Transendothelial lipoprotein exchange and microalbuminuria

Jan Skov Jensen\textsuperscript{a,b,\*}, Bo Feldt-Rasmussen\textsuperscript{a}, Kurt Svarre Jensen\textsuperscript{a,c}, Peter Clausen\textsuperscript{a}, Henrik Scharling\textsuperscript{b}, Børge Grønne Nordestgaard\textsuperscript{b,c}

\textsuperscript{a}Department of Nephrology and Endocrinology, The National University Hospital, Copenhagen, Denmark\textsuperscript{b}The Copenhagen City Heart Study, Bispebjerg University Hospital, Copenhagen, Denmark\textsuperscript{c}Department of Clinical Biochemistry, Herlev University Hospital, Copenhagen, Denmark

Received 28 October 2003; received in revised form 19 February 2004; accepted 23 February 2004
Available online 9 April 2004
Time for primary review 20 days

Abstract

Objective: Microalbuminuria associates with increased risk of atherosclerosis in individuals without diabetes. We hypothesized that transendothelial lipoprotein exchange is elevated among such individuals, possibly explaining increased intimal lipoprotein accumulation and thus atherosclerosis. Methods: Using an in vivo isotope technique, transendothelial exchange of low density lipoprotein (LDL) was measured in 77 non-diabetic individuals. Autologous 131-iodinated LDL was reinjected intravenously, and the 1-h fractional escape rate was calculated as index of transendothelial exchange. Results: There was no difference in transendothelial LDL exchange between subjects with microalbuminuria versus normoalbuminuria (mean (95% confidence interval) 3.8%/h (3.3–4.3%/h) versus 4.2%/h (3.7–4.7%/h); \( P = 0.33 \)). In contrast, there was a positive correlation between transendothelial LDL exchange and (logarithmically transformed) plasma insulin: \( \beta = 0.6 \) (95% CI: 0.1–1.1); \( R = 0.22; P < 0.05 \). This correlation was independent of age, sex, blood pressure, plasma concentration of lipoproteins, LDL size, body mass index, plasma volume, and use of medicine, and it was unlikely caused by altered hepatic LDL receptor expression, or glycosylation of LDL. Conclusion: In non-diabetic individuals, transendothelial LDL exchange is not associated with microalbuminuria, but possibly with hyperinsulinemia.

© 2004 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.

Keywords: Atherosclerosis; Capillary permeability; Insulin resistance; Lipoproteins; Lipid metabolism; Microalbuminuria

1. Introduction

Through the last decade, several population studies have documented that microalbuminuria, i.e., excretion of small amounts of albumin in urine, is associated with increased risk of atherosclerotic cardiovascular disease and death [1–7]. The pathophysiological mechanism behind this association has not yet been clearly elucidated, but it is likely that microalbuminuria operates independently of the conventional atherosclerotic risk factors such as hypertension, diabetes, hyperlipidemia, obesity, and smoking.

In selected groups of patients and controls, we have previously shown that microalbuminuria is a marker of a systemic transvascular leakiness of albumin as measured by the fractional escape rate of albumin from the intravascular space (FER\textsubscript{albumin}) [8–10]. We therefore hypothesized that this leakiness may include lipoproteins, thereby leading to increased lipoprotein deposition in the vessel wall, and thus accelerated atherogenesis [11,12].

We have developed a method to measure human fractional escape rate of low density lipoprotein from the intravascular space (FER\textsubscript{LDL}) using intravenous injection of radioactively labeled autologous LDL [13]. By this method, we investigated 77 non-diabetic individuals in order to test the hypothesis that microalbuminuria is associated with increased transvascular sieving of LDL; microalbuminuria was defined according to the results of our previous population study [5]. Furthermore, we measured LDL size, plasma volume, blood pressure, and plasma concentrations of lipoproteins and insulin as this could affect FER\textsubscript{LDL}. 
2. Methods

