Title
Lipoprotein particles in patients with pediatric Cushing disease and possible cardiovascular risks

Authors
Makri, Angeliki
Cheung, Anita
Sinaii, Ninet
et al.

Lipoprotein particles in patients with pediatric Cushing disease and possible cardiovascular risks

Angeliki Makri[1], **Anita Cheung**[1], **Ninet Sinaii**[2], **Alan T. Remaley**[3], **Maureen Sampson**[3], **Meg Keil**[1], **Elena Belyavskaya**[1], **Charalampos Lyssikatos**[1], **Maria De La Luz Sierra**[1], **Constantine A. Stratakis**[1], **Maya Lodish**[1,4]

[1]Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), National Institutes of Health, Bethesda, Maryland; [2]Biostatistics and Clinical Epidemiology Service, National Institutes of Health, Bethesda, Maryland; [3]National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, Maryland [4]Division of Pediatric Endocrinology, Department of Pediatrics, University of California San Francisco, San Francisco, California

Abstract

Background: Cardiovascular (CV) complications are the most significant cause of mortality in adults with Cushing disease (CD); little is known about CV risk factors in children with CD. Measurement of lipoprotein particles by nuclear magnetic resonance (NMR) spectroscopy is a novel technology to assess CV risk. The objective of the current study is to analyze the NMR lipid profile in pediatric CD patients before and 1 year after remission.

Methods: NMR lipid profile was obtained via the Vantera NMR analyzer, using frozen serum samples from 33 CD patients (mean age 13.8 ± 4.0 years) evaluated between 1997 and 2017 at the National Institutes of Health (NIH) Clinical Center (CC).

Results: GlycA, triglyceride-rich particles (TRLP medium and very small sizes), LDL particles (LDLP total and large size), HDL particles (HDLP total, medium and small sizes), total cholesterol, LDL cholesterol, HDL cholesterol, GlycA inflammatory biomarker, Apolipoprotein B and Apolipoprotein A1 concentrations showed statistically significant changes after remission of CD ($p<0.05$).

Conclusion: In our study population, most of the lipid variables improved post-CDremission, with the exception of HDL and apoA1, indicating that NMR lipoprotein profile may be a helpful tool in assessing the cardiovascular risk in pediatric patients with CD.

Correspondence: Angeliki Makri, MD, MSc, Building 10, Rm 2E-5140, 10 Center Drive, Bethesda, Maryland, 20892, Tel +1 301-827-1492, Fax +1 301-451-3959, angeliki.makri@gmail.com.

Disclosure Statement: The authors have nothing to disclose.

Category of study: Clinical Research Study

Introduction

Cushing disease (CD) is a rare disease in pediatrics, with an estimated incidence of 1 to 1.5 per million children per year [1]. It is caused by ACTH overproduction from the pituitary, most often due to an ACTH-secreting pituitary microadenoma. The prolonged hypercortisolism results in the typical Cushing syndrome (CS) signs and symptoms: truncal obesity, growth deceleration, skin changes, muscle weakness, and hypertension [2, 3].

Cardiovascular complications are the most significant cause of mortality in adult patients with CD, with an estimated cardiovascular mortality rate 4-5 times higher than the general population [4, 5]. A recent meta-analysis of 14 studies reports higher prevalence of markers of early atherosclerosis in 332 adult patients with CS compared to 462 controls [6]. Cardiovascular risk factors, including metabolic syndrome, atherosclerotic plaques, elevated low-density lipoprotein, and total cholesterol levels, may persist in adults even after surgical remission has been achieved [7].

Pediatric studies of cardiovascular involvement in CD are limited. Our group had previously showed that pediatric CD patients exhibit a significant improvement of hypertension after remission is achieved, however, they remain at risk for residual hypertension[8]. We also showed that pediatric CD patients are at increased risk for metabolic syndrome with more than 60% of our cohort exhibiting signs of insulin resistance based on HOMA-IR values [9].

Measurement of lipoprotein particles using nuclear magnetic resonance (NMR) spectroscopy is a novel technology to assess cardiovascular risk. NMR spectroscopy measures the signals generated by the terminal methyl groups on lipids; lipoprotein subclass concentrations are then derived from deconvolution of the lipid methyl signal. The Vantera Clinical Analyzer is the first NMR device cleared by the FDA for use as a clinical instrument; it measures particle concentrations for all lipoprotein classes (VLDL, LDL, HDL) and subclasses (large, medium, small), as well as particle sizes for VLDL, LDL and HDL [10].

The lipoprotein particles transport cholesterol and triglycerides in the bloodstream and are direct mediators of atherosclerosis; LDL particles initiate formation of cholesterol-rich foam cells and atherosclerotic plaque, while HDL particles potentially mediate the reverse cholesterol transport by inhibiting the oxidation of LDL particles and removing cholesterol from the foam cells for delivery back to the liver [11-13]. The small LDL particles are considered more atherogenic than the large LDL particles with previous studies showing that lower levels of LDL and HDL mean particle sizes are associated with insulin resistance [14, 15].Two recent large scale studies in obese, pre-diabetic, and type 2 diabetic adolescents and young adults showed that NMR lipid profile is a better indicator of vascular damage compared to the traditional lipid profile [16, 17].

