Insulin-Mediated Increases in the HDL Cholesterol/Cholesterol Ratio in Humans

Craig N. Sadur and Robert H. Eckel

The role of insulin in the regulation of lipoprotein cholesterol distribution was studied in 18 human volunteers who were of normal weight, normolipemic, and glucose-tolerant. We used the euglycemic clamp technique and made comparisons with six saline-infused controls. While total cholesterol and low density lipoprotein cholesterol levels fell similarly by 6 hours in both groups (p = NS), there was a greater decrease in triglyceride levels (p < 0.02) but less decrease in high density lipoprotein cholesterol levels (p < 0.02) in the insulin-infused group. These changes resulted in both a higher higher density lipoprotein cholesterol/total cholesterol ratio (p = 0.01) and a higher high density lipoprotein cholesterol/low density cholesterol ratio (p = 0.02) in the insulin-infused group. The timing of this effect of insulin implies a mechanism unrelated to high density lipoprotein turnover. Thus, insulin infusion during euglycemia appears to alter cholesterol distribution favorably in normal subjects.

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The role of insulin in the regulation of lipoprotein cholesterol metabolism is unclear. In vivo, diseases at the extremes of serum insulin concentration have been associated with alterations in lipoprotein cholesterol distribution. In obesity, a state where serum insulin is high, there are increases in total, very low density lipoprotein (VLDL), and low density lipoprotein (LDL) cholesterol and a decrease in high density lipoprotein (HDL) cholesterol. Type I diabetes mellitus, where serum insulin is low, is associated with altered lipoprotein metabolism which normalizes with improved glycemic control. In fact, some studies have demonstrated increased levels of HDL cholesterol, LDL cholesterol, or HDL cholesterol/LDL cholesterol ratios in insulin-treated diabetics. Although the lipoprotein alterations seen in the insulinopenic patient could have resulted from insulin deficiency alone, a direct effect of insulin on lipoprotein cholesterol metabolism has not been proven because other metabolic alterations are also corrected (e.g., ketonemia). Thus, a method to examine the insulin effect in a more direct manner would be beneficial. In the present study, the euglycemic clamp technique has provided a tool for assessment of the effect of insulin on lipoprotein cholesterol distribution in human subjects.

Methods

The study group included 18 subjects who received intravenous insulin and glucose. Each person was average in height and within 20% of ideal body weight according to the Metropolitan Life Insurance Company Standards. The body mass index (wt/ht²) was 2.06 × 10⁻³ ± 0.05 kg/cm² (x ± SEM). The subjects ranged in age from 20 to 45 years, x = 29.2 ± 1.4 years. There were 15 women and three men. The control group, composed of four women and two men, received intravenous infusions of 0.9% (normal) saline at approximately 100 to 150 ml/hr. They were also normal in height and weight with a body mass index of 2.17 × 10⁻³ ± 0.05 kg/cm² and with an age range of 23 to 40 years, x = 29.0 ± 2.5 years. Individuals were excluded if they were taking drugs with known effects on glucose or lipid metabolism, such as oral contraceptives, other estrogenic compounds, diuretics, or other antihypertensive medications. All subjects were free of acute or chronic illnesses. Two individuals were euthyroid while taking thyroxine for full thyroid hormone replacement. Physical examinations confirmed the normal...
Results

Baseline Data

The baseline data of both the 18 study subjects and the six control subjects are displayed in Table 1. Basal plasma glucose values in all subjects ranged from 71 to 99 mg/dl. Basal lipid determinations were similar in both high-insulin (n = 7) and low-insulin (n = 11) infusion groups. Whereas TG, HDL cholesterol, and HDL cholesterol/total cholesterol were similar in both the insulin and saline control groups, total cholesterol and LDL cholesterol were significantly higher in the control group (p < 0.05) while HDL cholesterol/LDL cholesterol was significantly lower in the control group (p < 0.05). These differences became insignificant when the data were matched for gender.

