Low-density lipoprotein cholesterol is associated with insulin secretion

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Abstract:

Objective: Pharmacological lowering of LDL cholesterol potently reduces cardiovascular risk while concurrently increasing type 2 diabetes risk. The aim of this study was to investigate the relationship between LDL cholesterol concentrations and insulin secretion and glucagon levels.

Methods: 3,039 subjects without cholesterol lowering therapy, but with increased risk for diabetes, underwent routine blood tests and a 5-point oral glucose tolerance test (OGTT). Glucagon concentrations, insulin secretion and insulin clearance indices were derived from the OGTT.

Results: There was no association between LDL cholesterol and fasting glucagon (p=0.7, β=-0.01) or post glucose load glucagon levels (p=0.7, β=-0.07), but we detected significant positive associations of LDL cholesterol and C-peptide-based indices of insulin secretion (AUC C-Peptide(0-30min)/AUC Glucose(0-30min): p=0.0001, β=0.06; AUC C-Peptide(0-120min)/AUC Glucose(0-120min): p<0.0001, β=-0.08). In contrast we found a negative association of insulin-based insulin secretion indices with LDL concentrations (Insulinogenic index: p=0.014, β=-0.04; disposition index: p=0.0005, β=-0.06). Though, LDL cholesterol levels were positively associated with insulin clearance assessed from C-peptide and insulin concentrations, both in the fasting state and post glucose load (p<0.0001, β=0.09 and p<0.0001, β=0.06, respectively).

Conclusion: As C-peptide based indices reflect insulin secretion independent of hepatic clearance, our results indicate lower insulin secretion in case of lesser LDL cholesterol. This could explain deteriorating glycemic control in response to cholesterol lowering drugs.

Keywords: LDL cholesterol, insulin secretion, type 2 diabetes, glucagon, insulin clearance
**Abbreviations:** CEACAM1, carcinoembryonic antigen-related cell adhesion molecule 1; DI, Disposition Index; HDL, high density lipoprotein; IGI, Insulinogenic Index; ISI Matsuda, Insulin sensitivity estimated as proposed by Matsuda and DeFronzo; LDL, high density lipoproteins; LDLR, LDL receptor; NGR, normal glucose regulation; OGTT, oral glucose tolerance test

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1. Introduction

Dyslipidemia is characterized by low levels of high density lipoproteins (HDLs), hypertriglyceridemia, high total and LDL cholesterol concentrations as well as an increased proportion of small dense lipoproteins (sdLDLs). Changes in lipoprotein particles and their concentrations, especially increased levels of pro-atherogenic LDL particles, play an important role in the development of cardiovascular diseases. It is well established that statin treatment is very effective in lowering LDL cholesterol levels and therefore in preventing cardiovascular events.\(^1\text{-}^3\)

Statins inhibit hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase), the key enzyme for cholesterol synthesis in the liver.\(^4\) Despite the beneficial effects on the cardiovascular system, statin therapy is unfortunately linked to increased risk for type 2 diabetes, especially in subjects prone to the disease.\(^5,6\) Recently, data of several meta-analyses of randomized, controlled trials with statins and population-based studies of subjects taking statins were summarized. In these studies, incidence rates for new onset diabetes mellitus range from 28% to 43%.\(^7\) Another review reported a range of 9-12% in two meta-analyses of statin trails and of 18-99% in five population-based studies.\(^8\) Swerdlow et al. tested if the observed effects is a consequence of the mode of action of statins—the inhibition of HMG-CoA reductase.\(^9\) Single nucleotide polymorphisms in the HMG-CoA reductase gene were therefore used as proxies for its inhibition by statins and were indeed associated with a higher risk for type 2 diabetes.\(^9\) Though, this associative study cannot differentiate between effects of lower LDL versus consequences of altered enzyme activity.

