Plasma sterol evidence for decreased absorption and increased synthesis of cholesterol in insulin resistance and obesity\textsuperscript{1–4}

Pathmaja Paramsothy, Robert H Knopp, Steven E Kahn, Barbara M Retzlaff, Brian Fish, Lina Ma, and Richard E Ostlund Jr

ABSTRACT

Background: The rise in LDL with egg feeding in lean insulin-sensitive (LIS) participants is 2- and 3-fold greater than in lean insulin-resistant (LIR) and obese insulin-resistant (OIR) participants, respectively.

Objective: We determined whether differences in cholesterol absorption, synthesis, or both could be responsible for these differences by measuring plasma sterols as indexes of cholesterol absorption and endogenous synthesis.

Design: Plasma sterols were measured by gas chromatography–mass spectrometry in a random subset of 34 LIS, 37 LIR, and 37 OIR participants defined by the insulin sensitivity index (S\textsubscript{I}) and by BMI criteria selected from a parent group of 197 participants. Cholesterol and plant sterols provide a measure of cholesterol absorption, and lathosterol provides a measure of cholesterol synthesis.

Results: The mean (\pm SD) ratio of plasma total absorption biomarker sterols to cholesterol was 4.48 \pm 1.74 in LIS, 3.25 \pm 1.06 in LIR, and 2.82 \pm 1.08 in OIR participants. After adjustment for age and sex, the relations of the absorption sterol–cholesterol ratios were as follows: LIS > OIR (P < 0.001), LIS > LIR (P < 0.001), and LIR > OIR (P = 0.11). Lathosterol-cholesterol ratios were 0.71 \pm 0.32 in the LIS participants, 0.95 \pm 0.47 in the LIR participants, and 1.29 \pm 0.55 in the OIR participants. After adjustment for age and sex, the relations of lathosterol-cholesterol ratios were as follows: LIS < OIR (P < 0.001), LIS < LIR (P = 0.03), and LIR < OIR (P = 0.002). Total sterol concentrations were positively associated with S\textsubscript{I} and negatively associated with obesity, whereas lathosterol correlations were the opposite.

Conclusions: Cholesterol absorption was highest in the LIS participants, whereas cholesterol synthesis was highest in the LIR and OIR participants. Therapeutic diets for hyperlipidemia should emphasize low-cholesterol diets in LIS persons and weight loss to improve S\textsubscript{I} and to decrease cholesterol overproduction in LIR and OIR persons.

INTRODUCTION

Obesity and insulin resistance portend a significant risk of atherosclerotic disease (1–4). Elevated triglycerides, moderate elevations in small dense LDL cholesterol, and decreased HDL cholesterol define the atherogenic dyslipidemia (combined hyperlipidemia) of insulin resistance (4–7). The dietary contribution to this dyslipidemia is poorly understood but likely relates to amounts of cholesterol absorbed and synthesized endogenously.

We previously reported that feeding 4 eggs daily for 1 mo was associated with a 2–3-fold greater rise in LDL cholesterol in LIS participants than in LIR and OIR participants, respectively (8). These observations suggest that LIR and OIR participants had less cholesterol absorption than did LIS participants. However, LIR and OIR participants had higher baseline concentrations of LDL cholesterol and non-HDL cholesterol than did LIS participants, which suggested higher cholesterol synthesis. Similarly, the rise in HDL cholesterol with egg feeding was also less in LIR and OIR than in LIS participants, which points to a common effect of cholesterol absorption on lipoproteins (8, 9).

Because cholesterol absorption and biosynthesis are difficult to measure directly, we measured circulating concentrations of several cholesterol-related sterols as biomarkers of these processes. Sitosterol, campesterol, and stigmasterol are plant sterols similar in structure to cholesterol that are not synthesized in mammals and must therefore, in part, reflect exogenous sterol absorption. Although the absolute amount of phytosterols absorbed is very low, their plasma concentrations are correlated with cholesterol absorption (10). Cholestanol, an endogenous 5–3-reduced metabolite of cholesterol, undergoes enterohepatic recirculation, and its plasma concentration is also correlated with cholesterol absorption (11). Lathosterol, an endogenous precursor of cholesterol, is correlated with cholesterol biosynthesis (12, 13). Mevalonate, an intermediate in the cholesterol biosynthetic pathway that has been shown to reflect acute changes in cholesterol synthesis, is increased in obesity (14).
in cholesterol biosynthesis, was not measured here because of the large diurnal variation in concentration and short plasma half-life (14). To simplify presentation of the results, we combined circulating sitosterol, campesterol, stigmasterol, and cholesterol into a single biomarker of cholesterol absorption called total absorption biomarker sterols in this manuscript and used circulating lathosterol as a measure of cholesterol biosynthesis.

