Metabolism Clinical and Experimental 96 (2019) 8–11

Contents lists available at ScienceDirect

Metabolism Clinical and Experimental

journal homepage: www.metabolismjournal.com

Fractional turnover of apolipoprotein(a) and apolipoprotein B-100 within plasma lipoprotein(a) particles in statin-treated patients with elevated and normal Lp(a) concentration

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ARTICLE INFO

Article history:
Received 15 February 2019
Accepted 10 April 2019

ABSTRACT

Context: Lipoprotein(a) [Lp(a)] is a highly atherogenic lipoprotein characterized by apolipoprotein(a) [apo(a)] covalently bounded to apoB-100 (apoB). However, the metabolism of apo(a) and apoB within plasma Lp(a) particles in patients on statins remains unclear.

Methods: The kinetics of Lp(a)-apo(a) and Lp(a)-apoB were determined in 20 patients with elevated Lp(a) (≥0.8 g/L; n = 10) and normal Lp(a) (≤0.3 g/L; n = 10) using stable isotope techniques and compartmental modeling. Plasma apo(a) concentration was measured using liquid chromatography–mass spectrometry. All patients were on statin therapy and were studied in the fasting state.

Results: The fractional catabolic rate (FCR) of Lp(a)-apo(a) was not significantly different from that of Lp(a)-apoB in statin-treated patients with elevated or normal Lp(a) ($P > 0.05$ in both). Lp(a)-apo(a) FCR was significantly correlated with Lp(a)-apoB in patients with elevated and normal Lp(a) concentrations ($r = 0.970$ and $r = 0.979$, respectively; all $P < 0.001$) with Lin's concordance test showing substantial agreement between the FCRs of Lp(a)-apo(a) and Lp(a)-apoB in patients with elevated and normal Lp(a) concentrations ($r_c = 0.978$ and $r_c = 0.966$, respectively).

Conclusion: Our data indicate that the apo(a) and apoB proteins within Lp(a) particles have similar FCR and are therefore tightly coupled as an Lp(a) holoparticle in statin-treated patients with elevated and normal Lp(a) concentrations.

1. Introduction

Lipoprotein(a) [Lp(a)] is a low-density lipoprotein (LDL)-like particle with one molecule of apolipoprotein(a) [apo(a)] covalently bound to apoB-100 (apoB) [1]. Epidemiological and genetic studies suggest that an elevated plasma level of Lp(a) is a strong, causal risk factor of atherosclerotic cardiovascular disease (ASCVD) [2,3]. Recent large clinical trials have shown that elevated Lp(a) remains a risk factor despite reduction of LDL-cholesterol with statins [4]. However, the metabolism of Lp(a) particles remains poorly understood.

Some, but not all, stable isotope studies in humans observed similar fractional catabolic rates (FCR) for Lp(a)-apo(a) and Lp(a)-apoB providing support for the intracellularly assembly and coupling of apo(a) and apoB within Lp(a) particles [5–8]. We recently reported similar FCRs of Lp(a)-apo(a) and Lp(a)-apoB in normolipidemic men, supporting tight coupling of the metabolism of both protein components of the Lp(a) particle [9]. However, we did not specifically examine the metabolism of apo(a) and apoB within Lp(a) particles in patients with elevated Lp(a) on statin therapy. Increased plasma concentration of Lp(a) is primarily driven by higher hepatic production of Lp(a) as a consequence of increased hepatic apo(a) mRNA concentration. Statins potentially decrease the hepatic synthesis and availability of apoB and consequently may affect the production of Lp(a) [10]. There is evidence that apo(a) recycles in the circulation following catabolism of the Lp(a) particle [1]. As a consequence of upregulation of the LDL receptor, statins may lead to greater relative clearance of apoB from plasma and could accelerate the recycling of apo(a) [10]. Statins could therefore theoretically bear on the coupling of the two protein constituents of Lp(a) particles [1]. Understanding the kinetics of Lp(a)-apo(a) and Lp(a)-apoB is fundamentally important to broaden our understanding of this complex cardiovascular risk factor and to advance the development of new interventions targeting elevated Lp(a).

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https://doi.org/10.1016/j.metabol.2019.04.010
0026-0495/

In the present study, we hypothesized that the fractional turnover of apo(a) and apoB within plasma Lp(a) particles is coupled in statin-treated patients with elevated and with normal plasma Lp(a) concentrations.

2. Materials and methods

2.1. Patients

Ten Caucasian patients aged between 18 and 70 years were recruited from the Lipid Disorder Clinics at Royal Perth Hospital (RPH). All patients had elevated plasma Lp(a) concentrations of ≥0.8 g/L, and were on statins and aspirin. Ten patients of similar clinical and biochemical characteristics with normal Lp(a) concentrations of ≤0.3 g/L were also recruited. The kinetics of Lp(a)-apo(a) and Lp(a)-apoB were determined using an intravenous bolus injection of $^2H_3$-leucine (chemical formula CH3CH(CD3)CH2CH(NH2)COOH) [9]. Patients provided informed written consent and the study was approved by the RPH Human Research Ethics Committee.