Other genetic studies detected loss-of-function mutations in PCSK9, the gene encoding proprotein convertase subtilisin/kexin type 9. These variants associate with lower LDL cholesterol levels and protect against coronary heart disease\(^10\) but were also linked to higher fasting glucose concentrations and an increased risk of type 2 diabetes in a mendelian randomization study.\(^11\) Accordingly, short term PCSK9 inhibitor therapy was found to be related to a significant elevation of plasma glucose levels and HbA1c.\(^12\) Finally, cross-sectional data from the Netherlands demonstrate, that patients with familial hypercholesterolemia show a significantly higher prevalence of type 2 diabetes than among unaffected relatives, with variability by mutation type.\(^13\)
Type 2 diabetes is characterized by insulin resistance and impaired insulin secretion from pancreatic beta-cells. Insulin resistance alone is insufficient to cause type 2 diabetes, as long as the β-cell remains able to compensate for the increased demand for insulin. Once this compensatory mechanism reaches its physiological limits, glucose levels raise and patients progress towards overt type 2 diabetes.

Mechanistic studies suggest an impact of LDL cholesterol on structure and function of pancreatic islets\textsuperscript{14-16}, however this has not jet been comprehensively studied in humans. LDL cholesterol and diabetes risk might either be directly link at the molecular level, e.g. in pancreatic islets, or this might be coincidence in a metabolic state that goes along with both lower LDL cholesterol and higher diabetes risk.

We therefore aimed to investigate the association between LDL cholesterol concentrations and the key pathogenic mechanism of type 2 diabetes, insulin secretion, in a cohort with increased risk for the disease.

2. Materials and Methods

Participants: Data from 3,039 white individuals from the southern part of Germany who had participated in the Tübingen Family Study were analyzed. The subjects participated in different studies on the pathogenesis of type 2 diabetes between 1993 and 2017. All participants underwent metabolic characterization including a detailed medical history and physical examination, routine blood tests (fasting state) and a 5-point OGTT (oral glucose tolerance test)\textsuperscript{17}. Selection of the present study cohort was based on the absence of treatment with cholesterol lowering drugs and the availability of complete clinical data. The subject characteristics are presented in Table 1.

OGTT: A 5-point 75 g OGTT was performed after an overnight fasting period of at least 8 hours, and venous blood samples were drawn at time-points 0, 30, 60, 90, and 120 min for the determination of plasma glucose, insulin and C-peptide. 1833 participants showed a normal glucose regulation (NGR), 1055 a prediabetes and 151 subjects fulfilled diagnostic criteria for diabetes. In a subset of 595 participants, fasting glucagon was measured, in 387 subjects glucagon was assessed at all time points.
Laboratory measurements: Plasma glucose was measured using YSI 2300 glucose analyzer (YSI, Yellow Springs, CO, USA). Serum insulin and C-peptide were determined by immunoassay with the ADVIA Centaur XP Immunoassay System (Siemens Healthcare Diagnostics, Erlangen, Germany). Glucagon was measured as previously described for the TUEF cohort\textsuperscript{18}. Total-, HDL-, LDL-cholesterol and triglycerides were measured on the ADVIA XPT Clinical Chemistry System (Siemens Healthcare Diagnostics, Eschborn, Germany). Glycated haemoglobin (HbA1c) measurements were performed in the central laboratory of the University Hospital of Tübingen using the Tosoh A1c analyzer HLC-723G8 (Tosoh Bioscience GmbH, Griesheim, Germany).