2.1. Subjects

We studied 77 subjects without diabetes mellitus (62 men and 15 women, age range 23–73 years). All had fasting blood glucose under 6.7 mmol/l. In order to compare subjects with presence of atherosclerosis with healthy controls, we included subjects who suffered from angiographically verified coronary heart disease (n=24) or from angiographically verified arterial insufficiency in the lower extremities (n=11), and clinically healthy subjects who did not receive any form of medication or had any familial history of atherosclerosis (n=42). Out of the 35 patients, 30 received acetyl salicylic acids, 21 calcium channel blockers, 9 angiotensin converting enzyme inhibitors, and 7 beta adrenoceptor blockers. All 77 participants were randomly recruited: the 35 patients from The National University Hospital in Copenhagen, and the 42 healthy individuals from the Copenhagen City Heart Study, a major epidemiological population study of cardiovascular disease and risk factors [14,15]. Out of these 77 participants, 26 exhibited microalbuminuria using the definition from our previous population study of subjects without diabetes: a urine albumin/creatinine concentration ratio above 0.65 mg/mmol in a morning urine sample [5]. All participants gave informed consent. The investigation conformed with the principles outlined in the Declaration of Helsinki and was approved by the Copenhagen and Frederiksberg Ethics Committee (file no. 01-302/97). The study was surveyed by the National Department of Isotope Pharmacy who permitted investigation of maximally two subjects per week.

2.2. Procedures

FER_{LDL} and FER_{ab} were measured by means of plasma decay curves during 1 h after intravenous injection of autologous 131-iodinated LDL (131I-LDL) and commercially available 125-iodinated human serum albumin (125I-albumin), respectively.

LDL was isolated from 100 ml of blood by sequential ultracentrifugation at 4 °C in solvent densities of 1.019 and 1.050 g/ml (to exclude lipoprotein(a)), respectively, with a Beckman 50.4 Ti rotor for at least 1.8 × 10⁸ g × min; 131-iodination of LDL was done with 18.5 MBq 131-iodine using iodine monochloride [16]. The iodination efficiency was 25 ± 5% (n = 54), which corresponds to 52 ± 13 cpm/ng LDL protein. In the labeled preparations, 98.6 ± 0.4% of the 131I radioactivity was precipitable with 15% (vol/vol) trichloroacetic acid (TCA), and 5.6 ± 0.5% of the 131I radioactivity was lipid-soluble, i.e., extractable into chloroform–methanol (1:1, vol/vol). In fixed-density ultracentrifugation analysis of labeled LDL in the presence of added carrier plasma, ≥ 96% of the total radioactivity was in the LDL density range of 1.019–1.063 g/ml. No evidence of fragmentation of the labeled LDL was detected using a 3% to 8% Tris–Acetate Gradient Gel followed by autoradiography. In all labeled preparations, test for sterility was negative, and <5 pg of pyrogens was detectable per milliliter sample.

The participants met at 0800 h after an 8 h fast and tobacco abstinence. A 17 G Teflon cannula was inserted in an antecubital vein in both arms, one for blood sampling and one for injection. After 30 min rest at recumbency, the preparation containing 131I-LDL (700 kBq) and 125I-albumin (500 kBq; code IFE-IT.23S or IFE-IT.20S, Isopharma, Kjeller, Norway (free 125I radioactivity < 1.5 ± 0.2%)) was injected intravenously. Venous blood samples of 10 ml were drawn without stasis in heparinized tubes before and at 10, 20, 30, 40, 50, and 60 min after injection. Proteins in plasma (3 ml) and doses (0.1 ml with 2.9 ml unlabeled plasma added) were precipitated at 4 °C with TCA at a final concentration of 15% (vol/vol). Following mixing and centrifugation, total radioactivity as well as radioactivity in the supernatant was counted for 20 min in a double-channel gamma counter (1282 Compugamma, LKB, Wallac, Turku, Finland). For both tracers, the TCA-precipitable radioactivity at each time point was plotted versus time after logarithmic transformation. FER_{LDL} and FER_{ab} (in %/h) were then calculated on the basis of the slopes (β) of the best linear curves fitted by the least-squares method using the formula (1 – 0eβt) × 100%, thus assuming that radioactivities declined monoexponentially with time (one-compartment system). Distribution volumes of LDL (DV_{LDL}) and albumin (DV_{ab} = plasma volume) were calculated from the amounts of injected radioactivities divided by the plasma radioactivities at time zero, as derived from the intercepts of the fitted lines for the two tracers, respectively. The obtained DV values were corrected for body surface area (m²) by the formula 0.007184 × weight⁰.⁴₂₅ × height⁰.⁷₂₅.