Table 1. Baseline Data Before Insulin or Saline Infusion

<table>
<thead>
<tr>
<th></th>
<th>Insulin</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>29.2 ± 1.4</td>
<td>29.0 ± 2.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>57.7 ± 2.2</td>
<td>63.8 ± 3.3</td>
</tr>
<tr>
<td>BMI x 10^-2 (kg/m^2)</td>
<td>2.06 ± 0.05</td>
<td>2.17 ± 0.05</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>84.2 ± 1.7</td>
<td>86.8 ± 2.6</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>159 ± 5</td>
<td>179 ± 8</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>88 ± 5</td>
<td>108 ± 6</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>74 ± 7</td>
<td>90 ± 11</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>56 ± 3</td>
<td>53 ± 6</td>
</tr>
<tr>
<td>HDL cholesterol/total cholesterol (%)</td>
<td>35 ± 2</td>
<td>30 ± 3</td>
</tr>
<tr>
<td>HDL cholesterol/LDL cholesterol (%)</td>
<td>67 ± 6</td>
<td>50 ± 4</td>
</tr>
</tbody>
</table>

Values are means ± SEM. TG = triglyceride; HDL = high density lipoprotein; LDL = low density lipoprotein.
Glucose

Saline infusion resulted in a gradual fall in plasma glucose over the 6-hour infusion period to 92% ± 3% of basal. While insulin was infused into study subjects, plasma glucose was maintained by a variable glucose infusion and ranged throughout the study from 94% to 103% of basal value.

Total Cholesterol

For total cholesterol and all remaining lipid measurements, no significant differences between insulin at low (40 mU/m² per minute) and high (120 mU/m² per minute) infusion rates were found. Thus, all insulin data are combined measurements for both insulin infusion rates. Although women appeared to have a greater decrease in total cholesterol by 6 hours during both the insulin infusions (men = -10 ± 2 mg/dl vs women = -21 ± 3 mg/dl) and the saline infusions (men = -9 ± 8 mg/dl vs women = -21 ± 5 mg/dl), these differences were not statistically significant. The combination of men and women, as shown in Figure 1A, revealed similar 6-hour declines in both infusion groups (saline = -17 ± 5 mg/dl vs insulin = -19 ± 3 mg/dl, p = ns).

LDL Cholesterol

As shown in Figure 1B, there were no significant differences between the two groups in the decreases in LDL cholesterol levels.

Triglycerides

TG levels decreased more in the study subjects than in the controls (Figure 1C). The p values for the differences in TG for the two groups at the 80-minute, 180-minute, and 380-minute time-points were < 0.01, < 0.05, and < 0.02, respectively. There was no gender difference in basal or subsequent TG changes for either saline or insulin infusions.

HDL Cholesterol

The change in HDL cholesterol is seen in Figure 1D. In both study subjects and controls, there was a fall in HDL cholesterol with the infusions, but the insulin-infused group had no further decrease after the first 80 minutes. Whereas the differences between the two groups were insignificant at 80-minute and 180-minute time-points, the difference at 380 minutes was statistically significant (p < 0.02). Again, there was no gender difference between responses during insulin and saline infusions.

Figure 1. Changes in total cholesterol (A), LDL cholesterol (B), triglycerides (C), and HDL cholesterol (D) are plotted by minutes during saline (○—○) and insulin (●—●) infusions. *p < 0.01, †p < 0.05, ‡p ≤ 0.02. Data are mg/dl (mean ± SEM).
HDL Cholesterol/Total Cholesterol

When expressed as the ratio of HDL cholesterol/total cholesterol, insulin infusion resulted in a higher absolute increase in the ratio at 380 minutes when compared to saline infusion, 2.8 ± 1.2% vs −1.2 ± 0.8%, respectively (Figure 2 A, p = 0.01). Once again, gender did not affect the changes that occurred during the infusions. In men, the 6-hour HDL cholesterol/total cholesterol ratio changed −2.0 ± 0.0% with saline infusion vs −2.0 ± 1.2% with insulin; in women, the change was −0.8 ± 1.3% vs 3.0 ± 1.4%.

HDL Cholesterol/LDL Cholesterol

As with HDL cholesterol/total cholesterol, the study group had a statistically significant increase in the HDL cholesterol/LDL cholesterol ratio by 380 minutes after the start of the infusion (Figure 2 B, p = 0.02).

