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Markers of cholesterol synthesis are elevated in adolescents and young adults with type 2 diabetes

Ivana Semova¹, Amy E. Levenson¹, Joanna Krawczyk¹, Kevin Bullock², Kathryn A. Williams^{1,3}, R. Paul Wadwa⁴, Philip R. Khoury⁵, Thomas R. Kimball⁵, Elaine M. Urbina⁶, Sarah D. de Ferranti⁷, David M. Maahs⁴, Lawrence M. Dolan⁸, Amy S. Shah⁸, Clary B. Clish², Sudha B. Biddinger¹

¹Division of Endocrinology, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts

²Broad Institute of MIT and Harvard, Cambridge, Massachusetts

³Biostatistics and Research Design Center, Boston Children's Hospital, Boston, Massachusetts

⁴Barbara Davis Center for Diabetes, University of Colorado School of Medicine, Aurora, Colorado

⁵Division of Cardiology, Cincinnati Children's Hospital Medical Center and University of Cincinnati, Cincinnati, Ohio

⁶Heart Institute, Cincinnati Children's Hospital Medical Center and University of Cincinnati, Cincinnati, Ohio

⁷Department of Cardiology, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts

⁸Division of Endocrinology, Cincinnati Children's Hospital Medical Center and University of Cincinnati, Cincinnati, Ohio

Abstract

Background: Changes in cholesterol absorption and cholesterol synthesis may promote dyslipidemia and cardiovascular disease in individuals with type 2 diabetes mellitus (T2DM).

Correspondence: Sudha B. Biddinger, Boston Children's Hospital, 3 Blackfan Circle, Boston, MA, 02115, USA. sudha.biddinger@childrens.harvard.edu.

Present address

Amy E. Levenson, Division of Pediatric Endocrinology, University of North Carolina School of Medicine, Chapel Hill, North Carolina
Thomas R. Kimball, Division of Cardiology, Children's Hospital of New Orleans, Louisiana State University Health Sciences Center, New Orleans, Louisiana

David M. Maahs, Division of Endocrinology, Department of Pediatrics, Stanford School of Medicine, Stanford Diabetes Research Center, Stanford University, Stanford, California

AUTHOR CONTRIBUTIONS

I.S., A.E.L., S.D.F., E.M.U., L.M.D., A.S.S., R.P.W., P.R.K., D.M.M., and S.B.B. designed the study; A.S.S., T.R.K., E.M.U., L.M.D. recruited participants; I.S., A.V.L., J.K. performed the experiments and/or analyzed the data; I.S., J.K., K.B., and C.B.C. performed LC-MS/MS and data analyses; K.A.W. performed the biostatistical analysis; I.S. and S.B.B. wrote the manuscript. All authors contributed to the review and editing of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPORTING INFORMATION Additional supporting information may be found online in the Supporting Information section at the end of this article.

Objective: To assess cholesterol synthesis and absorption in lean individuals, obese individuals, and individuals with T2DM.

Methods: We measured lathosterol and lanosterol (markers of cholesterol synthesis) as well as campesterol and β -sitosterol (markers of cholesterol absorption) in the serum of 15 to 26 years old individuals with T2DM (n = 95), as well as their lean (n = 98) and obese (n = 92) controls.

Results: Individuals with T2DM showed a 51% increase in lathosterol and a 65% increase in lanosterol compared to lean controls. Similarly, obese individuals showed a 31% increase in lathosterol compared to lean controls. Lathosterol and lanosterol were positively correlated with body mass index, fasting insulin and glucose, serum triglycerides, and C-reactive protein, and negatively correlated with HDL-cholesterol. In contrast, campesterol and β -sitosterol were not altered in individuals with T2DM. Moreover, campesterol and β -sitosterol were negatively correlated with body mass index, fasting insulin, and C-reactive protein and were positively correlated with HDL-cholesterol.

Conclusions: Adolescents and young adults with T2DM show evidence of increased cholesterol synthesis compared to non-diabetic lean controls. These findings suggest that T2DM may promote cardiovascular disease by increasing cholesterol synthesis, and provide additional rationale for the use of cholesterol synthesis inhibitors in this group.

