>
<tr>
<td>Creatinine clearance, ml/min</td>
<td>65 [57-78]</td>
<td>Serum creatinine, μmol/l</td>
<td>134 [106-149]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Proteinuria, g/24h</td>
<td>0.1 [0.0 - 0.2]</td>
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</table>

<table>
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<tr>
<th><strong>Blood pressure</strong></th>
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<tr>
<td>Systolic blood pressure, mmHg</td>
<td>145 ± 15</td>
<td>Diastolic blood pressure, mmHg</td>
<td>85 ± 11</td>
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<table>
<thead>
<tr>
<th><strong>Lipids</strong></th>
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<tbody>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>5.4 ± 0.9</td>
<td>LDL-cholesterol, mmol/l</td>
<td>3.3 ± 0.8</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/l</td>
<td>1.3 [0.9-1.7]</td>
<td>Triglycerides, mmol/l</td>
<td>1.7 [1.1-2.4]</td>
</tr>
</tbody>
</table>

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<tr>
<th><strong>Medication</strong></th>
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<tbody>
<tr>
<td>Anti-hypertensive</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-blocker, n (%)</td>
<td>6 (12)</td>
<td>ACE inhibitor, n (%)</td>
<td>20 (40)</td>
</tr>
<tr>
<td>A-II antagonist, n (%)</td>
<td>4 (7)</td>
<td>Calcium antagonist, n (%)</td>
<td>19 (38)</td>
</tr>
<tr>
<td>Diuretics, n (%)</td>
<td>22 (44)</td>
<td>Lipid lowering drugs</td>
<td></td>
</tr>
<tr>
<td>Statine, n (%)</td>
<td>37 (72)</td>
<td>[Immunosuppression]</td>
<td></td>
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<tr>
<td>Prednisolone dose, mg/d</td>
<td>10 [7.5-10]</td>
<td>Cyclosporine, n (%)</td>
<td>51 (100)</td>
</tr>
<tr>
<td>Trough-level, μg/l</td>
<td>109 [78-143]</td>
<td>Azathioprine, n (%)</td>
<td>10 (20)</td>
</tr>
<tr>
<td>Mycophenolate mofetil, n (%)</td>
<td>13 (25)</td>
<td>Trough-level, μg/l</td>
<td>1.5 [1.1 - 3.6]</td>
</tr>
<tr>
<td>Rapamycine, n (%)</td>
<td>1 (2)</td>
<td></td>
<td></td>
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</table>

*Parametric characteristics presented as mean ± SD*
*Non-parametric characteristics presented as median [interquartile range]*
and the majority (90%) had received a cadaveric allograft. Forty percent was overweight (BMI between 25-30 kg/m²) and 20% was obese (BMI > 30 kg/m²). Creatinine clearance was 65 [57-78] ml/min. Average blood pressure was 145/85 mmHg. The study sample did not differ significantly from the population from which it was recruited with respect to age, sex, time after transplantation, BMI, blood pressure, or renal allograft function (data not shown). Only proteinuria was significantly lower in the study sample (0.1 [0.0-0.2] vs 0.2 [0.0-0.5] g/24 h, \( P = 0.001 \)).

The hyperinsulinaemic euglycaemic clamp was performed with glucose concentrations of 5.04 [0.16 mmol/l during the last hour of the clamp. Insulin levels were raised to 550 [391 - 751] pmol/l, yielding an M-value of 4.9 ± 1.8 mg/kg/min and an M/I-value of 0.83 [0.57-1.39] mg/kg/min per pmol/L.

Fasting insulin was 16.5 [12.0-23.5] μU/ml; fasting glucose was 4.5 ± 0.6 mmol/L; HOMA 6.4 [5.2-9.3]; QUICKI 0.32 [0.30-0.34]; and McAuley’s index 5.4 ± 1.2. Correlation coefficients between the indices and the M/I-value were \( r = -0.56 \) for fasting insulin, \( r = -0.53 \) for HOMA, \( r = 0.52 \) for QUICKI and \( r = 0.61 \) for McAuley’s index, all at \( P < 0.01 \). Figures 1a-d show the regression analyses with 95% prediction intervals. Agreement was
reached for all indices. HOMA and QUICKI had two (4%) subjects outside the prediction interval. Fasting insulin and McAuley’s index had one subject outside the interval.