Miscellaneous

Insulin-stimulated adipose tissue lipoprotein lipase failed to correlate with changes in cholesterol distribution. There was also no relationship between the fall in serum triglyceride and the changes in HDL cholesterol. In addition, the amount of glucose infused did not predict the changes observed.

Discussion

In this study the euglycemic clamp technique has provided a tool for examination of the effect of insulin on lipoprotein cholesterol distribution in 18 normal subjects. Because the response was similarly affected at the two insulin infusion rates, 40 and 120 mU/m² per minute, the results from the two groups were combined.

In both saline-infused and insulin-infused subjects, a progressive decrease in total cholesterol occurred over 380 minutes. LDL cholesterol also fell similarly in the two groups during the same time course because of a greater decrease in TG levels but a smaller decrease in HDL cholesterol levels in insulin-infused subjects. Some caution in the interpretation of the calculated LDL cholesterol data is needed because the maintenance of VLDL composition was assumed but not proven. Because substantial intravenous volumes were infused in both groups, a dilutional effect is likely. The insulin effect on triglycerides, however, was more than dilutional and has been reported previously.

Most important, insulin administration reduced the decrease in HDL cholesterol level, particularly during the latter part of the infusion. This effect was not associated with the insulin-stimulatory effect on adipose tissue lipoprotein lipase activity, the decrease in triglyceride level, or the amount of glucose infused (data not shown). Moreover, when the data were expressed as the ratios of both HDL cholesterol/total cholesterol and HDL cholesterol/LDL cholesterol, insulin administration resulted in higher ratios than those found with normal saline infusions. Some investigators have felt that these ratios provide the most meaningful lipid assessment of antiatherogenic risk. Because the half-life of apo A-I and apo A-II is measured in days, the effects of the hormone on HDL production or clearance would be unlikely explanations for the differences noted between saline and insulin administration.

Possibly, an effect of insulin on cholesterol transfer between two lipoprotein particles played a role. In vitro studies have revealed that cholesterol can be transferred from HDL to VLDL, an effect mediated by a specific transfer protein. This phenomenon also occurs with triglyceride transferred from VLDL to HDL. Both these transfers are reversible and are probably independent of each other. Although HDL cholesterol, the HDL cholesterol/total cholesterol ratio, and the HDL cholesterol/LDL cho-
lesterol ratio. did not change significantly at the 80- and 180-minute time-points in the study group when compared to the control group, the 380-minute time-points revealed significant changes with the insulin infusion, consistent with the temporal course of lipid transfers described in vitro. Thus, perhaps insulin-mediated increases in the HDL cholesterol/total cholesterol and HDL cholesterol/LDL cholesterol ratios could be due to alterations in cholesterol transfer to or from HDL, preventing HDL cholesterol concentrations from falling during the infusion period.

The evidence that insulin affects cholesterol metabolism in humans is indirect. A favorable effect of insulin on cholesterol metabolism is best exemplified by Type I diabetics who demonstrate a reduction in total cholesterol after improvement of the hypoinsulinemic state. After insulinization, lipids return to normal. In fact, higher levels of circulating free insulin concentrations found in treated Type I diabetics may relate to higher levels of HDL cholesterol or HDL cholesterol/total cholesterol found in these patients.

An unfavorable effect of insulin on cholesterol metabolism would have also been possible. For instance, high carbohydrate diets, which have been associated with reductions in total and LDL cholesterol and have also resulted in increases in VLDL and decreases in HDL cholesterol. In addition, obese subjects who are hyperinsulinemic have increased total cholesterol, VLDL cholesterol, and LDL cholesterol with lower HDL cholesterol concentrations than normal-weight controls. Thus, although the present study was a short-term one and involved a nonphysiologic administration of insulin, we observed an antilipolytic influence of insulin on lipoprotein cholesterol distribution in normal human subjects. This effect was predominantly on HDL cholesterol and suggests that mechanisms independent from lipoprotein turnover may be important in mediating this phenomenon.

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References

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