The contribution of receptor mediated elimination of LDL from the intravascular compartment to FER_{LDL} during the 1-h blood sampling period was elucidated by comparing FER_{LDL} with FER_{Gly-LDL} in three humans without diabetes mellitus [13]. Glycosylated LDL is not recognized by LDL receptors [17,18], and thus the difference between FER_{LDL} and FER_{Gly-LDL} represents receptor elimination. Glycosylation of LDL was performed as previously described by others [17,19,20]. Autologous 131I-LDL (700 kBq) and autologous 125I-Gly-LDL (500 kBq) was reinjected under similar conditions as described above. Venous blood samples of 10 ml were drawn without stasis in heparinized tubes before and at 10, 20, 30, 40, 50, and 60 min after reinjection. Eleven additional blood samples were obtained during the subsequent 6 days. Plasma was precipitated with TCA and counted for radioactivity as described above. For both tracers, the logarithmically transformed TCA-
Precipitable radioactivity was plotted versus time, and FER\textsubscript{LDL} and FER\textsubscript{Gly-LDL} were calculated as previously described. Mean FER\textsubscript{LDL} was about 1%/h higher than mean FER\textsubscript{Gly-LDL} (3.6 ± 1.1%/h versus 2.6 ± 1.1%/h; \(P<0.05\)). Moreover, fractional catabolic rates, FCR\textsubscript{LDL} and FCR\textsubscript{Gly-LDL} (in %/h), were calculated according to Matthews using the formula \((C_1/\beta_1 + C_2/\beta_2)^{-1}\), where \(\beta_1\) and \(\beta_2\) are slopes and \(C_1\) and \(C_2\) intercepts of the late and initial linear curve fits, respectively [21]. Mean FCR\textsubscript{LDL} was about two times higher than mean FCR\textsubscript{Gly-LDL} \((P<0.01)\), documenting that Gly-LDL was indeed glycosylated.

The use of a one-compartment system for calculation of FER\textsubscript{LDL} was validated by comparing FER\textsubscript{LDL} with transvascular LDL permeability as described by Matthews [21], which takes into account an extravascular protein compartment, receptor mediated metabolism, and excretion. In eight subjects without diabetes, blood samples were collected every 10th minute during the first hour and subsequently once a day during the next week upon reinjection of autologous \(^{131}\text{I}\)-LDL. Transvascular LDL permeability using the multicompartment model was calculated by the formula \(C_1C_2(b_2 - b_1)^2/(C_1b_2 + C_2b_1)\) [21]. There was a positive correlation between FER\textsubscript{DL} using one-compartment kinetics and transvascular LDL permeability using multicompartment kinetics \((R^2 = 0.41; \ n = 8; \ \text{one-sided} \ \ P < 0.05)\). The equation for the linear correlation was one-compartment FER\textsubscript{LDL} = 0.43 \times \text{multicompartment FER\textsubscript{LDL}} + 2.8 (all in %/h). The overestimation of FER\textsubscript{LDL} by one-compartment kinetics was most pronounced in the lower range.

Finally, we correlated FER\textsubscript{LDL} to FCR\textsubscript{LDL} in 27 subjects, where FCR\textsubscript{LDL} was calculated by multicompartmenal analysis using the SAAM II software (SAAM Institute, Seattle, WA) [22]. There was no correlation between FER\textsubscript{LDL} and FCR\textsubscript{LDL} \((R < 0.01; \ n = 27; \ P = 0.99)\). Thus, this method cannot demonstrate that receptor elimination (which dominates FCR\textsubscript{LDL}) influences FER\textsubscript{LDL} measurements. Therefore, it is not very likely that metabolism, i.e., receptor-mediated elimination of LDL, contributes to FER\textsubscript{LDL} to any major degree, and certainly not more than approximately 28% suggested from the experiments described above.