2.2. Biochemical analyses

Plasma lipid, lipoprotein and apolipoprotein concentrations were determined by standard enzymatic methods as previously described [9,11]. Plasma Lp(a) was determined by an isoform-independent immunoassay (Quantia assay, Abbott Diagnostics) and expressed as g/L. Plasma apo(a) was determined by LCMS method (Proteomics International, Perth, WA) [9]. This LCMS method was validated against a reference immunoassay (r = 0.982) based on a monoclonal antibody directed to apo(a) (Northwest Lipid Metabolism and Diabetes Research Laboratories, University of Washington, Seattle, WA). The value to the assay calibrator was assigned by amino acid analysis using a purified Lp(a) and expressed in nmol/L [12].

2.3. Laboratory methods

Full details of methods, including isolation and measurement of tracer-tracee ratio (TTR) of Lp(a)-apo(a) and Lp(a)-apoB, compartment model, and calculation of kinetic parameters have been reported elsewhere [9]. At low levels of tracer incorporation, modeling tracer data as enrichment or TTR does not impact kinetic parameters, but enrichment should be used at high levels [13].

2.4. Statistical analysis

All analyses were performed using SPSS 21 (SPSS, Inc., Chicago). Skewed variables were log-transformed as appropriate. Data are presented as mean ± SD or geometric mean (95% confidence interval [CI]). Group characteristics were compared using chi-square statistics or independent *t*-tests for categorical and continuous variables, respectively. Lp(a)-apo(a) and Lp(a)-apoB FCRs were compared using the paired *t*-test. Association and agreement between the FCRs of Lp(a)-apo(a) and Lp(a)-apoB were examined using simple correlation, Bland-Altman analysis and Lin's concordance correlation. Statistical significance was defined at the 5% level using a 2-tailed test.

3. Results

3.1. Clinical and biochemical characteristics

Table 1 shows the clinical and biochemical characteristics of the patients. On average, they were middle-aged, overweight/obese, normotensive, non-diabetic and had normal lipid and lipoprotein concentrations. Patients with elevated Lp(a) levels had significantly higher Lp(a) and apo(a) concentrations compared to patients with normal Lp(a) levels ($P < 0.001$ for both). There were no significant differences in other clinical and biochemical characteristics ($P > 0.05$).

Table 1
Clinical and biochemical characteristics in the patients studied.

	Elevated Lp(a) (n = 10)	Normal Lp(a) (n = 10)
Age (years)	54.3 ± 12.1	56.0 ± 12.8
Male (%)	60.0	70.0
Body mass index (kg/m^2)	29.7 ± 6.57	29.2 ± 4.87
Systolic blood pressure (mm Hg)	132 ± 12.8	132 ± 8.75
Diastolic blood pressure (mm Hg)	75.0 ± 10.0	75.2 ± 5.55
Glucose (mmol/L)	5.37 ± 0.34	5.57 ± 0.47
Total cholesterol (mmol/L)	4.00 ± 0.87	4.58 ± 1.40
Triglycerides (mmol/L)	1.37 ± 0.85	1.23 ± 0.47
HDL-cholesterol (mmol/L)	1.15 ± 0.25	1.32 ± 0.40
LDL-cholesterol (mmol/L)	2.23 ± 0.72	2.68 ± 0.97
Total apolipoprotein B (g/L)	0.80 ± 0.24	0.89 ± 0.22
Lipoprotein(a) [g/L]	1.60 (1.25, 2.04)	0.11 (0.07, 0.20)*
Apo(a) [nmol/L]	260 (199, 339)	17.9 (10.7, 30.1)*

Data are presented as mean ± SD or geometric mean (95% confidence interval).
* $P < 0.001$ compared with elevated group.

3.2. Kinetic parameters of Lp(a)-apo(a) and Lp(a)-apoB

Fig. 1A and B show the tracer curves (expressed as TTR [%]) of Lp(a)-apo(a) and Lp(a)-apoB over time (0 to 96 h) following the intravenous infusion of $^2H_3$-leucine in statin-treated patients with elevated and normal Lp(a) concentrations. As seen, the two tracer curves of Lp(a)-apo(a) and Lp(a)-apoB had a similar contour and peak TTR in both groups, suggesting similar fractional turnover rates of apo(a) and apoB irrespective of Lp(a) concentration. The FCRs of Lp(a)-apo(a) and Lp(a)-apoB were similar in patients with elevated Lp(a) (0.46 [95%CI 0.37–0.57 pools/day] vs 0.46 [95%CI 0.37–0.58 pools/day], P = 0.861). There were also no significant differences in the FCRs of Lp(a)-apo(a) and Lp(a)-apoB in patients with normal Lp(a) concentration (0.47 [95%CI 0.38–0.58] pools/day vs 0.48 [95%CI 0.39–0.60] pools/day], P = 0.373).