We used these measures of cholesterol-related sterols in a subset of LIS, LIR, and OIR men and women from the parent egg-feeding study (8) to understand the role of cholesterol absorption and synthesis in obesity and insulin resistance. Specifically, we wished to understand whether differences exist to explain the discrepancy seen in the LDL-cholesterol response to egg feeding between insulin-sensitive and insulin-resistant individuals.

SUBJECTS AND METHODS

Study design

This study presents a cross-sectional evaluation of sterol markers of cholesterol absorption (sitosterol, stigmasterol, campesterol, and cholesterol) called total absorption biomarker sterols and cholesterol synthesis (lathosterol) in LIS, LIR, and OIR participants at baseline before beginning a cholesterol-feeding intervention.

Study participants

The parent study of 197 participants was a randomized, double-blinded, 3 period (1-mo duration for each) crossover trial that evaluated the effects of 0, 2, or 4 eggs daily on cholesterol. Specifically, the parent study evaluated the effects of insulin resistance with and without obesity on cholesterol and saturated sterols and cholesterol synthesis (lathosterol) in LIS, LIR, and OIR participants at baseline before beginning a cholesterol-feeding intervention.

TABLE 1

Baseline characteristics of LIS, LIR, and OIR participants

<table>
<thead>
<tr>
<th></th>
<th>LIS (n = 34)</th>
<th>LIR (n = 37)</th>
<th>OIR (n = 37)</th>
<th>P for across-group comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>50 ± 9(^2)</td>
<td>55 ± 12</td>
<td>54 ± 9</td>
<td>0.08</td>
</tr>
<tr>
<td>Women (%)</td>
<td>74</td>
<td>59</td>
<td>49</td>
<td>0.10</td>
</tr>
<tr>
<td>S(_l) \times 10(^{-4}) min(^{-1}) (μU/mL)(^6)</td>
<td>5.23 (4.78, 6.8)(^d)</td>
<td>3.24 (1.95, 3.8)(^d)</td>
<td>1.89 (1.36, 2.4)(^d)(^s)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>24.1 ± 2.2</td>
<td>24.1 ± 1.9</td>
<td>31.3 ± 4.1(^d)(^s)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>79.1 ± 9.7</td>
<td>82.6 ± 9.5</td>
<td>101.5 ± 12.6(^d)(^s)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.80 ± 0.08</td>
<td>0.82 ± 0.09</td>
<td>0.90 ± 0.09(^d)(^s)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Intraabdominal fat area, CT scan (cm(^2))</td>
<td>53.2 ± 30.8</td>
<td>89.4 ± 47.2(^d)</td>
<td>169.0 ± 78.6(^d)(^s)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Subcutaneous fat area, CT scan (cm(^2))</td>
<td>152 ± 52.8</td>
<td>183 ± 55.4(^d)</td>
<td>320 ± 147.5(^d)(^s)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total fat area, CT scan (cm(^2))</td>
<td>493 ± 95</td>
<td>564 ± 99(^d)</td>
<td>813 ± 182.2(^d)(^s)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)(^1)</td>
<td>0.93 (0.66, 1.46)</td>
<td>1.26 (0.92, 1.46)</td>
<td>1.35 (1.04, 1.35)(^d)</td>
<td>0.02</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.86 ± 1.0</td>
<td>5.41 ± 0.88(^d)</td>
<td>5.17 ± 0.93</td>
<td>0.048</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.81 ± 0.79</td>
<td>3.44 ± 0.75(^d)</td>
<td>3.23 ± 0.77</td>
<td>0.003</td>
</tr>
<tr>
<td>Non-HDL cholesterol (mmol/L)</td>
<td>3.34 ± 0.94</td>
<td>4.05 ± 0.89(^d)</td>
<td>4.01 ± 0.94(^d)</td>
<td>0.002</td>
</tr>
<tr>
<td>apo B (g/L)</td>
<td>0.086 ± 0.022</td>
<td>0.102 ± 0.022(^d)</td>
<td>0.102 ± 0.023(^d)</td>
<td>0.003</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.51 ± 0.39</td>
<td>1.36 ± 0.39</td>
<td>1.16 ± 0.67(^d)(^s)</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

\(^1\) ANOVA was used for continuous variables, and chi-square was used for categorical variables. apo, apolipoprotein; CT, computed tomography; LIS, lean insulin-resistant; LIR, lean insulin-sensitive; OIR, obese insulin-resistant; S\(_l\), insulin sensitivity index.