The goal of the current study was to assess the lipoprotein profile in pediatric patients with CD before treatment and 1 year after biochemical remission was achieved. We hypothesized that remission of CD would improve the NMR lipid variables. Furthermore, we investigated potential correlations between lipoprotein variables and clinical markers of CD severity, using the CD severity score and its individual components, as described previously [1].

Patients, Materials and Methods

Patient population

Out of 211 pediatric CD patients (<21 years old) evaluated under the research protocol 97-CH-0076 of the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) (January 1997 until August 2017), we identified 33 patients who remained in remission at 1 year after transsphenoidal surgery (TSS) and for whom frozen serum or plasma samples were available. The study was approved by the Investigational Review Board of NICHD and all patients and/or their parents gave written assent/consent. The NIH Clinical Research Information System was used to obtain medical records and clinical data. Samples were obtained at their initial evaluation and at the one-year post transsphenoidal resection (TSS) follow-up visit. For the purposes of this study, we defined remission of CD only based on biochemical criteria as follows: midnight cortisol <4.4mcg/dl and/or urinary free cortisol levels within the normal range at 12 months post-TSS.

NMR spectroscopy

NMR spectroscopy profile was obtained via the Vantera analyzer *(LipoScience/LabCorp Global Research Services)* at the Department of Laboratory Medicine at the NIH Clinical Center, using the LP4 algorithm. All the measurements were performed on the same analyzer. Between-day coefficient of variation is less than 11%, as determined in the performing lab. The following six categories of lipid variables were obtained (table 1): 1) Triglyceride-rich particles (TRLP-total, very large, large, medium, small and very small) are reported as nanomoles per liter with mean reference range valuesat 125.2±61.6, 0.4±1.0, 2.9±6.5, 17.9±16.2, 56.5±37.5 and 47.5±46.9 nmol/L respectively, 2) Low-density lipoprotein particle concentrations (LDLP-total, large, medium and small) are reported as nanomoles per liter with mean reference range values at 1454.0±393, 309.0±223, 676.0±405 and 469.0±431 nmol/L respectively, 3) High-density lipoprotein particle concentrations (HDLP-total, large, medium and small) are reported as micromoles per liter with mean reference range values at 24.0±3.0, 2.5±1.9, 7.7±2.7, 13.8±3.4 μmol/L respectively, 4) Mean particle sizes (triglyceride rich-TRLZ, low density lipoprotein-LDLZ and high density lipoprotein-HDLZ) are reported as nanometers with mean reference range values at 44.0±8.4, 21.0±0.5 and 8.98±0.44 nm respectively, 5) Lipids and apolipoproteins (total triglycerides-TG, total cholesterol-TC, TRL triglycerides-TRLTG, LDL cholesterol-LDLC, HDL cholesterol-HDLC, apolipoprotein B-apoB and apolipoprotein A1-apoA1) are reported as milligrams per deciliter with mean reference range values at 119.3±89.8, 193.8±36.5, 99.1±88.7, 110.5±30.7, 61.1±14.4, 87.1±23.6 and 156.8±27.8 mg/dl respectively, and finally 6) inflammatory biomarker GlycA (glycosylated acute phase proteins) is reported as micromoles per liter with mean reference range value at 402.4±65.8μmol/L.

Cushing disease severity score

The CD severity score was previously introduced by our group to quantify the clinically relevant manifestations of cortisol exposure in children. Seven individual factors are used in the scoring (degree of hypercortisolemia, impaired glucose tolerance, hypertension, height z-score, BMI z-score, time to diagnosis, and tumor size) and each of these factors has been

independently associated with worse outcome. A full description of the CD severity score is outlined in the paper by Gkourogianni et al [1].

Statistical analysis

All summary statistics are reported as mean ± SD. All reported p-values are two-tailed, with p<0.05 considered to be statistically significant. Paired comparisons utilizing either the paired t-test or non-parametric Wilcoxon signed-rank test were performed to compare lipoprotein variables in cured CD patients before and 1-year after TSS. In these paired comparisons, stepdown Bonferroni corrections for multiple comparisons were carried outfor groups of related measures,and corrected p-values are reported where applicable.Correlation analyses were done using Pearson's correlation coefficient or Spearman's rho, as appropriate, between CD severity score parameters and the patients' lipoprotein profile. Data were analyzed using SAS v9.4 (SAS Institute, Inc, Cary, NC).

Results

Patient population

Out of 211 pediatric CD patients (<21 years old) evaluated at NIH since 1997, we identified 33 patients (11 males, 22 females, mean age 13.8 ± 4.0years) who achieved biochemical remission at 1 year post-TSS and for whom pre- and post-surgery frozen serum or plasma samples were available. Pre- and post-surgery samples were drawn between 0-12 months before TSS, and 3-13 months (except two at 48 and 49 months) after TSS. Clinical and biochemical characteristics of CD patients are summarized in table 2.