Calculations: Insulin secretion indices were derived from the OGTT with insulin and C-peptide concentrations given in pmol/l, and glucose concentration given in mmol/l. AUCs of insulin, C-peptide, glucose and glucagon concentrations during the entire 120 min of the OGTT were calculated according to the trapezoid method as: \(0.5 \times (0.5 \times c_{0\text{min}} + c_{30\text{min}} + c_{60\text{min}} + c_{90\text{min}} + 0.5 \times c_{120\text{min}})\) with \(c\) = concentration. \(\text{AUC}_{C\text{-Peptide}(0-30\text{min})}/\text{AUC}_{Glucose(0-30\text{min})}\) was calculated as: \((C_{\text{Peptide}_{0\text{min}}} + C_{\text{Peptide}_{30\text{min}}})/(Glc_{0\text{min}} + Glc_{30\text{min}})^{19}\). Insulinogenic Index (IGI\textsubscript{0-30min}) was calculated as: \((\text{Ins}_{30\text{min}} - \text{Ins}_{0\text{min}})/(Glc_{0\text{min}} - Glc_{30\text{min}})^{19}\). Insulin sensitivity derived from the OGTT was estimated as proposed by Matsuda and DeFronzo (ISI Matsuda)\textsuperscript{20}: \(10000/\sqrt{(Glc_{0\text{min}} \times \text{Ins}_{0\text{min}} \times Glc_{\text{mean}} \times \text{Ins}_{\text{mean}})}\). Disposition Index (DI) was calculated as: IGI x ISI\textsuperscript{19}. Fasting insulin clearance was calculated as \(C_{\text{Peptide}_{0\text{min}}}/\text{Ins}_{0\text{min}}\) with \(C_{\text{Peptide}_{0\text{min}}}=\)fasting C-peptide, insulin clearance during the OGTT was calculated as \(\text{AUC}_{C\text{-Peptide}(0-120\text{min})}/\text{AUC}_{\text{Insulin}(0-120\text{min})}\).

Single cell data: Recently published single cell expression profiles\textsuperscript{21} were analyzed for expression of LDLR. A tSNE (t-distributed stochastic neighbor embedding) plot from 2544 pancreatic single cell data sets was generated. For this, we used the top 250 expressed genes with a perplexity parameter of 30 and a theta of 0.4. Then, we assigned each cell to a probable cell type based on the highest expression of the cell type specific marker genes. Next, we plotted the log-transformed LDL receptor (LDLR) expression on these cells.

Statistical analyses: Prior to statistical evaluation, variables with skewed distribution were loge-transformed. For multiple linear regression analysis, the standard least-squares method was applied and the variable of interest was used as dependent variable, LDL cholesterol concentration as independent variable, and sex, age, BMI and ISI Matsuda (for all insulin and C-peptide based...
secretion parameters except DI and gucagon secretion during the OGTT) as confounding variables. Data are presented as means ± SEM. For illustrative purposes we divided the cohort into LDL cholesterol quartiles and compared them by ANOVA (figure 1). A p value ≤ 0.05 was considered statistically significant. The statistical software package JMP 13.0.0 (SAS Institute Inc, Cary, NC, USA) was used. Single cell data were analyzed using R (version 3.6.1) and the Rtsne library (version 0.15).

3. Results

3.1. LDL receptor expression in human pancreatic islets

Basis for a possible effect of LDL cholesterol levels on human islets is the expression of LDL receptors in pancreatic tissue. We therefore enquired single cell data from Enge et al. and observed LDLR expression in all major endocrine cells types of the pancreas (figure 2).

3.2. Association between LDL cholesterol levels and glucagon concentrations

As LDLR expression was present in alpha cells, we first analyzed possible links to glucagon secretion at fasting as well as during an oral glucose tolerance test. There was no association between LDL cholesterol and fasting glucagon levels (p=0.7, β=-0.01 adjusted for sex, age and BMI, p=0.0369, β=0.09 unadjusted respectively). There was also no significant association between fasting LDL and glucagon secretion during the oral glucose tolerance test (p=0.2, β=-0.07 adjusted for sex, age and BMI, p=0.4, β=0.05 unadjusted respectively) (table 2). We detected no significant interaction with BMI (all p ≥ 0.5).

