EFFECT OF METFORMIN ON LIPOPROTEIN PARTICLE CONCENTRATION IN PEDIATRIC CARDIOMETABOLIC PATIENTS

Tara L. Dall,[1] Madhuri M. Vasudevan,[2] Christie M. Ballantyne,[2] Ray Pourfarzib,[3]

1. Advanced Lipidology, Delafield, WI; 2. Baylor College of Medicine and Methodist DeBakey Heart Center, Houston, TX; 3. LipoScience, Inc., Raleigh, NC

INTRODUCTION

As the incidence of childhood obesity continues to increase globally to near epidemic proportions, there is an associated increase in the prevalence of metabolic syndrome and Type 2 diabetes in adolescence. In the past 20 years, the prevalence of an adolescent with a body mass index (BMI) above the 95th percentile has increased by more than 50%.[1,2] Although statin therapy has been shown in small studies to improve insulin resistance, recent large randomized trials in adults showed no benefit.[3,4] Metformin therapy in adults with metabolic syndrome has favorable effects on glycemic control, weight and lipid parameters.[5] Metformin hydrochloride is an oral antihyperglycemic agent that has been prescribed for over 40 years. It is now available as an inexpensive generic in both short acting and long acting formulations.[6,7]

Despite the success of cholesterol screening and low-density lipoprotein cholesterol (LDL-C) lowering therapy in the diabetic population, it has been recognized that many patients with low or moderate LDL-C levels still experience CHD events.[8,9] The limitation of testing LDL-C is that cholesterol is only one type of lipid carried within low-density lipoprotein particle (LDL-P) "containers" and its measurement provides only an approximation of the numbers of LDL particles.[10]

To our knowledge, no study to date has directly addressed the question of the effect of metformin on LDL particle concentration measured by nuclear magnetic resonance (NMR) in the pediatric population with metabolic syndrome. Hence, the aim of this study was to examine the discordance between LDL-C and LDL-P in adolescent patients with increased cardiometabolic risk and to compare the effects of metformin plus TLC therapy versus TLC therapy alone on standard lipid profile measurements and lipoprotein particle number and size as measured through the *NMR LipoProfile®* test analyzer.

METHODS

Patient Selection and Study Design. A total of 78 pediatric patients were seen in the lipid clinic over the period of January 2006 through May 2008. A retrospective chart review was conducted in the office and 20 patients met the study criteria and were eligible for the analysis (Table 1). The criteria for analysis included patients with increased cardiometabolic risk and at least 2 available NMR lipoprotein data sets in the time course specified. Increased cardiometabolic risk was defined as having one or more of the following: diabetes, obesity, family history of premature cardiovascular disease, impaired fasting glucose or lipid abnormalities on traditional lipid panel or at least 2/3 metabolic markers on NMR lipoproteins (small dense LDL-P predominance, reduced large HDL P, excess large VLDL-P). The treating physician abstracted the data from the patient's medical records.

Defining Treatment Goals. All pediatric patients presenting to the lipid clinic received trial of therapeutic lifestyle changes as noted in table 2. Subjects were then assigned to receive either metformin with diet and exercise (TLC) or TLC alone. The protocol for assigning patients to one of these two groups is detailed in table 3.

The patients were seen at varying frequency, but most were seen every 4-8 weeks. Based on criteria listed in table 3 some patients were placed on metformin therapy in addition to TLC after initial dietary trial. All patients were started on once daily metformin 500 mg with titration to 500 mg twice daily as tolerated, typically in 2-4 weeks. Goal of treatment was weight loss and improvement in lipoprotein parameters with optimal LDL-P <1100 nmol/L. This is based on NCEP pediatric lipid guidelines of LDL-C <110 mg/dL.[11]

Laboratory Analysis. All lipid and lipoprotein particle analyses were conducted on the same plasma specimens by the same laboratory. Total cholesterol, triglycerides, and HDL cholesterol (HDL-C) were measured by standardized automated methods, and LDL-C was calculated by the Friedewald equation.12 Concentrations of LDL-P were determined by automated nuclear magnetic resonance spectroscopic at LipoScience.

Statistical Analysis. Nine subjects were selected to receive TLC, and 11 subjects were selected to receive metformin plus TLC. Homoscedastic student's t-test was used to compare the differences in lipoprotein parameters between TLC-treated subjects and metformin plus TLC-treated subjects at baseline visit. A paired student's t-test was used to compare the differences in lipoprotein parameters in TLC-treated patients before and after therapy, and compare metformin plus TLC-treated subjects before and after therapy.

RESULTS

Baseline Characteristics. In the metformin plus TLC and TLC group, the mean age was 12.5 and 12 years of age, respectively (Table 4). For all of the subjects (N= 20) at the initial visit there was discordance between LDL-P versus LDL-C and non-HDL-C (Table 5). Comparing LDL-C less than 110 mg/dL and LDL-P less than 1100 nmol/L, 68% of the subjects had LDL-C less than 110 mg/dL and 15% of the subjects had LDL-P less than 1100 nmol/L.