NMR spectroscopy

Six categories of NMR lipid variables were analyzed in 33 CD patients in remission and their values were compared before and after surgery. As pediatric normative data for the NMR lipid parameters are not available in the literature thus far, we compared the delta changes of each lipid variable before and after cure (table 3). There were robust, statistically significant effects on several lipid variables: GlycA (delta=−69.0, 95% CI −115.6 − −22.4), triglyceride rich particles TRLP (medium: delta = 6.2, 95% CI 1.9-10.5; and very small: delta = −27.1, 95% CI −44.1- −10.2), LDLP (total: delta = −271.6, 95% CI −465.4 − −77.7; and large: delta = −142.9, 95% CI −235.1 − −50.6), HDLP (total: delta = −3.6, 95% CI −5.6 − −1.6; medium: delta = −2.0,95% CI −3.2 − −0.7; and small: delta = −1.9,95% CI −3.1 − −0.8), TC (delta = −31.5, 95% CI −53.7 − −9.3), LDLC (delta = −23.1,95% CI −39.3 − −6.8), HDLC (delta = −7.5, 95% CI −13.2 − −1.9), ApoB(delta = − 16.5,95% CI −28.3 − −4.8) and ApoA1 (delta = −17.5, 95% CI −31.2 − −3.9).

CD severity score

CD severity score was calculated as following: degree of hypercortisolemia (24 hour UFC normal = 0; 24 hour UFC up to 3x normal = 1; 24 hour UFC > 3x normal = 2), impaired glucose tolerance (fasting plasma glucose FPG <100 = 0; FGP >= 100 = 1; FPG >=126 or diabetes mellitus diagnosis = 2), hypertension (SBP & DBP z-score both <2=0; SBP or DBP z score >= 2 =1; HTN-Tx = 2), height z-score (>−0.5 = 0; <=−0.5 = 1), BMI z-score (<2 = 0; >=2 = 1), time to diagnosis (<3 years = 0; >=3 = 1) and tumor characteristics (adenoma

<5 mm = 0; adenoma >=5 mm and or CS invasion = 1). Pre-TSS GlycA, TRLP (medium and very small sizes), LDLP (large size), HDLP (medium size), HDLC, ApoA1 showed statistically significant individual correlations with one or more of the following CD severity score variables: BMI z-score, 24-h urinary cortisol concentrations, midnight serum cortisol levels, early morning serum cortisol levels, and age at surgery.

Table 4 summarizes the correlations of individual NMR lipid variables with CD severity score variables that reached statistical significance and carry potential importance. Total HDL-particle, apolipoprotein A1 (surrogate of HDL) and HDL cholesterol are all considered to exert a protective cardiovascular effect and all showed a reverse correlation with pre-TSS morning serum cortisol levels (r=−0.36, r= −0.45 and r=−0.50, respectively), indicating that the higher the degree of hypercortisolemia, the worse the patient's HDL lipid profile (Figure 1a,1b, and 1d). Also, the very small triglyceride rich particles (VSTRLP), that act by inhibiting the protective mechanism of lipolysis, showed a positive correlation with the morning cortisol values (r=0.37) and also exhibited a robust post-TSS decrease by −27.1 (p=0.0162) (table 3). Thus, a higher cortisol level, which indicates more severe CD, is clinically correlated with a higher VSTRLP value (Figure 1c). Finally, GlycA, a novel inflammatory biomarker, that showed a robust postop decrease by −69.0 (p=0.0050), also exhibited a positive correlation with BMI z-score (r=0.47). The higher the BMI, the worse the CD, which clinically correlates with a higher GlycA level (Figure 2).

Discussion

This is the first study to our knowledge to report NMR spectroscopy data in pediatric patients with CD in order to assess their cardiovascular risk. We hypothesized that normalization of cortisol levels post-TSS would improve the patient's lipoprotein levels and thus have a positive effect on their atherogenic profile. In contrast to regular lipid profile that only measures lipoprotein concentrations, NMR has the ability to measure lipoprotein particle number, size, and concentration [12].

Clinical interpretation of the individual NMR lipid variables is not yet well-established in the literature. The total LDL-particle (LDLP) is approved by the FDA for clinical use in adults. Matyus et al examined the analytical performance of the NMR-based LDLP assay as per clinical laboratory standards institute (CLSI) guidelines and report mean LDLP value at 1193 ± 472 nmol/L (95% CI 457-2282) [10]. In our pediatric population, the mean pre-TSS value was 1,751.1± 578.5 and decreased to 1479.5 ± 506.2 post-surgery (p=0.0225), which represents a significant post-cure decrease; however it did not reach the normal adult reference range of <1,200nmol/L. 21% of our patients continued to have abnormal LDLP levels at 1 year follow up, implying that some lipid abnormalities may persist even after CD is cured. A recent study reported total LDLP values, as part of the NMR lipid profile, using the 400-MHz proton NMR analyzer at Liposcience, in 232 lean, 228 obese and 214 type 2 diabetics adolescents and young adults at 849.7 ± 248, 1102.6 ± 326.8 and 1257 ± 437.9nmol/L respectively [16]. Shah et al measured NMR lipoprotein data, using the LipoProfile-3 algorithm by LipoScience, in 118 obese normoglycemic and 96 obese prediabetic youth; mean total LDLP levels were1037 (834, 1266) and 1098 (898, 1353) nmol/L respectively [17]. As compared to both these studies, keeping in mind that