\(^2\) Mean ± SD (all such values).

\(^3\) Median; IQR in parentheses (all such values).

\(^4\) Significantly different from LIS, P < 0.016 (ANOVA; threshold for significance for multiple comparisons).

\(^5\) Significantly different from LIR, P < 0.016 (ANOVA; threshold for significance for multiple comparisons).

As described elsewhere (8), obesity, for the purposes of this study, was defined as a BMI (in kg/m\(^2\)) ≥27.5 and insulin resistance by an S\(_l\) < 4.2 \times 10^{-4} \text{min}^{-1} \text{(μU/mL)} determined by the frequently sampled intravenous glucose-tolerance test, using the minimal model of Bergman et al (15, 16). Participants who were obese and insulin sensitive were not included in the analysis due to low numbers of these participants in the parent study. Participants were excluded from the parent study if they had a total cholesterol concentration >300 mg/dL, triglyceride concentration >500 mg/dL, diabetes, blood pressure >150/100 mm Hg, coronary heart disease, peripheral vascular disease, anemia, or renal, liver, or unstable thyroid disease. They were also excluded if they were taking lipid-altering medications, β-blockers, thiazides, corticosteroids, or anticonvulsants. Oral contraceptive use was prohibited, but continuous postmenopausal hormone replacement therapy was allowed. Women with irregular menses were excluded. Participants with excessive alcohol use, history of serious mental illness, or inability to comply with study requirements were also excluded (8).

Participants were recruited by public advertising, and the study was approved by the University of Washington Human Subjects Review Committee. All participants gave written informed consent before participating.

Measurements

Detailed methods for conducting the frequently sampled intravenous glucose-tolerance test and for quantifying abdominal fat area by CT and plasma lipoproteins were as previously described (8). Plasma sterols were measured using gas chromatography–mass spectrometry. The concentration of plant sterols was determined by reference to standard curve of natural and deuterated soy sterols as described previously (11). Cholestanol and lathosterol in plasma were computed by reference to standard curves of natural cholesterol, lathosterol, and deuterated soy sterols (11).

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Statistics

Descriptive statistics included means ± SDs, medians and IQRs, and group percentages. Across-group comparisons between the LIS, LIR, and OIR groups were performed by ANOVA for continuous variables and by chi-square for categorical variables. A comparison of the association of sterol to cholesterol ratios between the LIS, LIR, and OIR participants was evaluated by univariate and multivariate linear regression. A Bonferroni-corrected P value <0.016 was considered significant for multiple comparisons (LIR compared with LIS, OIR compared with LIS, and LIR compared with OIR). The interaction between obesity (as defined by BMI) and insulin sensitivity (by SI) for each sterol was evaluated. Pairwise correlations were performed to assess the correlation between each sterol and markers of obesity (BMI, waist circumference, and abdominal fat area as determined by CT) and insulin sensitivity (by SI) as well as fasting triglycerides, non-HDL cholesterol, and apo B concentrations. A P value <0.05 was considered significant for the interaction. STATA 10.2 (StataCorp) was used to analyze the data.

RESULTS

Baseline characteristics

No significant differences were observed in age or sex among the 34 LIS, 37 LIR, and 37 OIR participants (Table 1). By definition, LIR participants had lower insulin sensitivity (SI) than did the LIS participants. Likewise, the OIR participants had both a higher BMI and a lower SI than did LIS participants. OIR participants also had a greater waist circumference, waist/hip ratio, and intraabdominal, subcutaneous, and total abdominal fat areas by CT than either LIS or LIR participants. Although LIR and LIS participants had similar waist circumference, LIR participants had higher intraabdominal, subcutaneous, and total abdominal fat areas than did the LIS participants.

Triglycerides were not significantly different between the LIS and LIR but were significantly different between the OIR and LIS participants. Non-HDL cholesterol and apo B were elevated to similar levels in LIR and OIR participants compared with LIS (Table 1). Total plasma cholesterol and LDL-cholesterol concentrations were highest in the LIR group and were significantly greater than in LIS participants. Total plasma cholesterol and LDL cholesterol were not significantly different between OIR and LIR or between OIR and LIS participants. Only HDL cholesterol showed stepwise lower concentrations in LIR and OIR than in LIS participants, and the differences were significant between the OIR and LIS and between the OIR and LIR participants.