At baseline, there were no significant differences in lipid levels between those selected for treatment with TLC alone, versus those selected for treatment with metformin plus TLC, except for LDL-C, which was higher (p=0.037) in the metformin plus TLC-treated group (average LDL-C 114.45 mg/dL) compared to the LDL-C level in the TLC treated group (average LDL-C 85.75 mg/dL). In the TLC-treated group, small LDL-P decreased by 21% (p=0.022) after an average 5.4 months of therapy. In the metformin plus TLC-treated group, after an average 6.7 months of therapy, large HDL-P increased by 47 % (p=0.037), LDL particle number decreased by 34% (p=0.002), small LDL-P decreased by 57% (p=0.002), LDL particle size increased by 5% (p=0.001), LDL-C decreased by 14% (p=0.025), total cholesterol decreased by 8% (p=010), and Non-HDL-C decreased by 16% (p=0.001). In the metformin plus TLC treated group, although there was a 21% increase in HDL-C, this did not reach clinical significance. No significant differences were found in TG levels, large VLDL-P, glucose, HbA1C or weight before and after therapy in the metformin plus TLC-treated group (Table 6).

During therapy, total LDL-P decreased 34% in the metformin treated group (p=0.002). Small LDL-P ($p < 0.005$), LDL-C ($p < 0.05$), and Non-HDL-C ($p < 0.005$) also decreased in the metformin treated patients (-57%, -14%, -16%, respectively). Large HDL-P value increased by 47% ($p < 0.05$).

In the TLC group at baseline, the mean values for LDL-C, non-HDL-C, and LDL-P were approximately at the 10th, 30th, and 55% percentile of the population, respectively. As expected, the mean values for LDL-C, non-HDL-C and LDL-P were also lower compared to the metformin plus TLC group (86 mg/dL, 129 mg/dL, 1457 nmol/L, respectively). Furthermore, there was no significant change in the TLC group for all lipid and lipoprotein values except a significant drop in the small LDL-P (-21%, $p < 0.05$).

CONCLUSION

The obesity epidemic and associated comorbidities in children is particularly concerning as these patients are likely to have long term cardiovascular and diabetes risk. Based on our study findings many of these children were noted to have increased atherogenic risk as defined by high number of LDL particles that were not identified by traditional LDL cholesterol measurements. Treatment of lipid disorders in children has to date focused on TLC and statin therapy. Statins are proven to reduce LDL but do not prevent diabetes. Metformin has been shown to slow progression to diabetes in previously published studies. Metformin has also been shown have beneficial effects on lipids as well as long term CV event reduction as studied in the adult population.[7]

Strong evidence now exists in the benefit of LDL-P measurement in management of CVD. What we have found in our adult population is that patients with predominantly small LDL particles and total LDL particles in excess have favorable lipoprotein/lipid changes with treatment of insulin resistance with agents such as metformin or pioglitazone in addition to TLC. If LDL-P, triglycerides, and HDL normalize with this treatment option, a need for additional higher cost lipid lowering agents may not be necessary.

Moreover, the discordance in LDL-C and LDL-P as well as Non-HDL-C and LDL-P is expected in the setting of metabolic syndrome or diabetes.[13,14] Metformin plus TLC was very effective in decreasing small LDL-P, increasing large HDL-P and decreasing large VLDL-P values which is also associated with a decrease in total LDL-P, weight loss, and often drop in triglycerides and non-HDL-C. With the rising epidemic of pediatric obesity and comorbities of metabolic syndrome, diabetes, and dyslipidemia, we need to find alternative clinical approaches. Treatment of dyslipidemia in children currently is focused on TLC and statins. However, the underlying cause of dyslipidemia in these children is often insulin resistance. Therefore, we believe this condition can be cost effectively treated with TLC and Metformin to lower their lipids and improve their weight loss.

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TABLE 1 : Patient Selection Criteria

- Pediatric population- ages 4 to 18 years of age
- Minimum of 2 visits
- Baseline and follow-up NMR LipoProfile test results available for analysis
- Diagnosis of increased cardiometabolic risk defined as having any one of the following: diabetes, obesity, impaired fasting glucose or lipid abnormalities on traditional lipid panel or at least 2/3 metabolic markers on NMR (small dense LDL-P predominance, reduced large HDL P, excess large VLDL-P), family history of premature cardiovascular disease

TABLE 2 : TLC - Patient Management Protocol (Specific TLC Recommendations)

First Visit:

- Parents and patients were informed that this was family program and it was expected that everyone in the family make changes and child should not be singled out
- Read labels on every item purchased; patients were given a detailed handout on reading labels that was specific to looking for added sugar, high fructose corn syrup, sat fat and partially hydrogenated oil
- Avoid High fructose corn syrup, limit added sugar to no more than 4-6 grams added sugar per serving
- Avoid partially hydrogenated oils and limit sat fats to no more than 3 grams per serving
- Increase vegetable intake: Assignments for trying one new vegetable every week and reporting back what the vegetable was, how it was cooked and whether child liked it or not.
- Exercise: based on an assessment of each individual patient according to likes, current activity levels, family schedules and financial situation
- Limit sedentary activities including television, computer not being used for homework, video games limited to no more than 1 hour on school nights and no more than 2 hours on weekend days

Second Visit:

- Limit portions- used division of 9" plate into fourths; half with non-starchy vegetable; ¼ protein 6 oz; ¼ grain or starchy vegetable
- Insoluble fiber intake: age plus 5 grams minimum and soluble fiber 10 grams
- Incorporating legume type beans into diet
- Incorporating nuts 1-2 ounces daily

TABLE 3 : Criteria for Patient Selection for Treatment with Metmormin + TLC vs. TLC Only

Any one of the following:

- low HDL, <40 mg/dL males <50 mg/dL females and elevated triglycerides >150 mg/dL
- elevated small particles where the majority of the total LDL-P were small particles
- family history of diabetes
- age (children were not started on metformin strictly because < 10 years of age)
- physical exam showing acanthosis nigricans, a clinical sign of insulin resistance

TABLE 4 : Baseline Characteristics for the Study Participants

Characteristic	Metformin + TLC (n = 11)	TLC (n = 9)
Age - yr (mean)	12.5	12
Female sex (%)	55%	63%
LDL-P (nmol/L)	1808	1457
Small LDL-P (nmol/L)	1325	1114
LDL-P size (nm)	20.5	20.6
LDL-C (mg/dL)	114	86
Large HDL-P (nmol/L)	6.77	6.1
HDL-C (mg/dL)	41	41
Non-HDL-C (mg/dL)	151	129
Large VLDL (nmol/L)	6.1	2.8
Triglyceride (mg/dL)	185	177
Weight (lb)	178	189
BMI	30.8	30.5
HbA1C	5.6	5.4
Glucose	84	82

TABLE 5 : Percent of Subjects with Discordance Between LDL-C, Non-HDL-C, and LDL-P Goals and their Perspective Percent Population Distribution During Initial Visit

(N = 20)	Percent to Goal Initial Visit	Mean Values at Initial Visit	Framingham Pop. Distribution
LDL-C < 110 mg/dL	68%	102 mg/dL	~ 20%
LDL-P < 1100 nmol/L	15%	1650 nmol/L	~ 70%

TABLE 6 : Percent Change of Various Values Between the Metformin + TLC vs. TLC Group from Baseline to Follow-Up visit

Characteristic	Metformin + TLC (n = 11) Baseline Follow-Up Visit (% change, p < 0.05)	TLC (n = 9) Baseline Follow-Up Visit (% change, p < 0.05)	Characteristic	Metformin + TLC (n = 11) Baseline Follow-Up Visit (% change, p < 0.05)	TLC (n = 9) Baseline Follow-Up Visit (% change, p < 0.05)
LDL-P (nmol/L) (% change)	1808 - 1201 (-34%, p < 0.005)	1457 - 1283 (-12%, NS)	Large VLDL (nmol/L) (% change)	6.1 - 3.0 (-51%, NS)	2.8 - 6.2 (122%, NS)
Small LDL-P (nmol/L) (% change)	1325 - 574 (-57%, p < 0.005)	1114 - 880 (-21%, p < 0.05)	Triglyceride (mg/dL) (% change)	185 - 146 (-21%, NS)	177 - 202 (14%, NS)
LDL-P size (nm) (% change)	20.5 - 21.4 (5%, p < 0.002)	20.6 - 20.9 (2%, NS)	Weight (lb) (% change)	178 - 166 (-7%, NS)	189 - 185 (-2%, NS)
LDL-C (mg/dL) (% change)	114 - 98 (-14%, p < 0.05)	86 - 95 (11%, NS)	BMI (% change)	30.8 - 29.5 (-4%, p < 0.05)	30.5 - 29.8 (-2%, NS)
Large HDL-P (nmol/L) (% change)	6.77 - 10.7 (47%, p < 0.05)	6.1 - 7.2 (17%, NS)	HbA1C (% change)	5.6 - 5.4 (-2%, NS)	5.4 - 5.5 (1%, NS)
HDL-C (mg/dL) (% change)	41 - 49.6 (21%, NS)	41 - 43 (5%, NS)	Glucose (% change)	84 - 86 (1%, NS)	82 - 90 (10%, NS)
Non-HDL-C (mg/dL) (% change)	151 - 127 (-16%, p < 0.002)	129 - 134 (4%, NS)			