3.3. Association between LDL cholesterol levels and insulin secretion

We next analyzed the relation of LDL with insulin secretion from pancreatic beta cells and detected statistically significant positive associations of LDL cholesterol and C-peptide based indices of insulin secretion (AUC\textsubscript{C-Peptide(0-30min)}/AUC\textsubscript{Glucose(0-30min)}: p=0.0001, β=0.06; AUC\textsubscript{C-Peptide(0-120min)}/AUC\textsubscript{Glucose(0-120min)}: p<0.0001, β=0.08, figure 1). This remained significant after adjustment of LDL cholesterol for HDL cholesterol (AUC\textsubscript{C-Peptide(0-30min)}/AUC\textsubscript{Glucose(0-30min)}: p=0.0002, β=0.06; AUC\textsubscript{C-Peptide(0-120min)}/AUC\textsubscript{Glucose(0-120min)}: p<0.0001, β=0.09).
Adjusting LDL cholesterol for fasting blood glucose levels or the area under the blood glucose curve during the OGTT revealed comparable association (all \( p \leq 0.002 \)). Additionally, we found significant interaction between LDL cholesterol and glucose tolerance on insulin secretion (\( \text{AUC}_{\text{C-Peptide}(0-30\text{min})}/\text{AUC}_{\text{Glucose}(0-30\text{min})} \), \( p=0.0035, \beta=-1.28 \) and \( p=0.03, \beta=-0.97 \), respectively). This interaction remained significant after adjusting for sex, age, BMI, and insulin sensitivity, at least for \( \text{AUC}_{\text{C-Peptide}(0-120\text{min})}/\text{AUC}_{\text{Glucose}(0-120\text{min})} \) (\( p=0.0246, \beta=-0.79 \)). Therefore, we stratified our cohort by glucose tolerance. While C-peptide-based insulin secretion was not linked with LDL cholesterol in subjects with prediabetes or treatment-naïve diabetes (all \( p \geq 0.2 \)) there was a significant association in those with normal glucose regulation (\( p<0.0001, \beta=0.09; \text{AUC}_{\text{C-Peptide}(0-120\text{min})}/\text{AUC}_{\text{Glucose}(0-120\text{min})} \), \( p<0.0001, \beta=0.1 \) adjusted for sex, age, BMI, and ISI Matsuda). Though, no interaction with sex was present (\( p=0.9; \text{AUC}_{\text{C-Peptide}(0-120\text{min})}/\text{AUC}_{\text{Glucose}(0-120\text{min})} \), \( p=0.3 \)), indicating a comparable relation in both sexes. We also found no interaction with BMI (\( p=0.8; \text{AUC}_{\text{C-Peptide}(0-120\text{min})}/\text{AUC}_{\text{Glucose}(0-120\text{min})} \), \( p=0.6 \)). In contrast to the C-peptide-based indices, we found a negative association between LDL concentrations and insulin secretion when analyzing insulin-based insulin secretion indices (Insulinogenic index: \( p=0.014, \beta=-0.04 \); disposition index: \( p=0.0003, \beta=-0.06 \), figure 2). This remained significant after adjustment for HDL cholesterol (Insulinogenic index: \( p=0.0121, \beta=0.04 \); disposition index: \( p=0.0005, \beta=-0.06 \) (table 2). For the insulin-based insulin secretion indices, we detected no significant interaction with glucose tolerance, sex, or BMI (all \( p \geq 0.1 \)).

### 3.4. Association between LDL cholesterol levels and insulin clearance

After secretion into the portal vein, insulin undergoes hepatic extraction as well as peripheral clearance. In comparison, C-peptide is not extracted by the liver, but reaches the systemic circulation to a full extent before being cleared in the kidneys. As insulin and C-peptide show different elimination kinetics, we next performed an analysis for estimates of fasting and post-load insulin clearance. LDL cholesterol levels were directly associated with both fasting insulin clearance as well as clearance during the OGTT (\( p<0.0001, \beta=0.09 \) and \( p<0.0001, \beta=0.06 \), respectively) (table 2). This relation did
not interact with glucose tolerance (fasting insulin clearance: p= 0.06; clearance during OGTT: p=0.9)
or BMI (fasting insulin clearance: p=0.8; clearance during OGTT: p=0.96).

4. Discussion

Lowering LDL cholesterol levels has beneficial effects on the cardiovascular system but is unfortunately linked to increased risk for type 2 diabetes. Based on previous experimental data \(^{14-16}\), we hypothesized that LDL – cholesterol is linked via its receptor to pancreatic islet function. Therefore, we now investigated the relevance for humans and addressed whether LDL cholesterol levels are linked to insulin secretion from pancreatic beta cells or to alpha cell function. Such a relation could contribute to the widely observed association between LDL-cholesterol lowering and diabetes risk.