3.3. Correlation and agreement between Lp(a)-apo(a) and Lp(a)-apoB FCR

The FCRs of Lp(a)-apo(a) and Lp(a)-apoB were highly correlated in patients with elevated and normal Lp(a) concentrations (Fig. 1C and D, r = 0.970 and 0.979, respectively, $P < 0.001$ for both). As shown in Fig. 1E and F, all values fall within the limits of agreement, consistent with substantial agreement between FCRs of Lp(a)-apo(a) and Lp(a)-apoB in patients with elevated and normal Lp(a) concentrations (r_c = 0.978 and r_c = 0.966, respectively).

4. Discussion

Our major finding was that the fractional turnover of apo(a) and apoB proteins within Lp(a) were similar, reflecting a tight coupling of these protein components, in statin-treated patients with elevated Lp(a) concentration. We also confirm this observation in statin-treated with normal Lp(a) concentration.

Previous tracer studies reported inconsistent findings regarding the metabolism of the apo(a) and apoB proteins constituents of Lp(a) particles. In a recent study of healthy normolipidemic men with a wide range of Lp(a) concentrations, we concurred with other reports showing similar turnover rates of apo(a) and apoB within Lp(a) [9]. These findings were consistent with observations in diabetic and/or renal patients [5–8]. Other studies have found that the turnover of apo(a) was slower than apoB within the Lp(a) particle [14,15]. These investigations were, however, carried out in the fed state, which may increase the exchange of apo(a), between Lp(a) and triglyceride-rich lipoproteins (TRLs) particles. We have extended previous reports by examining the metabolism of both moieties of Lp(a) in patients receiving statin therapy across a broad range of Lp(a) concentration, and particularly in those with elevated Lp(a).