Keywords

cardiovascular disease risk; cholesterol synthesis; type 2 diabetes; youth

1 | INTRODUCTION

Obesity and type 2 diabetes mellitus (T2DM) have reached epidemic proportions in our society.¹ Cardiovascular disease (CVD) is the leading cause of death in T2DM, and the excess CVD risk conferred by T2DM remains despite the remarkable therapeutic advances over the past few decades.² Of particular concern is the increasing number of youth and young adults with T2DM.³ Though it had previously been suggested that youth may be protected from the development of CVD, it has become clear that they can develop hypertension, arterial stiffness, and dyslipidemia with increased total cholesterol (TC), LDL-cholesterol (LDL-C), and ApoB levels, as well as atherogenic lesions.^{4–10} Thus, an earlier age of onset of T2DM could portend earlier and more severe CVD.^{11,12}

The exact mechanisms by which T2DM promotes CVD are still not clear. While hyperglycemia has long been assumed to play a central role, glucose lowering does not consistently reduce CVD risk,^{13,14} raising the possibility that other factors are involved. Cholesterol plays a central role in the pathogenesis of atherosclerosis, and prior studies in adults have shown T2DM to be associated with increased cholesterol synthesis.^{15–19} We hypothesized that younger individuals with T2DM would also show increased cholesterol synthesis. To test this, we measured markers of cholesterol synthesis, as well as markers of cholesterol absorption, in a cohort of lean, obese, and T2DM adolescents and young adults.

2 | METHODS

2.1 | Participants

Adolescents and young adults with T2DM were previously recruited as part of the Type 2 Cardiovascular Disease Study conducted at Cincinnati Children's Hospital Medical Center from 2005 until 2010.^{20,21} Lean and obese control individuals were recruited for a longitudinal study of the natural history of obesity and insulin resistance in an urban school district in Cincinnati which used the same methods for assessment of cardiovascular risk factors.²² For this study, 285 participants (age 15–26 years) were randomly selected from the lean, obese, and T2DM groups, and were frequency-matched for age and sex. T2DM was defined by the criteria of the American Diabetes Association and included only islet cell antibody-negative individuals (Barbara Davis Center for Diabetes, Aurora, Colorado). Obese controls had body mass index (BMI) ≥95th percentile and lean controls had BMI <85th percentile, which was defined by age- and sex-specific BMI percentiles. For participants older than 20 years, age was set to 19.99 years to calculate the BMI percentile. All obese individuals underwent oral glucose tolerance testing to exclude subjects with sub-clinical T2DM. Participants who reported taking lipid-lowering medications such as statins were excluded from this analysis. Prior to study enrollment, written consent was obtained from individuals ≥18 years old, or from a parent/guardian for individuals <18 years old. Written assent was obtained for individuals <18 years old according to guidelines by the institutional review board at Cincinnati Children's Hospital Medical Center. The study design was approved by the local institutional review boards at Cincinnati Children's Hospital Medical Center and Boston Children's Hospital.

2.2 | Anthropometric measures and laboratory assays

All participants had an in-person visit conducted in Cincinnati, during which demographics, anthropometrics, medication history, and a fasting (>10 h) blood sample were collected. Weight and height were each measured twice and the average was reported. Assays for measuring fasting plasma glucose, insulin, triglycerides (TG), TC, LDL-C, HDL-cholesterol (HDL-C), high-sensitivity C-reactive protein (Hs-CRP), and hemoglobin A1c (HbA1c) have previously been described.²³ Briefly, plasma glucose was measured with Hitachi model 704 glucose analyzer (Roche Hitachi, Indianapolis, Indiana), plasma insulin was measured by radioimmunoassay, lipids were measured in an NHLBI/Center for Disease Control and Prevention-standardized laboratory, Hs-CRP was measured by enzyme-linked immunosorbent assay, and HbA1c was measured by a high-performance liquid chromatography technique.

2.3 | Sterol sample preparation and mass spectrometry analysis

Cholesterol synthesis and absorption markers were measured as previously described.²⁴ Briefly, sterols were extracted from serum, derivatized to picolinyl esters, and analyzed using a targeted liquid chromatography tandem mass spectrometry method on an Agilent 6495 triple quadrupole mass spectrometer (the intra-assay coefficients of variation were 7.1%–12.4%). Samples (10 µL) were injected onto a Hypersil GOLD column (150 × 2.1 mm, 3 µm, Thermo Electron) and eluted with a flow rate of 300 µL/min with acetonitrile/methanol/water (40/40/20, v/v/v) with 0.1% acetic acid (mobile phase A) for 0.5 min,

followed by linear gradient to acetonitrile/methanol/water (45/45/10, v/v/v) with 0.1% acetic acid (mobile phase B) over 19.5 min and held for 21 min. Mass spectrometer settings were: 3.5 kV, ionization voltage; 200°C, gas temperature; 14 L/min, gas flow; 40 psi, nebulizer pressure; 325°C, sheath gas temperature; 11 L/min, sheath gas flow; 0.5 kV, nozzle voltage; 150, high pressure RF; 90, low pressure RF. Mass spectra were acquired using electrospray ionization in the positive ion mode and using dynamic multiple reaction monitoring scanning. Collision energies and precursor-to-product ion transitions were determined using derivatized authentic reference standards. Spectrum peak integration was performed using Agilent MassHunter software (Agilent, G3336AA); normalization was performed to quality control pooled serum sample and presented as arbitrary units (A.U.).