The correlation coefficients between the indices and clamp-assessed insulin resistance did not change significantly after stratification along the median of age and renal allograft function. However, a difference was observed after stratification for BMI and gender. In the lower BMI (<26.0 kg/m²) and female sex groups, the correlations of fasting insulin, HOMA, and QUICKI with clamp-assessed insulin resistance lost statistical signifi-
cance (data not shown). Only McAuley index remained significantly correlated with M/I-values in all subgroup analyses (low BMI group r=0.41, p<0.05; high BMI group r=0.63, p<0.01; males r=0.64, p<0.01; females r=0.60, p<0.01). No effect modification was found for BMI and gender in the linear regression analyses.

Putative determinants of insulin resistance were analyzed, first univariately and later multivariately in a backward linear regression model. Table 2 shows that only fasting insulin, BMI, HDL-cholesterol, fasting triglycerides, and waist circumference were univariately associated with the M/I-value. All other putative variables did not reach the p≤0.10 level; specifically: gender, age, post-transplant weight gain, LDL-cholesterol, use of lipid lowering drugs, systolic and diastolic blood pressure, blood pressure medication (diuretics, β-blocker, angiotensin inhibitor or angiotensin receptor blocker and total number of anti-hypertensive drugs), fasting glucose, creatinine clearance, daily prednisolone dosage, cyclosporine trough-levels, mycophenolate mofetil or azathioprine use, cold and warm ischaemia times, delayed graft function, HLA-mismatches, cold and warm ischaemia times, CMV-seropositivity of donor and recipient, and acute rejection treatment with high dose corticosteroids or monoclonal antibodies.

Variables that were significantly associated with M/I values were entered together with age and gender in a backward linear regression model. The crude model was subsequently tested for interaction terms, higher order regression, and goodness-of-fit with ANOVA. These subsequent models were not significantly better, so the crude model was accepted as the final model. In this model, only log transformed insulin (β -0.59, 95%CI [-0.96, -0.22], p=0.002), log transformed fasting triglycerides (β -0.33 95%CI [-0.64, -0.01], p=0.04), and log transformed BMI (β -1.22, 95%CI [-2.27, 0.00], p=0.05) remained independently associated with M/I values (R²=0.44, F-test=12.2, dF 47, p<0.001) as shown in table 2.

**Discussion**

The present study shows that four commonly used insulin resistance indices, based on risk factors for insulin resistance in non-transplant populations, are valid estimates of clamp-assessed insulin resistance in a stable renal transplant outpatient population. Incidence and prevalence of cardiovascular disease are high in the renal transplant population.¹ ² Insulin resistance is an independent risk factor for cardiovascular mor-
tality in the general population and has been hypothesized to play a role in the development of chronic renal allograft dysfunction as well. Consequently, validated insulin resistance indices are needed to study the role of insulin resistance in the development of cardiovascular morbidity and mortality. Blood fasting based indices have the advantage that they are practical and easy to use for large scale epidemiological studies.

The finding that the McAuley index, which consists of fasting triglycerides and fasting insulin, performed best, was additionally supported by our multivariate linear regression analyses which revealed that only fasting insulin, fasting triglycerides, and BMI were associated with insulin resistance in the long-term after renal transplantation. HOMA and QUICKI yielded weaker correlations and lesser agreement in comparison with both McAuley and fasting insulin, but did compare similarly to each other. This is most likely due to the fact that they are mathematically comparable. The presence of glucose in the HOMA and QUICKI indices clearly did not increase the strength of the association with insulin resistance compared to fasting insulin alone. This finding was additionally supported by the fact that glucose was not associated with clamp-assessed insulin resistance in the linear regression analysis. This lack of significant relationship is probably caused by the fact that the current study population was non-diabetic.