Plasma sterol concentrations

No significant physiologic variation in total absorption biomarker sterols or lathosterol concentrations was found between participant groups. CVs for total absorption biomarker sterols were 0.39, 0.38, and 0.38 for the LIS, LIR, and OIR participants, respectively. CVs for lathosterol were 0.45, 0.49, and 0.43 for the LIS, LIR, and OIR participants, respectively. Unadjusted plasma sterol concentrations are depicted in box plots for each group in Figure 1. Total absorption biomarker sterols progressively decreased with increasing BMI and decreasing insulin sensitivity (P = 0.02 for the comparison between LIS and LIR participants, P < 0.001 for the comparison between LIS and OIR participants, P = 0.05 for the comparison between LIR and OIR participants), and lathosterol progressively increased with increasing BMI and decreasing insulin sensitivity (P = 0.013 for the comparison between LIS and LIR participants, P < 0.001 for the comparison between LIS and OIR participants, and P = 0.03 for the comparison between LIR and OIR participants).

Ratios of plasma sterol to cholesterol

Mean ratios of plasma sterol to cholesterol, unadjusted for baseline variables, are shown in Table 2. Plasma measures of the ratio of total absorption biomarker sterols to cholesterol (cholesterol, campesterol, stigmasterol, sitosterol, and their total) showed stepwise lower values from the LIS to LIR to OIR groups, with the exception of stigmasterol, which was present at very low concentrations. In contrast, the plasma lathosterol-cholesterol ratio trended higher in the LIR and OIR participants than in the LIS participants.
participants, significantly so for the OIR compared with the other 2 groups (Table 2).

Plasma total absorption biomarker sterols/cholesterol ratios and lathosterol/cholesterol ratios adjusted for age and gender for the LIS, LIR and OIR groups vs. each of the other 2 groups are shown in Figure 2. The downward trend in total plasma sterol/cholesterol ratio and the upward trend in lathosterol/cholesterol ratio confirm the unadjusted values in Table 2. Age- and sex-adjusted sterol/cholesterol ratios are provided elsewhere (see Supplemental Table 1 under “Supplemental data” in the online issue.

Correlations

Univariate correlations of plasma sterols and indexes of insulin sensitivity, obesity, and lipoproteins (n = 108) are presented in Table 3 and Figure 3. S_I was positively associated with total absorption biomarker sterol concentrations—a measure of increasing cholesterol absorption (r = 0.35, P = 0.002). Conversely, the lathosterol correlation with S_I was inverse (r = −0.40, P < 0.001), which indicated increasing cholesterol synthesis with decreasing insulin sensitivity. Both relations were linear.

Total cross-sectional abdominal fat area measured by CT was negatively correlated with total absorption biomarker sterol concentrations (r = −0.32, P = 0.002), which indicated decreased cholesterol absorption with increasing abdominal obesity. Conversely, lathosterol concentrations were positively associated with abdominal fat area (r = 0.39, P < 0.001), which indicated increasing cholesterol synthesis with increasing abdominal obesity. Other measures of obesity (BMI and waist circumference) were correlated and at similar levels (Table 3).

With regard to plasma lipids and lipoprotein associations with total absorption biomarker sterols concentrations, triglycerides, non-HDL cholesterol, apo B, and HDL cholesterol were not significantly correlated. In contrast, plasma triglyceride, non-HDL cholesterol, and apo B concentrations were highly positively correlated with increased synthesis (r = 0.52–0.61, all P < 0.001). HDL cholesterol was not significantly correlated with total absorption biomarker sterols or lathosterol.