coefficient variations between machines may be a consideration, our patient population had much higher LDLP values even after CD was cured, implying that the dyslipidemia associated with CD might be more severe than the degree of dyslipidemia associated with obesity or type 2 diabetes mellitus.

All LDL particles decreased post-cure, however, only the total and large LDL particles were statistically significant. Furthermore, very small TRLP, a very proatherogenic form of triglyceride-rich particles, showed a significant post-TSS decrease by −27.1 (p=0.0162). The GlycA NMR signal is a novel inflammatory biomarker that reflects the aggregated levels of several glycosylated acute phase proteins and is associated with increased cardiovascular disease risk in adults [18]. In our study, GlycA levels decreased post-cure, indicating an improvement in the patients' atherogenic profile after cortisol normalization. Importantly, BMI z-score showed a positive correlation with GlycA levels (r=0.47), however, we interpret this result with caution as obesity itself may contribute to the abnormal GlycA regardless of the CD. In our study, the mean BMI z-score improved from 1.88±1.00 pre-surgery to 1.61±0.78 post-surgery, indicating that obesity was still a consideration despite cortisol normalization. Jago et al recently analyzed by NMR spectroscopy GlycA levels in 1664 adolescents and showed that GlycA was significantly higher in higher BMI groups [19].

While most of the lipid markers (TRLP, LDLP, TC, LDL, apoB) in our study changed in a cardiovascular advantageous direction in the post-op samples, this did not hold true for the HDL particles and apoA1, which both decreased post-cure of CD. We do not have a reasonable explanation for the paradoxical worsening of the HDL markers after normalization of the cortisol levels, however this seems to be in accordance with prior studies reporting a variable effect of Cushing syndrome on HDL cholesterol; different studies have shown both increases and decreases of the HDL and apoA1 levels [20, 21]. Of note, the effect of Cushing syndrome on LDL cholesterol and apoB is consistent among different series; LDL cholesterol is increased in patients with Cushing syndrome, and it often correlates with the severity of the disease [20-22].

Limitations of our study include the relatively small patient population and the retrospective design of the study that did not allow us to calculate cardiovascular risk and HOMA-IR scores, as we did not routinely get insulin levels and/or waist circumference during CD evaluation. Furthermore, the retrospective nature of the study in combination with the rarity of CD in pediatrics accounted for the lack of consistency at the post-surgery sampling times among our study participants, as most patients were referred to our center by primary hospitals across the country. Study limitations also includes lack of a control or comparator population for variables that may affect lipid measurements over time, such as pubertal change during the study period, race/ethnicity, family history, diet, exercise, body composition and concomitant and conditions (including genetic associations).

NMR spectroscopy is an emerging technology in the field of lipidiology; thus its superiority in addressing cardiovascular disease parameters as compared to traditional lipid concentrations, has not yet been established in the pediatric population. However, recent studies have demonstrated that NMR lipid profiles are more strongly associated with cardiovascular risk factors in adolescents and young adults compared to conventional lipid

profiles [16,17]. Urbina et al [16] compared NMR and traditional lipid profiles in 214 lean, 228 obese, and 214 T2 diabetic patients (10-24 years old), and found that while LDL concentration was similar in the obese and diabetic group, the LDL size measured by NMR, was able to differentiate the 3 groups (p 0.0001). Using general linear models, that included patient demographics, risk factors and traditional or NMR lipid parameters and a composite vascular function score, the authors demonstrated that NMR lipid profiles were better in predicting vascular damage and were more strongly associated to vascular function, as compared to traditional lipid profiles. Shah et al [17] compared NMR and traditional lipid profiles in 96 obese youth with prediabetes and 118 obese normoglycemic controls and report that while conventional lipid profiles were not significantly different between groups, patients with prediabetes had higher levels of small LDL-P and HDL-P, as measured by NMR.

CD although rare, can serve as a model to study the effect of excess cortisol on cardiovascular disease. In our cohort of 33 CD pediatric patients who achieved biochemical remission at 1 year post-TSS, there was a significant post-cure reduction in most of the lipid variables that were measured, with the exception of the HDL and ApoA1 markers, despite the fact that most patients were peri-pubertal, and there is a well-documented tendency of lipid profile worsening in puberty. Our hypothesis is that the improvement in the lipid profile was due to a combination of lowering the cortisol levels and the BMI. Approaching dyslipidemia in patients with CD is challenging; even with normalization of the cortisol levels, lipid markers might remain elevated long after remission was achieved, suggesting that dyslipidemia in this population should be a life-long concern, with appropriate follow-up.