Urine concentration of albumin was measured by a micro ELISA method [23], and urine concentration of creatinine by a photometric method (Vitros Crea Slide, Johnson & Johnson, Rochester, US; coefficient of variation: 3%). Plasma concentration of insulin was measured by a two-site fluoroenzymometric assay (AutoDELFIATM Insulin, Wallac Oy, Turku, Finland). Plasma concentrations of total-, LDL- and HDL-cholesterol, triglycerides, and creatinine were all measured by commercially available assays (Roche Diagnostics, Mannheim, Germany) using a Hitachi analyzer. Plasma concentration of C-reactive protein (CRP) was measured by a highly sensitive nephelometric method (Behring Diagnostica, Germany). LDL particle size was measured by non-denaturing pore gradient gel electrophoresis as previously described [13,24]. All blood samples were drawn after an 8-h fast and tobacco abstinence. Systolic and diastolic blood pressures were measured by auscultation using a manometer and an appropriately sized cuff. Body mass index \((\text{kg/m}^2)\) was calculated as weight/height\(^2\).

2.3. Statistical analysis

Comparisons between groups were performed by Student’s \(t\) test or the \(\chi^2\) test. Factors associated with FER\textsubscript{LDL} were analyzed by multiple linear regression analysis. Plasma concentrations of triglycerides, insulin, CRP, and creatinine, and urine albumin/creatinine ratio were log-transformed.

Table 1

<table>
<thead>
<tr>
<th>Characteristics of study subjects</th>
<th>Normoalbuminuria ((n = 51))</th>
<th>Microalbuminuria ((n = 26))</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine albumin/creatinine (mg/mmol)</td>
<td>0.32 (0.30–0.36)</td>
<td>1.76 (1.17–2.65)</td>
<td></td>
</tr>
<tr>
<td>Plasma insulin (pmol/l)(^1)</td>
<td>32 (27–38)</td>
<td>33 (26–42)</td>
<td>0.81</td>
</tr>
<tr>
<td>Men (%)</td>
<td>78</td>
<td>85</td>
<td>0.52</td>
</tr>
<tr>
<td>Age (years)</td>
<td>53 (49–56)</td>
<td>57 (54–60)</td>
<td>0.09</td>
</tr>
<tr>
<td>Coronary heart disease (%)</td>
<td>33</td>
<td>27</td>
<td>0.57</td>
</tr>
<tr>
<td>Peripheral arterial disease (%)</td>
<td>10</td>
<td>23</td>
<td>0.15</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>37</td>
<td>58</td>
<td>0.09</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>120 (115–125)</td>
<td>129 (122–136)</td>
<td>0.03</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>73 (70–76)</td>
<td>78 (73–83)</td>
<td>0.11</td>
</tr>
<tr>
<td>Body mass index ((\text{kg/m}^2))</td>
<td>25.4 (24.4–26.4)</td>
<td>25.5 (24.4–26.6)</td>
<td>0.90</td>
</tr>
<tr>
<td>Plasma total cholesterol (mmol/l)</td>
<td>5.1 (4.8–5.4)</td>
<td>5.0 (4.6–5.4)</td>
<td>0.59</td>
</tr>
<tr>
<td>Plasma LDL cholesterol (mmol/l)</td>
<td>3.3 (3.0–3.6)</td>
<td>3.1 (2.7–3.5)</td>
<td>0.46</td>
</tr>
<tr>
<td>Plasma HDL cholesterol (mmol/l)</td>
<td>1.25 (1.13–1.37)</td>
<td>1.22 (1.06–1.38)</td>
<td>0.73</td>
</tr>
<tr>
<td>Plasma triglycerides (mmol/l)</td>
<td>1.0 (0.8–1.2)</td>
<td>1.1 (0.9–1.5)</td>
<td>0.41</td>
</tr>
<tr>
<td>Plasma C-reactive protein (mg/l)</td>
<td>1.1 (0.8–1.5)</td>
<td>1.1 (0.6–2.0)</td>
<td>0.97</td>
</tr>
<tr>
<td>Plasma creatinine (mmol/l)(^1)</td>
<td>87 (84–91)</td>
<td>88 (83–93)</td>
<td>0.84</td>
</tr>
</tbody>
</table>

Continuous variables are shown by means or geometric means \(^1\) with 95% confidence intervals in parentheses.
prior to the analyses, because of non-normal distribution. \( P \) values < 0.05 were significant.

3. Results

The 26 subjects with microalbuminuria were characterized by higher systolic blood pressure and smaller LDL size compared with subjects with normoalbuminuria (Tables 1 and 2).