We also examined whether BMI modified the association of insulin sensitivity (as measured by S_I) with total absorption

### TABLE 2
Unadjusted ratios of plasma sterol to cholesterol in the LIR, LIS, and OIR participants (total n = 108)

<table>
<thead>
<tr>
<th>Sterol</th>
<th>LIS (n = 34)</th>
<th>LIR (n = 37)</th>
<th>P value LIR vs LIS</th>
<th>OIR (n = 37)</th>
<th>P value OIR vs LIR</th>
<th>P value OIR vs LIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorption markers (µg/mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>1.29 ± 0.29</td>
<td>1.06 ± 0.23</td>
<td>&lt;0.001</td>
<td>0.97 ± 0.23</td>
<td>0.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Campesterol</td>
<td>2.59 ± 1.02</td>
<td>1.92 ± 0.64</td>
<td>&lt;0.001</td>
<td>1.68 ± 0.65</td>
<td>0.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>0.086 ± 0.031</td>
<td>0.050 ± 0.018</td>
<td>&lt;0.001</td>
<td>0.068 ± 0.025</td>
<td>0.002</td>
<td>0.003</td>
</tr>
<tr>
<td>Sitosterol</td>
<td>1.81 ± 0.71</td>
<td>1.27 ± 0.42</td>
<td>&lt;0.001</td>
<td>1.08 ± 0.43</td>
<td>0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total absorption biomarker sterols</td>
<td>4.48 ± 1.74</td>
<td>3.25 ± 1.06</td>
<td>&lt;0.001</td>
<td>2.82 ± 1.08</td>
<td>0.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Synthesis marker (µg/mg)</td>
<td>0.71 ± 0.32</td>
<td>0.95 ± 0.47</td>
<td>0.034</td>
<td>1.29 ± 0.55</td>
<td>0.002</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

1 All values are means ± SDs. Data were analyzed by linear regression. LIR, lean insulin-resistant; LIS, lean insulin-sensitive; OIR, obese insulin-resistant.

2 Significant for multiple comparisons, P < 0.016 (linear regression).

3 Total absorption biomarker sterols = cholestanol, campesterol, stigmasterol, and sitosterol.

### TABLE 3
Correlations of total absorption biomarker sterols and lathosterol with indexes of obesity and lipoproteins (n = 108)

<table>
<thead>
<tr>
<th></th>
<th>Total absorption biomarker sterols</th>
<th>Lathosterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r²</td>
<td>P</td>
</tr>
<tr>
<td>Obesity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>−0.31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>−0.23</td>
<td>0.015</td>
</tr>
<tr>
<td>Lipoproteins (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>apo B</td>
<td>0.06</td>
<td>0.60</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.09</td>
<td>0.34</td>
</tr>
</tbody>
</table>

1 Total absorption biomarker sterols (indexes of absorption) = cholestanol, campesterol, stigmasterol, and sitosterol.

2 Lathosterol (cholesterol precursor, indicator of endogenous fatty acid synthesis).

3 r = Spearman correlation coefficient; data obtained by linear regression.

4 apo B, apolipoprotein B.
biomarker sterols or lathosterol. The $P$ value for the interaction of BMI on the association of $S_I$ with total absorption biomarker sterols was $P = 0.03$ and with lathosterol was $P = 0.05$. Therefore, BMI had an independent and multiplicative effect on the relation of insulin sensitivity to total absorption biomarker sterols with a borderline effect on the relation of insulin sensitivity to lathosterol.

**DISCUSSION**

In this analysis, we showed that the phytosterols campesterol and sitosterol, the nonphytosterol cholestanol, and total biomarker absorption sterols were progressively lower in the OIR than in the LIR participants and in the LIR than in the LIS participants. These results suggest diminished cholesterol absorption with increasing insulin resistance and obesity. We previously showed that the LIR and OIR participants had one-third to one-half the rise in LDL cholesterol, respectively after consuming 4 eggs daily for 1 mo than did the LIS participants (8). A similar effect, a diminished effect of feeding or restricting dietary cholesterol in obesity, was seen previously in $\geq 10$ studies (see reference 17 for a review), beginning with Bronsgeest-Shoute et al (18) and Beynen and Katan (19). The novelty of our current findings was the finding that cholesterol absorption markers were lower, whereas cholesterol synthesis markers were higher in this cohort of insulin-resistant participants who had one-third to one-half the rise in LDL cholesterol with egg feeding.