All cells in the islets of Langerhans express LDL receptors. While glucagon levels were unrelated to LDL cholesterol, we observed significant associations with insulin secretion. Notably, C-peptide based indices were positively, but insulin-based ones were negatively associated with LDL cholesterol. This was due to an association of LDL cholesterol with insulin clearance.

In 1997 Grupping et al. showed that human islet \(\beta\)-cells isolated from donor pancreas express LDL binding sites that fulfill the properties of LDL receptors \(^{23}\). In 2017 Enge et al. provided single-cell data from human pancreatic cells \(^{21}\). Using this dataset, we identified \(LDLR\) expression in all major endocrine cells. Alpha cells secrete glucagon, which works antagonistic to insulin. Additionally, post challenge changes in glucagon contribute to glycaemia after an OGTT and are therefore quiet important in regulating postprandial glucose metabolism \(^{18}\). Since the hormone affects blood glucose levels and as single cell data showed a \(LDLR\) expression on \(\alpha\)-cells, we first investigated the association between LDL cholesterol levels and glucagon secretion. As we did not find links between LDL cholesterol and glucagon, this hormone is most likely not involved in increased diabetes risk in case of LDL lowering therapy. Though, the function of LDL-receptors on \(\alpha\)-cell biology remains to be determined.
Insulin is synthesized and released from pancreatic β-cells. When glucose levels rise, insulin and its cleavage fragment C-peptide are released in equimolar amounts. The ability to secret sufficient amounts of insulin is the most important component for a physiological control of glucose concentrations in the body. We detected a significant, positive association between fasting LDL concentrations and C-peptide based estimates for insulin secretion. As these indices reflect insulin secretion independent of hepatic clearance, they are the superior estimates of insulin secretion in our current setting. Our results indicate that higher LDL cholesterol levels could promote insulin secretion from pancreatic β-cells. This link could explain the observations in various clinical studies where lowering of LDL levels by statins therapy results in a deterioration of glucose control. Though, Natali et al. detected no link of LDL cholesterol and insulin secretion. This study is however not entirely comparable to our current analysis as the sample size was smaller and persons with higher cholesterol concentrations were excluded. Of note, potential LDL effects on insulin secretion appear to be affected by glucose control and seem to be blunted in prediabetes and diabetes. Thus, LDL lowering therapy might raise diabetes risk especially in yet metabolically healthy persons. In case of already impaired glycaemia other pathomechanisms might superimpose LDL effects on pancreatic beta cells.

Recently, Klimentidis et al. identified 31 genetic loci which are associated with lower circulating LDL cholesterol and increased diabetes risk. The identified variants are linked with genes that affect de novo fatty acid synthesis, hepatic lipid uptake, and export and insulin action. Of note, five of the identified loci were associated with insulin secretion, including SLC2A2. This gene encodes the glucose transporter 2 (GLUT2), which is essential for post-load hepatic glucose uptake that will subsequently enter hepatic de novo lipogenesis. Further genetic loci like C2CD4A/B, MICAL3, HNF1A-OASL and GIPR were previously described to be associated with insulin secretion and have now been linked to circulating LDL cholesterol. Molecular mechanisms that mediate this association remain unknown up to now.

While HDL cholesterol and triglycerides are also associated with insulin secretion, the link between LDL cholesterol and insulin secretion was independent of this potential confounders in our current analysis.
While our data on LDL cholesterol and C-peptide based analyses of insulin secretion are well in line with previous findings on diabetes risk, we unexpectedly detected an inverse correlation for the tested insulin-based estimates of insulin secretion (IGI and DI). Thus, our results at first glance appear controversial. As insulin and C-peptide undergo different elimination mechanisms with a high hepatic first pass clearance of insulin \(^{31}\), we next investigated possible links of LDL cholesterol and insulin clearance that could potentially explain these contrary results. Indeed, fasting and stimulated insulin clearance were both directly associated with LDL concentrations. Accordingly, with increasing LDL cholesterol concentrations, more insulin is extracted by the liver, explaining the inverse correlation of insulin-based and C-peptide-based estimates for insulin secretion. In humans, the liver is the most LDLR-abundant organ and accounts for more than 70% of the total LDL clearance from the plasma \(^{32}\). We therefore hypothesize that hepatic mechanisms that decrease circulating LDL cholesterol levels, concurrently enhance the liver’s insulin clearance capacity. The carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1), a transmembrane glycoprotein could possibly link LDL cholesterol levels and insulin clearance. Animal research suggests a link between CEACAM1, key component of hepatic insulin clearance, and lipid metabolism in the liver. However the detailed molecular pathways and their potential link to LDL metabolism still remain elusive \(^{33,34}\).