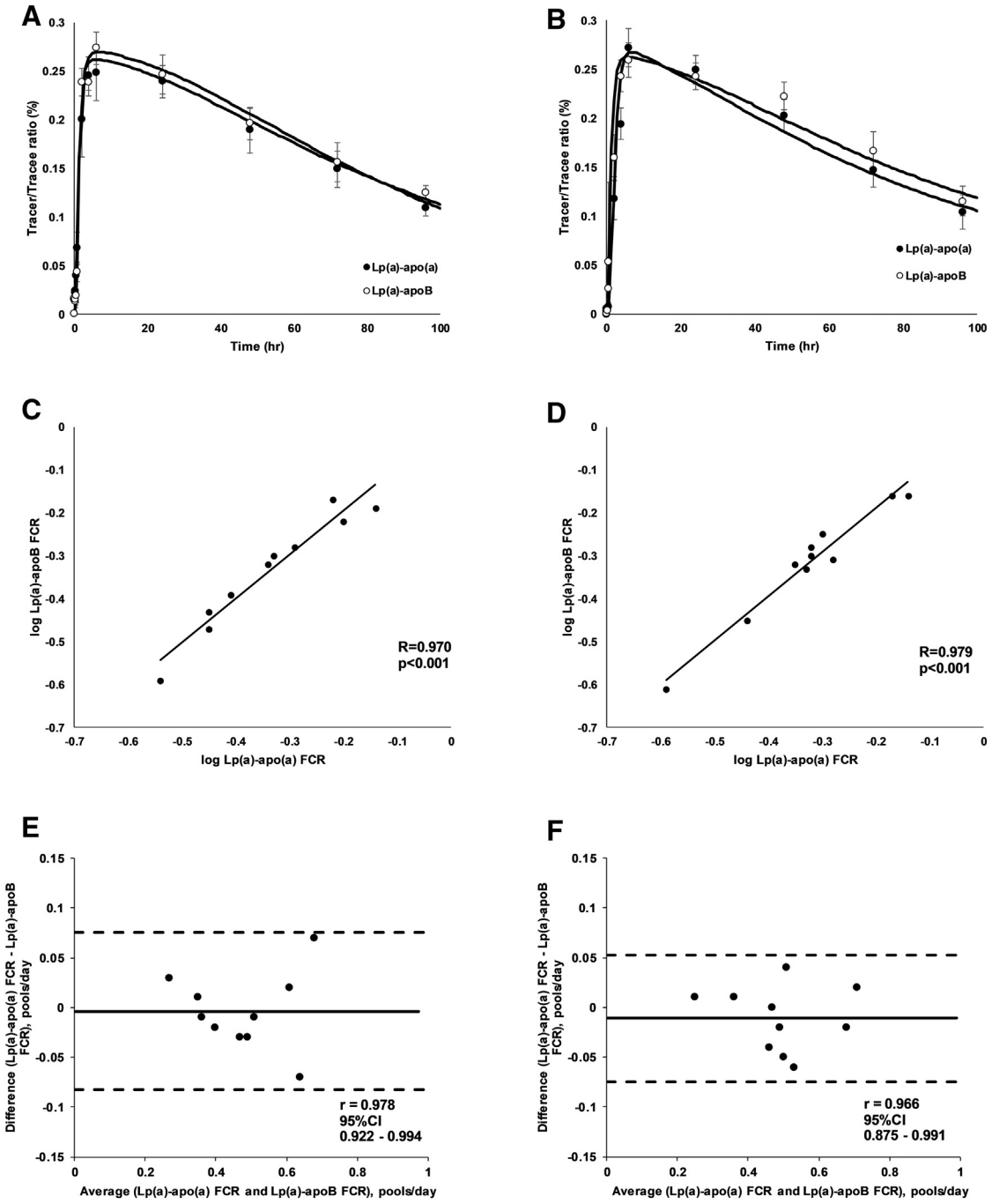

Fig. 1. Lp(a)-apo(a) and Lp(a)-apoB kinetic findings in elevated (A, C, E) and normal (B, D, F) Lp(a) patients including tracer-tracee ratio (%) (A, B), association of Lp(a)-apo(a) and Lp(a)-apoB FCR (C, D) and agreement of Lp(a)-apo(a) and Lp(a)-apoB FCR with 95% confidence interval (CI) of limitations of agreement (E, F).

It is well recognized that apo(a) and apoB components of Lp(a) are synthesised in the liver [1]. However, the exact site of assembly and transport of apo(a) and apoB components of Lp(a) are still not well understood. Some studies using isolated hepatocytes or HepG2 cells support the assembly of Lp(a) within the liver [16,17]. Patients with autosomal recessive abetalipoproteinemia have been shown to have reduced Lp(a), suggesting intracellular assembly of Lp(a) [18]. We found that the tracer curves for both protein components of Lp(a) were superimposable, supporting the former concept that apo(a) and apoB are assembled within or on the surface of hepatocytes [5–8].

In the present study, we found that the FCRs of Lp(a)-apo(a) and Lp (a)-apoB were highly correlated in patients with elevated and normal Lp(a) concentrations. This observation provides evidence that apo (a) and apoB within the Lp(a) particle are tightly coupled during its residence in the circulation and are cleared together as a holoparticle, refuting the notion of apo(a) recycling [10]. Our findings are consistent with previous kinetic studies carried out in the fasting state [5–9].

There are limitations to this study. The sample size was small, but comparable to previous kinetic investigations addressing the coupling of apo(a) and apoB [5–8,14,15]. We did not measure the turnover of Lp(a)-apo(a) and Lp(a)-apoB isolated from the LDL-HDL fractions. However, we previously found no significant differences between Lp (a)-apo(a) kinetic data derived from whole plasma and LDL-HDL fractions [9]. We did not measure the kinetics of both apo(a) isoforms because our gel separation method is not sensitive for isolating the minor apo(a) isoform, especially in patients with low apo

(a) concentration. Measurement of LDL-apoB kinetics would provide additional information for its association with the transport of Lp (a) particles [9].

Using tracer kinetics we provide evidence to support the concept that in statin-treated patients the FCRs of apo(a) and apoB with Lp (a) particles are similar and are tightly coupled. A recent kinetic finding of no effect in Lp(a) production with mipomersen suggests that the availability of apoB in the liver is unlikely to be rate-limiting for the assembly and production of Lp(a) particles [19]. Kinetic studies of specific inhibitors of apoB or apo(a) synthesis on the turnover of Lp(a)-apo (a) and Lp(a)-apoB may help further define the coupling of apo (a) and apoB within Lp(a) particles [19,20]. The metabolism of Lp(a)-apo(a) and Lp(a)-apoB in familial combined hyperlipidemia and familial hypercholesterolemia with elevated Lp(a) also merits investigation.

Acknowledgements

We would like to thank the nursing staff of the Clinical Research Studies Unit of the School of Medicine (Royal Perth Hospital, University of Western Australia) for providing expert clinical assistance.

Funding information

This work was supported by the National Health Medical Research Council. EMO is a Heart Foundation Future Leader Fellow (Award ID:100422).

Conflicts of interest

The authors declare no conflict of interest.

Contributions of authors

GFW, PHRB and EMMO designed the study. LM, DCC and GFW conducted the study and drafted the manuscript. LM and DCC analyzed the data. LM, DCC and PHBR undertook mathematical modeling of Lp (a) kinetics. All authors reviewed and approved the manuscript.

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