Correlations between the indices and M/I-values were significant, irrespective of age and renal allograft function. In contrast, fasting insulin, HOMA, and QUICKI did not correlate significantly with M/I-values in females and in the non-obese (low BMI) subgroups. However, further analyses by linear regression analyses could not demonstrate any significant effect modification of gender and degree of obesity. McAuley’s index was the only index that remained significantly correlated with clamp-assessed insulin resistance in all stratified analyses; again showing that it performed best.

A previous study validated insulin resistance indices in renal transplant recipients at ten weeks post-transplant. In that study, McAuley’s index performed best of all indices based on fasting blood parameters as well. That study did not only find BMI and triglycerides associated with insulin resistance, but daily prednisolone dose and active CMV-infection as well. The explanation for this difference may lie in the time period after transplantation in which that study was performed. The period immediately after transplantation is characterized by high doses of immunosuppression to prevent and treat acute rejection. The consequence of high-doses immunosuppression are opportunistic infections. In that particular study at ten weeks post-transplant, cyclosporine trough-levels were more...
than double the levels compared to our study (242 ± 60 vs 108 ± 42 μg/l). Cyclosporine is thought to increase insulin resistance and reduce insulin secretion. Additionally, daily prednisolone dosage was almost double in Hjelmesæth’s study compared to ours (15 ± 7 vs 8.7 ± 2.0 mg/day). This difference may be of influence because the same group recently showed that a reduction in the daily prednisolone dose from 15 [10-30] mg/day to 9 [5-12.5] mg/day was accompanied by an average decrease in insulin resistance of 24%19. Moreover, the majority of participants in that study had received methylprednisolone boluses of 125 to 500 mg/day for 4 to 5 consecutive days for treatment of acute rejection episodes.6 As mentioned before, Hjelmesæth found active cmv-infection to be associated with insulin resistance as well. Although this finding may constitute an epiphenomenon of the immunosuppression, CMV may add directly to an insulin resistant state through release of cytokines such as TNF-α.20, 21

When immunosuppression is tapered and opportunistic infections become less prevalent in the long-term after transplantation, obesity may become a more predominant factor that influences insulin resistance. Most renal transplant recipients experience at least a 10% weight gain after transplantation.22 In Hjelmesæth’s study, average BMI was 23.5 ± 3.8 kg/m² at three months post-transplant. Our study subjects had a similar BMI of 23.7 ± 3.4 kg/m² at 1 month post-transplant, which increased to 26.6 ± 3.8 kg/m² at the time they participated in this study. Since obesity is an important determinant of insulin resistance, this weight gain might have a large effect on insulin resistance. The inclusion of BMI in an estimate of insulin resistance could further increase accuracy of such an index as was shown in our multivariate linear regression analyses.

The present study had some limitations however. All of our subjects had a cyclosporine-based immunosuppressive regimen and relatively preserved renal allograft function. We wanted to study a homogenous population as cyclosporine is thought to influence insulin secretion as well as resistance. It remains unknown if our findings are applicable to subjects on other immunosuppressive regimens, and less preserved renal allograft function. Both cyclosporine treatment and impaired renal function have been shown to be associated with hypertriglyceridemia.23, 24 Consequently, our finding that McAuley’s index, which includes triglycerides in its equation, correlated and agreed strongest with clamp-assessed insulin resistance, may only hold true for renal transplant recipients on a cyclosporine-based immunosuppressive regimen with relatively preserved renal allograft function. However, since both cyclosporine trough-levels, and renal allograft function were not found associated with
M/I-values in this study, and since fasting insulin, BMI and triglycerides appeared to be the only determinants of insulin resistance in the long-term, we hypothesize that our results may be generalized to subjects on other immunosuppressive regimens, and to subjects with less preserved renal allograft function.

In conclusion, all insulin resistance indices investigated in this study were valid estimates of clamp-assessed insulin resistance in a stable renal transplant population. Only fasting insulin, triglycerides, and BMI were independently associated with insulin resistance. This underscores our finding that the McAuley’s index performed best in the present population with a cyclosporine-based immunosuppressive regimen and relatively preserved renal function.

**References to Chapter 2**