The current results of lower plasma concentrations of total absorption biomarker sterols in the LIR and OIR participants suggest that decreased cholesterol absorption as a cause of the diminished effect of dietary cholesterol manipulation is generalizable to insulin-resistant and obese states. Conversely, the elevated lathosterol concentrations in the OIR compared with the LIS and LIR participants were consistent with enhanced endogenous cholesterol synthesis with increasing insulin resistance and obesity. The strength of the positive correlations with lathosterol suggests that increased cholesterol biosynthesis may, in part, have caused the higher concentrations of apo B–containing lipoproteins in the LIR and OIR participants, despite diminished cholesterol absorption. Impaired LDL removal via the LDL

**FIGURE 3.** Scatter plots of the associations of the total absorption biomarker sterols (cholestanol, campesterol, stigmasterol, and sitosterol) and lathosterol with $S_I$, abdominal fat, plasma triglycerides, and non-HDL cholesterol. $n = 34$ for LIS participants, $n = 37$ for LIR participants, and $n = 37$ for OIR participants. Linear regression was used to obtain the data. CT, computed tomography; LIR, lean insulin-resistant; LIS, lean insulin-sensitive; OIR, obese insulin-resistant; $S_I$, insulin sensitivity index.
cholesterol ratio and an increased campesterol-cholesterol ratio (28). Furthermore, interplay between cholesterol synthesis and sterol pools and appears to be independently regulated (27). Absorbed cholesterol in a larger plasma and total body cholesterol feeding in obesity and insulin resistance is dilution of absorbed intestinal sterols back into the intestinal lumen by increased delivery of hepatic oxysterols into the intestine (depicted in Figure 4) (24–26).

An alternative explanation for a lesser cholesterol rise after cholesterol feeding in obesity and insulin resistance is dilution of absorbed cholesterol in a larger plasma and total body cholesterol pool (17). However, circulating cholesterol is not related to body cholesterol pools and appears to be independently regulated (27). Furthermore, interplay between cholesterol synthesis and sterol absorption markers is seen in statin treatment where inhibition of cholesterol synthesis is associated with a decreased lathosterol-cholesterol ratio and an increased campesterol-cholesterol ratio (28).

When evaluating the effect of BMI on the relation between S1 and total absorption biomarker sterols or lathosterol, we showed a significant interaction of BMI on the relation of insulin sensitivity and cholesterol absorption and a borderline significant interaction for the relation of insulin sensitivity with cholesterol synthesis. These findings suggest that obesity has an independent effect on cholesterol absorption and possibly synthesis beyond reduced insulin sensitivity. Whereas obesity is associated with decreased insulin sensitivity, obesity does not always equate with insulin resistance, because there are obese people who remain relatively insulin sensitive despite increased weight, whereas those who become insulin resistant with minimal weight gain (29, 30). The effect of obesity on the combined hyperlipidemia (elevated LDL cholesterol, elevated triglycerides, and low HDL cholesterol) seen in insulin resistance through cholesterol absorption and synthesis warrants further research. Improvement of LDL cholesterol is the primary goal in reducing the risk of future cardiovascular disease (31).

Our study had some limitations. First, the definitions of LIS, LIR, and OIR were created when designing the parent study at a time when the current definitions of overweight and obesity were not in place. These subgroup definitions based on a BMI cutoff of 27.5 and insulin sensitivity and resistance based on S1 have proven very valuable in defining the effects of differences in body size and insulin sensitivity (8, 35–38), but may not be fully applicable to obese individuals as defined today. Second, we did not perform measurements of total body fat, but focused on fat distribution in the abdominal area. Thus, whereas we also have other anthropometric data that have provided important information, we cannot comment on whether total body adiposity would have been associated with similar findings. Last, our findings are based on cross-sectional data; therefore, causality cannot be assumed. However, the relations we found do suggest the likelihood that cholesterol absorption is decreased and cholesterol synthesis is increased in obesity and insulin resistance, although these would need to be validated by using the metabolic balance method.

In summary, insulin resistance and obesity are associated with diminished cholesterol absorption, whereas leanness is associated with increased cholesterol absorption. These findings may explain why we observed one-half to one-third the increase in LDL cholesterol with egg feeding in the LIR and OIR individuals, respectively, compared with those who were OIR (8). The results underscore a greater importance of diet and lifestyle for decreasing endogenous cholesterol and lipoprotein synthesis for the management of the atherogenic (combined) dyslipidemia of insulin resistance and obesity, whereas dietary cholesterol

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In summary, insulin resistance and obesity are associated with diminished cholesterol absorption, whereas leanness is associated with increased cholesterol absorption. These findings may explain why we observed one-half to one-third the increase in LDL cholesterol with egg feeding in the LIR and OIR individuals, respectively, compared with those who were OIR (8). The results underscore a greater importance of diet and lifestyle for decreasing endogenous cholesterol and lipoprotein synthesis for the management of the atherogenic (combined) dyslipidemia of insulin resistance and obesity, whereas dietary cholesterol
restriction is a higher priority for the management of hypercholesterolemia in LIS persons.

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