2.4 | Statistical analysis

Values are reported as mean $\pm$ SD (range) or number (% of total), unless stated otherwise. Bivariate Spearman correlations were calculated between cholesterol synthesis and absorption markers and potential covariates (BMI, BMI z-score, fasting insulin and glucose, HbA1c, TG, TC, LDL-C, HDL-C, Hs-CRP). Statistical significance was determined by one-way ANOVA (for group or within-sex group comparisons) or Student's *t*-test (for sex comparisons), and *P* value lower than .05 was considered significant for all analyses; *n* = 285 total, except for the following measurements: fasting insulin (*n* = 240), fasting glucose (*n* = 277), HbA1c (*n* = 95; only measured in individuals with T2DM), TG (*n* = 276), TC (*n* = 275), LDL-C (*n* = 268), HDL-C (*n* = 276), and Hs-CRP (*n* = 169). Multivariable regression analysis was performed to determine and adjust for differing effects of covariates. Factors included in the analysis were group, sex, fasting insulin, and fasting glucose; in addition, two-way interactions were also tested in the models. BMI was not included as a covariate because of its innate relationship to the composition of the groups.

3 | RESULTS

3.1 | Demographic, anthropometric, and laboratory data by study group

The groups were similar in age and sex by study design (Table 1). BMI, BMI z-score, fasting insulin (all *P* < .001), and Hs-CRP (*P* = .004) were higher across groups from lean to obese to T2DM individuals, while fasting glucose and plasma lipid levels were only higher in T2DM subjects (all *P* < .001).

3.2 | Cholesterol synthesis and absorption markers

Serum levels of lathosterol and lanosterol (cholesterol synthesis markers), were 51% to 65% higher in individuals with T2DM compared to lean controls (*P* < .001) (Table 1 and Figure 1A,B). In obese individuals, lathosterol was 31% higher when compared to lean controls (*P* < .001), while lanosterol was not significantly different. These group differences were independent of fasting insulin and glucose (data not shown). Campesterol and β -sitosterol (cholesterol absorption markers) were not significantly different between the groups (Figure 1C,D).

In the complete cohort (*n* = 285), one or both cholesterol synthesis markers were positively correlated with BMI, BMI z-score, fasting insulin and glucose, HbA1c, TG, TC, LDL-C,

and Hs-CRP, but negatively correlated with HDL-C (Table 2). The absorption markers were also positively correlated with HbA1c, TC, and LDL-C; however, they were negatively correlated with BMI, BMI z-score, fasting insulin, and Hs-CRP, and positively associated with HDL-C (Table 2). Thus, with the exception of HbA1c, TC, and LDL-C, the cholesterol synthesis and absorption markers showed reciprocal associations with other metabolic parameters. The multivariable analyses showed that group differences remained after inclusion of sex, fasting insulin, fasting glucose, and the group and sex two-way interactions in the models (Supplemental Table 1).

3.3 | Sex differences

The levels of the cholesterol synthesis and absorption markers were similar in males and females (data not shown). However, the positive correlation of lanosterol with HbA1c was found only in females, whereas the negative correlations of campesterol and β -sitosterol with BMI, BMI z-score, fasting insulin, and Hs-CRP reached significance only in males (Supplemental Table 2).

4 | DISCUSSION

We find that cholesterol synthesis markers are higher in adolescents and young adults with T2DM than in lean individuals, and positively correlated with BMI, TG, hyperinsulinemia, hyperglycemia, and inflammation. In contrast, the absorption markers are not different between individuals with T2DM and their lean controls, and are negatively correlated with these same traits.

These changes in an adolescent and young adult cohort are largely similar to those previously reported, though primarily in older adults. Cholesterol synthesis markers, in most studies, were found to be increased in individuals with T2DM and associated with higher BMI, higher TG, and lower HDL-C.^{15–19,25,26} Similarly, in most prior studies, cholesterol absorption markers were not altered in individuals with T2DM, positively associated with glycemic control and cholesterol levels, and negatively associated with BMI, serum insulin, and inflammation.^{19,27–31}

Nonetheless, discrepancies exist in the literature. First, a few studies did not find an increase in cholesterol synthesis markers in individuals with T2DM.^{28,29} This could be due to the smaller size of their cohorts ($n = 16$ or 42 individuals), or their inclusion of individuals on lipid-lowering medications.^{28,29} In addition, several studies have shown individuals with T2DM to have lower cholesterol absorption.^{15–19} This could be because of their inclusion of individuals with CVD and related complications, or a lack of female subjects.^{15–19}

An important limitation of the present study is the use of serum markers of cholesterol synthesis and absorption rather than isotope tracer measurements which directly assess cholesterol synthesis and absorption.^{32–34} However, such direct measurements are not practical in a cohort of this size and serum markers of synthesis and absorption correlate well with direct measurements.^{35–39}

In summary, cholesterol synthesis appears to be increased even in adolescents and young adults with T2DM. Drugs to inhibit cholesterol synthesis are commonly used and exceedingly effective in reducing CVD in adults with T2DM.^{40–42} These data highlight the need to further evaluate the effectiveness and safety of these drugs in younger T2DM populations.⁴³

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIGURE 1.