There were no differences in FER\(_{LDL}\) or FER\(_{ab}\) between subjects with microalbuminuria versus normoalbuminuria (Table 2). FER\(_{LDL}\) was, in general, lower than FER\(_{ab}\) (4.1%/h (95% CI: 3.7–4.5%/h) versus 5.6%/h (5.2–6.0%/h); \( P < 0.01 \)).

In regression analysis including all participants (\( n = 77 \)), FER\(_{LDL}\) was positively correlated with FER\(_{ab}\) (\( \beta = 0.7 \) (95% CI: 0.5–0.9); \( R = 0.70 \); \( P < 0.001 \)) and ln\(_{C0}\) (plasma insulin) (\( \beta = 0.6 \) (95% CI: 0.1–1.1); \( R = 0.22 \); \( P < 0.05 \)) after correction for age, sex, blood pressures, lipoproteins, LDL size, body mass index, urine albumin/creatinine, C-reactive protein, D\(_{ab}\), and use of medicine. There was no correlation between FER\(_{LDL}\) and urine albumin/creatinine (\( R = -0.07 \); \( P > 0.05 \)), LDL size (\( R = -0.03 \); \( P > 0.05 \)), plasma LDL (\( R = -0.32 \); \( P > 0.05 \)), systolic blood pressure (\( R = -0.01 \); \( P > 0.05 \)), and diastolic blood pressure (\( R = -0.04 \); \( P > 0.05 \)).

Subjects with angiographically verified coronary or peripheral atherosclerosis (\( n = 35 \)) had similar FER\(_{LDL}\) as clinically healthy controls (\( n = 42 \)) (4.0%/h (95% CI: 3.4–4.6%/h) versus 4.2%/h (3.7–4.7%/h); \( P > 0.05 \)).

4. Discussion

Both microalbuminuria [1–7] and hyperinsulinemia [25–27] have been considered as determinants of atherosclerotic cardiovascular disease in subjects without diabetes mellitus. The underlying pathophysiological mechanisms of action are not clear, but a systemic transvascular leakiness of macromolecules including albumin and lipoproteins has been proposed [10,28,29]. The present clinical study suggests that microalbuminuria was not associated with elevated FER\(_{LDL}\). Thus, the well-established predictive effect of microalbuminuria on atherosclerotic cardiovascular disease [1–7] may likely be mediated through other mechanisms. If both microalbuminuria and hyperinsulinemia are present, the risk of atherosclerosis is highly increased [3]. As a secondary observation an independent relationship between hyperinsulinemia and increased FER\(_{LDL}\) from the intravascular compartment was observed in the present study. This is in accordance with our previous observations of elevated FER\(_{LDL}\) in patients with type 1 or type 2 diabetes [13,30], conditions with exogenous and endogenous hyperinsulinemia, respectively. If elevated FER\(_{LDL}\) leads to increased intimal influx and deposition of lipoproteins in arteries [11,12], our finding may contribute to explain the potential atherogenic impact of hyperinsulinemia. Taken together, our study suggests that the atherogenic effect of microalbuminuria and hyperinsulinemia is mediated through different mechanisms of action.

We have previously shown that FER\(_{LDL}\) is elevated in patients with manifest diabetes mellitus suggesting a vasculo- toxic effect of hyperglycaemia per se [13,30]. The present observation suggests that FER\(_{LDL}\) is also elevated in the prediabetic state, perhaps due to hyperinsulinemia or high plasma glucose in the non-diabetic range (glucose tolerance test was not performed). An alternative possibility is that FER\(_{LDL}\) reflects metabolism of LDL rather than transendothelial exchange, although FER\(_{LDL}\) was measured during only 1 h upon injection of labeled LDL. However, we found no relationship between FER\(_{LDL}\) and FCR\(_{LDL}\), as calculated by the SAAM II method [22]. We furthermore studied the contribution of receptor elimination to FER\(_{LDL}\) by measuring FER\(_{Gly-LDL}\) and FER\(_{LDL}\) simultaneously, as glycosylated LDL is not recognized by LDL receptors [17,18], as confirmed by the significantly lower FCR\(_{Gly-LDL}\) than FCR\(_{LDL}\) in our experiments. In non-diabetic individuals, FER\(_{Gly-LDL}\) was about 1%/h lower than FER\(_{LDL}\), indicating that receptor elimination contributes to FER\(_{LDL}\) by about 1%/h only (corresponding to about one third of FER\(_{LDL}\)). However, there was no significant inverse relationship between plasma LDL concentration and FER\(_{LDL}\), and the association between FER\(_{LDL}\) and plasma insulin was independent of plasma LDL. Together, these observations indicate that metabolism cannot fully explain the positive correlation between FER\(_{LDL}\) and plasma insulin. Furthermore, hyperinsulinemia may be associated with a tendency towards a higher diurnal blood glucose profile. This may have led to an underestimation the correlation between FER\(_{LDL}\) and plasma insulin, since individuals with normal/low plasma insulin may have higher rates of receptor elimination of LDL.