Among the limitations is the use of estimates for insulin secretion and clearance. As both physiological processes are complex and influenced by several further components, insulin secretion and clearance can be over- or underestimated. Furthermore the results are only based on a cross-sectional analysis and did not apply the gold standard measurements of insulin secretion but used well-established estimates from the OGTT. Further prospective analyses are necessary in an experimental setting, were LDL-cholesterol levels are actively decreased by cholesterol lowering drugs. Additionally, only Caucasians were included into this analysis and we cannot rule out that ethnicity affects the described associations. Nevertheless, the observed effects are not likely to outweigh the benefits of LDL lowering strategies in patients with increased LDL cholesterol levels. On the other hand, our data may suggest that benefits and risks in patients with reduced insulin secretory capacity should be carefully evaluated before commencing an LDL lowering strategy.

Taken together, our current results demonstrate that all major endocrine cells show LDLR expression in the pancreas. While we did not find an association between LDL cholesterol levels and glucagon
secretion from pancreatic α-cells, a positive association was observed for LDL concentrations and C-peptide based estimates for insulin secretion. Decreased insulin secretion in case of lower LDL cholesterol could underlie the observation of deteriorated glycemic control in response to LDL lowering drugs. The observed inverse correlation of LDL cholesterol concentrations and insulin-based estimates for insulin secretion is a result of enhanced insulin clearance in case of higher LDL levels. CEACAM1 as key component of hepatic insulin clearance could possibly link hepatic insulin clearance and LDL metabolism in the liver, while molecular mechanisms are not identified so far. Accordingly, our data suggest that LDL cholesterol levels and insulin secretion and clearance might be directly linked. A detailed understanding of the underlying complex biology will aid the way to novel approaches to preserve beta cell function and prevent diabetes in patients who require LDL-cholesterol lowering therapy.
Data Availability Statement: Some or all data generated or analyzed during this study are included in this published article or in the data repositories listed in References.

Author Contributions: CD researched and analyzed data and drafted the manuscript together with MH. RW researched and analyzed data. AP was responsible for laboratory measurements. JH and AF acquired data. HUH, AF, ALB contribute to discussion of the results, NS and MH researched data, and supervised the project. All authors contributed to the discussion and approved the final version of the manuscript prior to submission.

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Figure 1: LDL cholesterol levels and insulin secretion

Displayed are C-peptide based insulin secretion parameter (A) AUC$_{\text{C-Peptide}(0-30\text{min})}/$AUC$_{\text{Glucose}(0-30\text{min})}$, (B) AUC$_{\text{C-Peptide}(0-120\text{min})}/$AUC$_{\text{Glucose}(0-120\text{min})}$ and insulin based secretion parameter (C) Insulinogenic Index, (D) Disposition Index after stratification of participants in LDL cholesterol quartiles. Bars represent means±SEM. P-values are for comparison of LDL quartiles by ANOVA for illustrative purpose while p-values from continuous models are reported in the text.

Figure 2: LDLR expression across pancreatic cells.