In conclusion, NMR lipoprotein profile may serve as a marker of cardiovascular risk in pediatric patients with CD. Further studies are needed to see if lipoprotein markers are associated with preclinical markers of atherosclerosis in a larger cohort.

Acknowledgments

References

1. Gkourogianni A, Sinaii N, Jackson SH. et al., Pediatric Cushing disease: disparities in disease severity and outcomes in the Hispanic and African-American populations. Pediatric Research, 2017 82(2): p. 272–277. [PubMed: 28422946]

2. Stratakis CA, Cushing Syndrome in Pediatrics. Endocrinology and Metabolism Clinics of North America, 2012 41(4): p. 793–803. [PubMed: 23099271]

3. Holst JM, Horvath-Puho E, Jensen RB, et al., Cushing's syndrome in children and adolescents: a Danish nationwide population-based cohort study. European Journal of Endocrinology, 2017 176(5): p. 567–574. [PubMed: 28179451]

4. Dekkers OM, Horvath-Puho E, Jorgensen JO, et al., Multisystem morbidity and mortality in Cushing's syndrome: a cohort study. J Clin Endocrinol Metab, 2013 98(6): p. 2277–84. [PubMed: 23533241]

5. Etxabe J and Vazquez JA, Morbidity and mortality in Cushing's disease: an epidemiological approach. Clin Endocrinol (Oxf), 1994 40(4): p. 479–84. [PubMed: 8187313]

6. Lupoli R, Ambrosino P, Tortora A, et al., Markers of atherosclerosis in patients with Cushing's syndrome: a meta-analysis of literature studies. Ann Med, 2017 49(3): p. 206–216. [PubMed: 27763781]

7. Faggiano A, Pivonello R, Spiezia S, et al., Cardiovascular risk factors and common carotid artery caliber and stiffness in patients with Cushing's disease during active disease and 1 year after disease remission. J Clin Endocrinol Metab, 2003 88(6): p. 2527–33. [PubMed: 12788849]

8. Lodish MB, Sinaii N, Patronas N, et al., Blood pressure in pediatric patients with Cushing syndrome. J Clin Endocrinol Metab, 2009 94(6): p. 2002–8. [PubMed: 19293264]

9. Libuit LG, Karageorgiadis AS, Sinaii N, et al., A gender-dependent analysis of Cushing's disease in childhood: pre- and postoperative follow-up. Clin Endocrinol (Oxf), 2015 83(1): p. 72–7. [PubMed: 25388128]

10. Matyus SP, Braun PJ, Wolak-Dinsmore J, et al., NMR measurement of LDL particle number using the Vantera® Clinical Analyzer. Clinical Biochemistry, 2014 47(16-17): p. 203–210. [PubMed: 25079243]

11. Jeyarajah EJ, Cromwell WC, and Otvos JD, Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. Clin Lab Med, 2006 26(4): p. 847–70. [PubMed: 17110242]

12. Mora S, Advanced lipoprotein testing and subfractionation are not (yet) ready for routine clinical use. Circulation, 2009 119(17): p. 2396–404. [PubMed: 19414657]

13. Mackey RH, Mora S, Bertoni AG, et al., Lipoprotein particles and incident type 2 diabetes in the multi-ethnic study of atherosclerosis. Diabetes Care, 2015 38(4): p. 628–36. [PubMed: 25592196]

14. Garvey WT, Kwon S, Zheng D, et al., Effects of Insulin Resistance and Type 2 Diabetes on Lipoprotein Subclass Particle Size and Concentration Determined by Nuclear Magnetic Resonance. Diabetes, 2003 52(2): p. 453–462. [PubMed: 12540621]

15. Wang J, Stancakova A, Soininen P, et al., Lipoprotein subclass profiles in individuals with varying degrees of glucose tolerance: a population-based study of 9399 Finnish men. J Intern Med, 2012 272(6): p. 562–72. [PubMed: 22650159]

16. Urbina EM, McCoy CE, Gao Z, et al., Lipoprotein particle number and size predict vascular structure and function better than traditional lipids in adolescents and young adults. J Clin Lipidol, 2017 11(4): p. 1023–1031. [PubMed: 28826565]

17. Shah AS, Davidson WS, Gao Z, et al., Superiority of lipoprotein particle number to detect associations with arterial thickness and stiffness in obese youth with and without prediabetes. J Clin Lipidol, 2016 10(3): p. 610–8. [PubMed: 27206949]

18. McGarrah RW, Kelly JP, Craig DM, et al., A Novel Protein Glycan-Derived Inflammation Biomarker Independently Predicts Cardiovascular Disease and Modifies the Association of HDL Subclasses with Mortality. Clin Chem, 2017 63(1): p. 288–296. [PubMed: 27811210]

19. Jago R, Drews KL, Otvos JD, et al., Novel measures of inflammation and insulin resistance are related to obesity and fitness in a diverse sample of 11-14 year olds: The HEALTHY Study. Int J Obes (Lond), 2016 40(7): p. 1157–63. [PubMed: 27143035]

20. Feingold K, B.E., Grunfeld C, The Effect of Endocrine Disorders on Lipids and Lipoproteins, C.G. De Groot LJ, Dungan K, et al., editors, Editor. 2017 2 24: Endotext [Internet] South Dartmouth (MA).