Abundance of markers for cholesterol synthesis and absorption in individuals with type 2 diabetes mellitus and lean and obese non-diabetic controls. Data are presented as mean $\pm$ SEM. A.U., arbitrary units; L, lean; O, obese; T2DM, type 2 diabetes mellitus. Statistical significance is based on one-way ANOVA for group comparisons (* $P < .05$)

TABLE 1

Demographics and clinical parameters of individuals with type 2 diabetes mellitus (T2DM), and lean and obese non-diabetic controls

Subject characteristics	Lean	Obese	T2DM
Age (yr)	20.6 ± 2.1	21.1 ± 2.4	20.5 ± 2.2
n (% female)	98(59)	92 (57)	95 (59)
BMI (kg/m ²)	23.3 ± 4.2	31.8 ± 7.7	37.1 ± 10.5 ^a
BMI z-score	0.1 ± 0.9	1.5 ± 0.7	1.9 ± 0.8 ^a
Fasting insulin (mU/mL)	13.3 ± 6.6	17.8 ± 8.4	20.3 ± 12.5 ^a
Fasting glucose (mg/dL)	87 ± 8	90 ± 7	169 ± 86 ^a
HbA1c (%)	ND	ND	8.8 ± 3.2
TG (mg/dL)	83.8 ± 33.6	84.7 ± 42.0	133.6 ± 88.4 ^a
TC (mg/dL)	163.6 ± 28.9	163.5 ± 33.0	187.5 ± 38.8 ^a
LDL-C (mg/dL)	92.4 ± 25.1	94.7 ± 27.5	115.6 ± 31.4 ^a
HDL-C (mg/dL)	54.8 ± 13.3	51.8 ± 9.9	44.8 ± 11.1 ^a
Hs-CRP (mg/dL)	1.6 ± 3.1	4.2 ± 7.6	4.5 ± 4.3 ^a
Campesterol (A.U.)	1.00 ± 0.35	1.03 ± 0.42	1.01 ± 0.44
β-Sitosterol (A.U.)	1.00 ± 0.41	1.06 ± 0.52	1.03 ± 0.51
Lathosterol (A.U.)	1.00 ± 0.44	1.31 ± 0.63	1.51 ± 0.60 ^a
Lanosterol (A.U.)	1.00 ± 0.51	1.18 ± 0.61	1.65 ± 0.89 ^a

Note: Values are reported as mean ± SD or n (% of total).

Abbreviations: A.U., arbitrary units; BMI, body mass index; HbA1c, hemoglobin A1c; HDL-C, high density lipoprotein cholesterol; Hs-CRP, high-sensitivity C-reactive protein; LDL-C, low density lipoprotein cholesterol; ND, not determined; TC, total cholesterol; TG, triglycerides.

^aMarks a significant difference between groups.

TABLE 2

Correlations with clinical parameters in the complete cohort

Subject characteristics	Lathosterol	Lanosterol	Campesterol	β -Sitosterol
BMI (kg/m ²)	.51 (<.001)	.45 (<.001)	-.17 (.003)	-.17 (.004)
BMI z-score	.51 (<.001)	.44 (<.001)	-.19 (.002)	-.18 (.002)
Fasting insulin (mU/mL)	.31 (<.001)	.24 (<.001)	-.16 (.016)	-.14 (.032)
Fasting glucose (mg/dL)	.30 (<.001)	.30 (<.001)	-.02 (.795)	-.02 (.689)
HbA1c (%)	.14 (.169)	.30 (.003)	.27 (.009)	.21 (.037)
TG (mg/dL)	.46 (<.001)	.38 (<.001)	-.10 (.090)	-.10 (.094)
TC (mg/dL)	.38 (<.001)	.43 (<.001)	.25 (<.001)	.23 (<.001)
LDL-C (mg/dL)	.35 (<.001)	.39 (<.001)	.26 (<.001)	.23 (<.001)
HDL-C (mg/dL)	-.19 (.002)	-.17 (.004)	.13 (.030)	.15 (.016)
Hs-CRP (mg/dL)	.44 (<.001)	.40 (<.001)	-.16 (.037)	-.20 (.008)

Note: Values are presented as Spearman correlation coefficients (*P*-value); bold-type font indicates statistical significance, *P* < .05.