Recently published single cell expression profiles [21] were analyzed for expression of LDLR. A tSNE (t-distributed stochastic neighbor embedding) plot from 2544 pancreatic single cell data sets was generated. (A) We assigned each cell to a probable cell type based on the highest expression of the cell type specific marker genes indicated in the figure legend in brackets. (B) Next, we plotted the log-transformed LDLR expression on these cells with high expression indicated by dark red color.
Table 1: Characteristics of the study participants

| Characteristics                                      | n    |
|------------------------------------------------------|------|
| n                                                    | 3039 |
| Sex (f/m)                                            | 2001/1038 |
| Age (years)                                          | 42 ± 0.25 |
| BMI (kg/m²)                                          | 31.2± 0.2 |
| WHR<sup>a</sup>                                      | 0.88 ± 0.004 |
| Fasting blood glucose (mmol/l)                       | 5.31 ± 0.01 |
| 2h blood glucose (mmol/l)                            | 6.69 ± 0.04 |
| Fasting glucagon<sup>b</sup> (pg/ml)                  | 69.23 ± 1.06 |
| AUC<sub>G</sub>Glucagon<sup>c</sup> (pg/ml)           | 115.18 ± 2.03 |
| HbA1c<sup>d</sup> (%)                                | 5.5 ± 0.01 |
| glucose tolerance (NGR/prediabetes/diabetes)         | 1833/1055/151 |
| Total cholesterol<sup>e</sup> (mg/dl)                 | 194.64 ± 0.70 |
| LDL cholesterol<sup>f</sup> (mg/dl)                   | 119.20 ± 0.61 |
| HDL cholesterol<sup>g</sup> (mg/dl)                   | 53.74 ± 0.26 |
| Triglycerides<sup>h</sup> (mg/dl)                     | 124.49 ± 2.14 |
| Liver fat content<sup>i</sup> (%)                    | 6.8± 0.2 |
| Insulin sensitivity<sup>j</sup> (ISI Matsuda)         | 12.05 ± 0.16 |
| IGI<sub>0-30min</sub><sup>k</sup>                    | 190.84 ± 3.96 |
| AUC<sub>C-Peptide(0-30min)/AUC<sub>Glucose(0-30min)</sub></sup> | 190.09 ± 1.37 |
| AUC<sub>C-Peptide(0-120min)/AUC<sub>Glucose(0-120min)</sub></sup> | 299.38 ± 1.84 |
| Disposition Index<sup>n</sup>                         | 1969 ± 70 |
| Fasting insulin clearance<sup>o</sup> (C-Pep<sub>0min</sub>/Ins<sub>0min</sub>) | 9.16 ± 0.10 |
| Insulin clearance during OGTT<sup>p</sup>             | 4.80 ± 0.04 |

Data are means ± SEM; data were available for the following numbers of subjects: <sup>6</sup>N = 3002; <sup>6</sup>N = 595; <sup>6</sup>N = 385; <sup>6</sup>N = 2913; <sup>6</sup>N = 3038; <sup>6</sup>N = 3012; <sup>6</sup>N = 3013; <sup>6</sup>N = 3037; <sup>6</sup>N = 998; <sup>6</sup>N = 2971 ; <sup>6</sup>N = 2982; <sup>6</sup>N = 2963; <sup>6</sup>N = 2945, <sup>6</sup>N = 2959, <sup>6</sup>N= 2968, <sup>6</sup>N = 2919. WHR: waist-to-hip ratio; NGR: normal glucose regulation.
Table 2: Association of LDL cholesterol levels with glucagon, insulin secretion and insulin clearance