21. Arnaldi G, Scandali VM, Trementino L, et al., Pathophysiology of Dyslipidemia in Cushing's Syndrome. Neuroendocrinology, 2010 92(1): p. 86–90. [PubMed: 20829625]

22. Mancini T, Kola B, Mantero F, Boscaro M, Arnaldi G, High cardiovascular risk in patients with Cushing's syndrome according to 1999 WHO/ISH guidelines. Clin Endocrinol (Oxf), 2004 61(6): p. 768–77. [PubMed: 15579193]
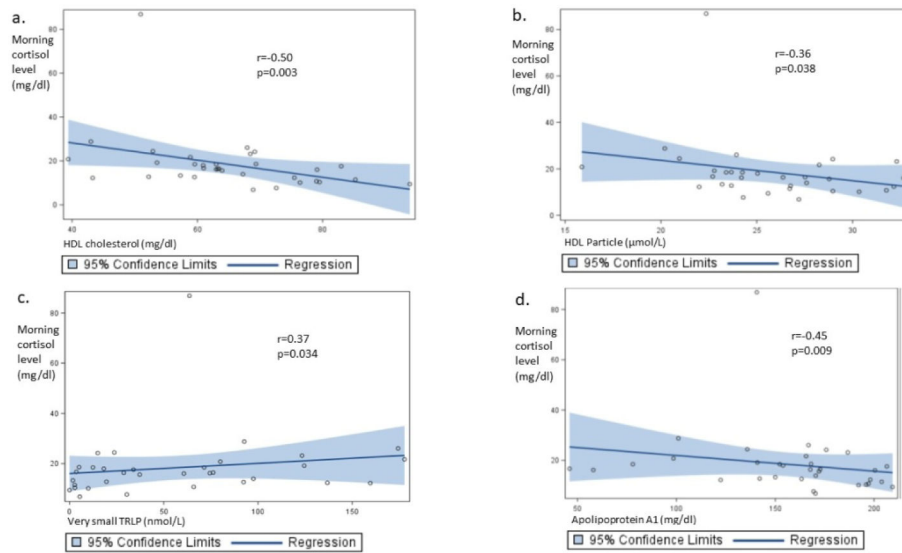
Figure 1.
Clinically relevant correlations between lipoprotein variables and morning cortisol levels before transsphenoidal surgery. a-d: HDL cholesterol, HDL-particle, very small triglyceride rich particles, and apolipoprotein A1, showed a significant correlation with morning serum cortisol levels (r=−0.50, r=−0.36, r = 0.37, and r= −0.45 respectively).
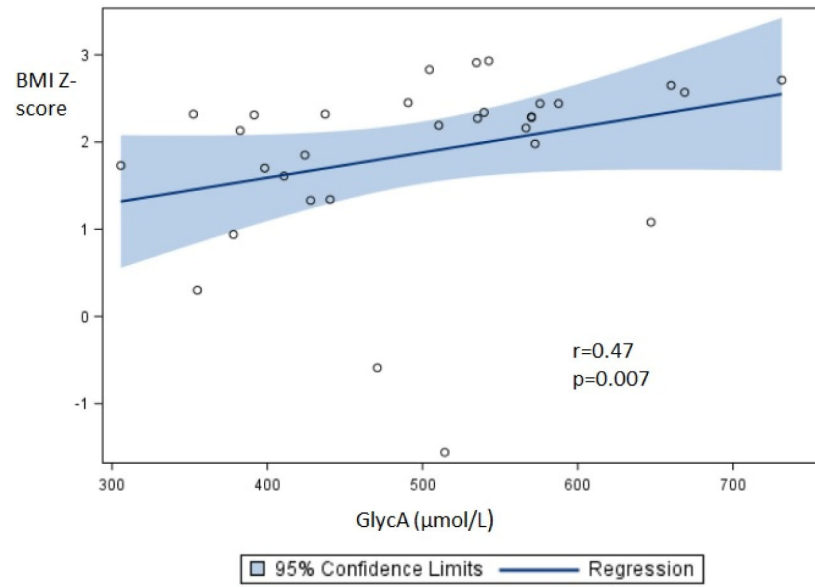
Figure 2:
Pre-transsphenoidal surgeryGlycA showed a positive correlation with BMI z-score (r=0.47)

Table 1:

NMR lipid variables, clinical and biochemical characteristics at pre- and post- transsphenoidal surgery (TSS) in 33 patients with Cushing's disease in remission