Yet, another possibility is that differences in LDL size may explain the correlation between FER\(_{LDL}\) and hyperinsulinemia. Small LDL enters faster into the vessel wall than large LDL [16], and small LDL is associated with increased risk of coronary heart disease [31]. This possibility is, however, unlikely since LDL size was not (inversely)
correlated with FER_{LDL}, and the association between FER_{LDL} and plasma insulin was independent of LDL size.

Because FER_{LDL} was also independent of both systemic arterial blood pressure and endothelial surface area as reflected by the plasma volume, we suggest the elevated FER_{LDL} in subjects with hyperinsulinemia to result mainly reflected by the plasma volume, we suggest the elevated arterial blood pressure and endothelial surface area as correlated with FER_{LDL}, and the association between i.e., fasting plasma insulin, and FER_{LDL}, the true association
tent correlation between a rough marker of insulin resistance, more sensitive measures than fasting plasma insulin in this
from increased transvascular permeability. This could be a consequence of endothelial cell death [32–34] or damage due to insulin resistance [29,35,36]. The main proportion of transendothelial LDL exchange probably takes place in the capillaries [37]. However, there exists indirect evidence for similar exchange of albumin and lipoproteins in capillaries and arteries [38]. Furthermore, the tight positive correlation between FER_{LDL} and FER_{albumin} observed in this study is analogous with the correlation between the exchange of LDL and albumin across the arterial wall in rabbits [39]. The significantly lower FER_{LDL} than FER_{albumin} is in accordance with the three times larger size of LDL particles than of albumin. Microalbuminuria may not be the best marker for atherosclerotic organ damage although it may be useful in patients with diabetes mellitus. Since the study population included patients with coronary or peripheral arterial disease, a number of other tools for evaluation of organ damage such as electrocardiographic findings, carotid arterial intima/media ratio, echocardiographic findings, vascular MRI, intravascular imaging, SAA, markers for platelet activation and so on could also have been included in this study. We did not, however, include any of these measurements, but used other markers of atherosclerotic organ damage: coronary angiography (n = 24) and angiography of lower extremities (n = 11); FER_{LDL} was not elevated in those with angiographically verified atherosclerosis.

Unfortunately, we did not assess insulin resistance by more sensitive measures than fasting plasma insulin in this study. However, since it was possible to detect an independent correlation between a rough marker of insulin resistance, i.e., fasting plasma insulin, and FER_{LDL}, the true association between insulin resistance and FER_{LDL} may be even stronger.

In conclusion, this human in vivo study of non-diabetic subjects has shown that microalbuminuria is not associated with the fractional escape rate of LDL particles from the intravascular compartment. Furthermore, the study suggested that hyperinsulinemia is associated with increased fractional escape rate of LDL independently of age, sex, blood pressure, plasma concentration and size of LDL, body mass index, plasma volume, and use of medicine. This latter observation may contribute to explain the atherogenic effect of insulin.

Acknowledgements

The study was funded by the Danish Heart Foundation; the Danish Diabetes Association; the Novo Nordisk Foundation; the Copenhagen University Hospitals’ (H:S) Research Foundation; the Danish Medical Association’s

Research Foundation; the A.P. Møller Foundation for the Advancement of Medical Science; Bayer; the Eli Lilly Diabetological Research Foundation; the Danish Foundation of Fight Against Circulatory Diseases; the Boserup Foundation; the Aage and Johanne Louis-Hansen Foundation; the Karl G. Andersen Foundation; the Kathrine and Vigo Skovgaard Foundation; the Jacob Madsen Foundation; the Lauritz Peter Christensen Foundation; the P. A. Messerschmidt Foundation; the Kønig-Petersen Foundation; and the Bjørnø Foundation.

References