|                                      | p unadj. | β ± SE | r²     | p adj.  | β ± SE | r²     | β ± SE | p adj.  | β ± SE | r²     | β ± SE | p adj.  |
|--------------------------------------|----------|--------|--------|---------|--------|--------|--------|---------|--------|--------|--------|---------|
|                                      |          |        |        | (age, sex, BMI) |        |        | (age, sex, BMI, ISI Matsuda) |        | (age, sex, BMI, ISI Matsuda, HDL) |        |        | (age, sex, BMI, ISI Matsuda, HDL) |        |
| Association of LDL cholesterol with glucagon secretion                      |          |        |        |          |        |        |          |        |        |        |        |          |
| Fasting glucagon levels            | 0.0369   | 0.0862 ± 0.0541 | 0.0057 | 0.7274 | -0.0138 ± 0.0632 | 0.1584 | 0.5283 | -0.0240 ± 0.050 | 0.2239 | 0.6318 | -0.0191 ± 0.0522 | 0.1601 | 0.5302 | -0.0240 ± 0.0902 |
| Glucagon secretion during OGTT     | 0.38     | 0.045 ± 0.0627 | -0.0006 | 0.15    | -0.074 ± 0.0618 | 0.1266 | 0.1616 | -0.0711 ± 0.0616 | 0.1342 | 0.1441 | -0.075 ± 0.0622 | 0.1244 | 0.17   | -0.0702 ± 0.0620 |
| Association of LDL cholesterol with C-peptide based insulin secretion indices |          |        |        |          |        |        |          |        |        |        |        |          |
| AUC C-Peptide(0-30min)/AUC Glucose(0-30min) | <0.0001  | 0.0733 ± 0.0239 | 0.0054 | <0.0001 | 0.0785 ± 0.0224 | 0.1990 | 0.0001 | 0.0643 ± 0.0214 | 0.2779 | <0.0001 | 0.0741 ± 0.0223 | 0.2111 | <0.0001 | 0.0741 ± 0.0233 |
| AUC C-Peptide(0-120min)/AUC Glucose(0-120min) | <0.0001  | 0.1112 ± 0.0379 | 0.0071 | <0.0001 | 0.0936 ± 0.0213 | 0.0820 | <0.0001 | 0.0767 ± 0.0205 | 0.1556 | <0.0001 | 0.0892 ± 0.0212 | 0.0908 | 0.0244 | 0.0369 ± 0.03386 |
| Association of LDL cholesterol with insulin based insulin secretion indices |          |        |        |          |        |        |          |        |        |        |        |          |
| Insulinogenic index (IGI)          | 0.1550   | -0.0262 ± 0.0485 | 0.0030 | 0.1043 | -0.0292 ± 0.0474 | 0.1307 | 0.014  | -0.043 ± 0.0456 | 0.2002 | 0.0706 | -0.0324 ± 0.0472 | 0.1360 | 0.0121 | -0.0435 ± 0.0456 |
| Disposition index                  | <0.0001  | -0.1781 ± 0.0033 | 0.0003 | -0.063 ± 0.1307 | -#     | -#     | -#     | 0.0005 | -0.0607 ± 0.1360 | -#     | -#     | -#     |
### Association of LDL cholesterol with insulin clearance indices

|                         | (DI)   | 0.0528 | 0.0508 | 0.0507 | 0.0507 |
|-------------------------|--------|--------|--------|--------|--------|
| **Fasting insulin Clearance** | 0.4227 | -0.0148 ± 0.0292 | 0.0002 | -0.062 ± 0.0273 | 0.1991 | <0.0001 | 0.094 ± 0.0221 | 0.4861 | 0.0002 | 0.0635 ± 0.0273 | 0.1989 | <0.0001 | 0.0916 ± 0.022 | 0.4913 |
| **Insulin clearance during OGTT** | <0.0001 | -0.0863 ± 0.0278 | 0.0070 | 0.3566 | 0.0151 ± 0.0246 | 0.2642 | <0.0001 | 0.055 ± 0.0157 | 0.6215 | 0.2181 | 0.020 ± 0.0244 | 0.2723 | <0.0001 | 0.0538 ± 0.0157 | 0.6225 |

For multiple linear regression analysis the standard least-squares method was applied with LDL cholesterol concentration as independent variable. P-values were given unadjusted (first column) or adjusted for covariates age, sex and BMI. ^ISI Matsuda was added as an additional covariate for all parameters except DI (second and third column). The last two columns show p-values after additional adjustment for HDL cholesterol concentrations. Betas are given ± SE.
Figure 1

A

B

C

D

AUC C-reactive protein (mg/L)/LDL cholesterol (mg/dL)

AUC C-reactive protein (mg/L)/LDL cholesterol (mg/dL)

Insulinogenic index (AU)

Disposition index (AU)

1st quartile
2nd quartile
3rd quartile
4th quartile

1st quartile
2nd quartile
3rd quartile
4th quartile

1st quartile
2nd quartile
3rd quartile
4th quartile

1st quartile
2nd quartile
3rd quartile
4th quartile

p = 0.0163

p = 0.0009

p = 0.0496

p = 0.0001