Variable	Definitions	Reference Range (5th – 95th %ile)	Pre-TSS Value (mean ± SD)	Post-TSS value (mean ± SD)
Triglyeride-Rich Particles (nmol/L)				
TRLP	Total TRLP	42 – 239	120.9 ± 66.8	103.3 ± 47.8
VLTRLP	Very Large TRLP	0 – 1.6	0.26 ± 0.23	0.24 ± 0.35
LTRLP	Large TRLP	0 – 12.8	4.4 ± 5.1	4.0 ± 5.3
MTRLP	Medium TRLP	0.3 – 48.4	8.5 ± 10.2	14.7 ± 11.2
STRLP	Small TRLP	7.3 – 124.4	49.5 ± 25.4	53.3 ± 26.2
VSTRLP	Very Small TRLP	0 – 142.3	58.2 ± 53.8	31.1 ± 33.4
LDL Particles (nmol/L)				
LDLP	Total cLDLP	891 – 2150	1751.1 ± 578.5	1479.5 ± 506.2
LLDLP	Large LDLP	17 – 748	450.3 ± 279.7	307.4 ± 140.0
MLDLP	Medium LDLP	0 – 1377	843.9 ± 662.2	752.7 ± 560.4
SLDLP	Small LDLP	13 – 1318	456.9 ± 435.4	419.4 ± 501.0
HDL Particles (µmol/L)				
HDLP	Total cHDLP	19.2 – 29.3	26.0 ± 4.0	22.4 ± 4.3
LHDLP	Large HDLP	0.2 – 6.3	1.9 ± 1.7	2.2 ± 1.6
MHDLP	Medium HDLP	3.7 – 12.6	9.4 ± 2.5	7.4 ± 2.0
SHDLP	Small HDLP	8.1 – 19.6	14.7 ± 3.3	12.8 ± 3.6
Mean Particle Sizes (nm)				
TRLZ	TRL Size	33.8 – 60.9	46.9 ± 7.4	45.6 ± 7.9
LDLZ	LDL Size	20.1 – 21.7	21.09 ± 0.52	21.04 ± 0.41
HDLZ	HDL Size	8.3 – 9.8	8.95 ± 0.34	9.03 ± 0.32
Lipids and Apolipoproteins (mg/dL)				
TG	Total Triglycerides	43 – 276	107.2 ± 58.6	115.6 ± 61.6
TC	Total Cholesterol	140 – 256	222.8 ± 59.7	191.3 ± 50.9
TRLTG	TRL Triglycerides	25 – 238	84.9 ± 54.6	96.4 ± 58.2
LDLC	LDL Cholesterol	63 – 163	135.1 ± 48.9	112.0 ± 38.4
HDLC	HDL Cholesterol	41 – 88	65.0 ± 12.5	57.4 ± 12.9
ApoB	Apolipoprotein B	53 – 127	110.6 ± 36.3	94.1 ± 27.9
ApoA-1	Apolipoprotin A-1	116 – 209	156.7 ± 41.6	139.2 ± 43.1
Inflammatory Biomarker (µmol/L)				
GlycA	GlycA	307 – 524	508.6 ± 110.3	439.6 ± 109.1
Clinical and Biochemical Characteristics				
Height Z-score	cm	n/a	−0.69 ± 1.51	−0.75 ± 1.19
BMI Z-score	kg/m2	n/a	1.88 ± 1.00	1.61 ± 0.78
SBP Z-score	mmHg	n/a	1.37 ± 1.12	0.45 ± 1.17

Variable	Definitions	Reference Range (5th – 95th %ile)	Pre-TSS Value (mean ± SD)	Post-TSS value (mean ± SD)
DBP Z-score	mmHg	n/a	0.44 ± 1.16	−0.23 ± 0.83
Midnight Serum Cortisol	mcg/dl	<4.4mcg/dl	17.36 ± 14.51	1.30 ± 0.70
Morning Serum Cortisol	mcg/dl	5-25mcg/dl	18.42 ± 13.39	4.77 ± 3.91
24-hour Urinary Free Cortisol	mcg/dl	4.0-56mcg/24h	340.10 ± 514.91	6.60 ± 9.03
Fasting plasma glucose	mg/dl	70-99mg/dl	111.09 ± 36.56	91.63 ± 13.64

Table 2:

Demographic data, clinical and biochemical characteristics at diagnosis of 33 patients with CD

Patient characteristics	mean ± SD **
Age (years)	13.8 ± 4.0
Gender (n, M/F)	11 / 22
BMI z-score (kg/m2) [*]	1.88 ± 1.00 (2.27, 1.61 - 2.44)
Height z-score (cm)	−0.69 ± 1.51
Tanner stage at diagnosis	3.2 ± 1.5
Systolic BP z-score (mmHg)	1.37 ± 1.12
Diastolic BP z-score (mmHg)	0.44 ± 1.16
Midnight serum cortisol (mcg/dl) [*]	17.36 ± 14.51 (13.35, 9.55 – 21.35)
Morning serum cortisol (mcg/dl) [*]	18.42 ± 13.39 (16.35, 12.35 – 19.2)
24-hour Urinary Free Cortisol (mcg/24 hr) [*]	340.10 ± 514.91 (175.5, 87.6 – 421.9)
Fasting plasma glucose (mg/dl) [*]	111.09 ± 36.56 (105.0, 91.0 – 114.0)
Time to diagnosis (years)	2.71 ± 1.88
Tumor size (mm) [*]	6.61 ± 5.04 (6.00, 4.00 - 8.00)
CD severity score [*]	5.67 ± 1.95 (6.0, 5.0 – 7.0)
Time to post-TSS sampling (months after TSS) [*]	9.72 ± 10.35 (7.0, 6.0-10.0)

[*]
not approximately normally distributed variables, for which the median with the interquartile ranges (25th-75th percentiles) are also reported into parentheses.

Table 3:

NMR lipid profile changes from pre- to post- transsphenoidal surgery (TSS) in 33 patients with Cushing's disease in remission

Variable			P-value	95% CI
	Triglyeride-RichParticles(nmol/L)			
TRLP	Total TRLP	−17.6	0.1147	−39.7 – 4.5
VLTRLP	VeryLarge TRLP	−0.03	0.1171	−0.18 – 0.12
LTRLP	Large TRLP	−0.4	0.5226	−2.6 – 1.9
MTRLP	Medium TRLP	6.2	0.0305[*]	1.9 – 10.5
STRLP	Small TRLP	3.7	0.4098	−5.4 – 12.9
VSTRLP	Very Small TRLP	−27.1	0.0162[*]	−44.1 – −10.2
	LDL Particles(nmol/L)			
LDLP	Total cLDLP	−271.6	0.0225[*]	−465.4 – −77.7
LLDLP	Large LDLP	−142.9	0.0140[*]	−235.1 – −50.6
MLDLP	Medium LDLP	−91.2	0.3985	−308.3 – 125.9
SLDLP	Small LDLP	−37.5	0.1585	−225.0 – 150.0
	HDL Particles(μmol/L)			
HDLP	Total cHDLP	−3.6	0.0004[*]	−5.6 – −1.6
LHDLP	Large HDLP	0.3	0.1549	−0.1 – 0.7
MHDLP	Medium HDLP	−2.0	0.0064[*]	−3.2 – −0.7
SHDLP	Small HDLP	−1.9	0.0009[*]	•3.2 – −0.8
	Mean Particle Sizes (nm)			
TRLZ	TRL Size	−1.3	0.3728	−4.3 – 1.6
LDLZ	LDL Size	−0.04	0.6269	−0.22 – 0.14
HDLZ	HDL Size	0.08	0.1001	−0.02 – 0.18
	Lipids and Apolipoproteins(mg/dL)			
TG	Total Triglycerides	8.4	0.4561	−13.8 – 30.7
TC	Total Cholesterol	−31.5	0.0069[*]	−53.7 – −9.3
TRLTG	TRL Triglycerides	11.5	0.2553	−9.5 – 32.5
LDLC	LDL Cholesterol	−23.1	0.0068[*]	−39.3 – −6.8
HDLC	HDL Cholesterol	−7.5	0.0102[*]	−13.2 – −1.9
ApoB	Apolipoprotein B	−16.5	0.0073[*]	−28.3 – −4.8
ApoA-1	Apolipoprotein A1	−17.5	0.0133[*]	−31.2 – −3.9
	InflammatoryBiomarker(μmol/L)			
GlycA	Glycosylated acute phase proteins	−69.0	0.0050[*]	−115.6 – −22.4

[*] statistically significant at p<0.05, corrected for multiple comparisons as applicable

Table 4:

Statistically significant and potentially important correlations of lipid variables with Cushing's disease (CD) severity score variables

Lipid Profile Variable	CD Severity Score Variable	Correlation Coefficient (r) *	95% CI	P-value
Medium TriglycerideRichparticle (MTRLP)	Total severity score	−0.40	−0.65 – −0.07	0.020
	24h Urinary free cortisol	−0.40	−0.66 – −0.07	0.020
	Midnightserum cortisol	−0.36	−0.62 – −0.02	0.041
VerysmallTriglycerideRichparticle (VSTRLP)	Morningserum cortisol	0.37	0.03 – 0.63	0.034
Large LDL-particle (LLDLP)	Age at surgery	−0.39	−0.65 – −0.06	0.023
Total HDL-particle (HDLP)	Morningserum cortisol	−0.36	−0.63 – −0.02	0.038
Medium HDL-particle (MHDLP)	BMI z-score	0.46	0.13 – 0.70	0.008
HDL mean particlesize (HDLZ)	Age at surgery	−0.40	−0.66 – −0.07	0.020
HDL colesterol (HDLC)	Morningserum cortisol	−0.50	−0.72 – −0.19	0.003
Apolipoprotin A-1(ApoA1)	Morningserum cortisol	−0.45	−0.69 – −0.12	0.009
GlycA	BMI z-score	0.47	0.14 – 0.71	0.007

*
Correlation coefficients are either Pearson's or Spearman's, as applicable; 95% Confidence Intervals (CIs) and p-values are based on Fisher's z transformation.