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Lipolysis, and not hepatic lipogenesis, is the primary modulator of triglyceride levels in streptozotocin-induced diabetic mice

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Abstract

Objective—Diabetic hypertriglyceridemia is thought to be primarily driven by increased hepatic *de novo* lipogenesis. However, experiments in animal models indicated that insulin deficiency should decrease hepatic *de novo* lipogenesis and reduce plasma triglyceride levels.

Approach and Results—To address the discrepancy between human data and genetically altered mouse models, we investigated whether insulin deficient diabetic mice had triglyceride changes that resemble those in diabetic humans. Streptozotocin (STZ)–induced insulin deficiency increased plasma triglyceride levels in mice. Contrary to the mouse models with impaired hepatic insulin receptor signalling, insulin deficiency did not reduce hepatic triglyceride secretion and *de novo* lipogenesis-related gene expression. Diabetic mice had a marked decrease in postprandial TG clearance, which was associated with decreased lipoprotein lipase (LpL) and PPARα mRNA levels in peripheral tissues and decreased LpL activity in skeletal muscle, heart and brown adipose tissue. Diabetic heterozygous LpL knockout mice had markedly elevated fasting plasma triglyceride levels and prolonged postprandial TG clearance.

Conclusion—Insulin deficiency causes hypertriglyceridemia by decreasing peripheral lipolysis and not by an increase in hepatic TG production and secretion.

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Keywords

lipoprotein lipase; hypertriglyceridemia; diabetes

Introduction

Dyslipidemia is a major risk factor for cardiovascular disease (CVD) in both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM)[1, 2]. Diabetic dyslipidemia is associated with high plasma triglycerides (TG), low HDL cholesterol and increased small dense LDL-cholesterol particles[3]. According to a National Health and Nutrition Examination Survey, over 30% of people with T2DM have TG levels >2.258 mmol/l (200 mg/dl)[4]. Patients with T1DM also have increased TG levels, especially with poor glucose management[1]. Possible causes of hypertriglyceridemia in patients with diabetes mellitus are increased hepatic VLDL production and/or defective removal of TG-rich lipoproteins (chylomicrons and VLDL).

A number of rodent models have been developed to explain the relationship between insulin actions and TG. It has been postulated that hyperinsulinemia associated with T2DM drives hepatic *de novo* TG synthesis via induction of sterol response element binding protein (SREBP)-1c[5]. Consistent with this hypothesis, lack of insulin action in the liver due to ablation of hepatic insulin receptors and Akt deficiency in mice prevented hepatic TG production, reduced liver TG secretion and led to low circulating TG levels[6–8.] According to this hypothesis, humans with poorly managed T1DM should show reduced hepatic TG production and plasma TG levels. Likewise, insulin therapy in T2DM should also drive greater liver TG production. However, the opposite has been found: In fact, plasma TG concentrations are increased in patients with T1DM[9, 10.] Moreover, treatment of T2DM patients with insulin results in systemic hyperinsulinemia, but reduced TG levels and decreased hepatic lipid accumulation[11]. Studies in diabetic rodents also conflict with conclusions derived from mice with genetic modifications in the insulin-signalling pathway. Viral destruction of pancreatic islet cells in mice leads to hypertriglyceridemia[12] and re-feeding of insulin deficient mice increased lipogenic gene expression, suggesting that regulation of *de novo* synthesis is independent of insulin[13]. These data suggest that diabetic hypertriglyceridemia is not primarily caused by defective insulin signalling leading to increased hepatic fatty acid synthesis. The objective of this study was specifically to determine whether the effects of impaired insulin signalling on hepatic triglycerides production found with genetic modifications were also evident in mice with insulin deficiency.

In this report, we show that insulin deficiency in mice leads to increased plasma TG levels and defective removal of postprandial TG. This type of diabetic hypertriglyceridemia was not associated either with reduced mRNA levels of *de novo* TG synthesis-related genes or with decreased hepatic TG production. LpL mRNA was significantly reduced in skeletal muscle, white adipose tissue (WAT) and heart. Furthermore, LpL activity was decreased in skeletal muscle, brown adipose tissue (BAT) and heart. In addition, diabetes further increased plasma TG in animals with a genetic LpL defect. Our data support human studies

and suggest that significant hypertriglyceridemia in insulin deficient diabetes is primarily due to changes in lipolysis and substrate return to the liver.signalling

Material and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

STZ-induced diabetes causes hypertriglyceridemia in mice

Two weeks after induction of insulin deficiency by intraperitoneal STZ administration, diabetic mice displayed marked hyperglycemia (6.66 ± 0.5 mmol/l vs. 25.55 ± 0.72 mmol/l) (Table 1A). Concomitantly, these mice had significantly elevated plasma TG levels (1.42 ± 0.09 versus 0.82 ± 0.03 mmol/l in non-diabetic mice). Hypertriglyceridemia persisted after 6 weeks of STZ diabetes (1.99 ± 0.18 versus 0.91 ± 0.06 mmol/l). In contrast, total plasma cholesterol levels and HDL cholesterol did not change at either time point. As expected, STZ-diabetic mice lost weight compared to non-diabetic control animals. Changes in TG were largely caused by increased VLDL TG (1.33 ± 0.09 mmol/l vs. 0.71 ± 0.02 mmol/l) (Table 1B). Plasma FFA were increased at both 3 and 6 weeks. Note that the baseline plasma FFA levels were higher in older mice. Plasma FFA showed a positive correlation with plasma TG levels in STZ-diabetic mice, whereas plasma FFA and TG did not significantly correlate with body weight (Supplement IA–C).

Insulin deficiency does not change hepatic *de novo* lipogenesis and hepatic TG secretion

To assess whether insulin deficiency and circulating glucose levels affect hepatic TG production, we quantified TG secretion in mice treated with STZ and a lipase inhibitor, P407. STZ-induced diabetes did not alter TG secretion compared to non-diabetic wild type controls (Figure 1A). In line with this, hepatic gene expression of *de novo* lipogenesis genes (*Fasn, Acc1, Dgat2, Scd1, Elovl6*) showed no significant difference between the diabetic mice and healthy controls after 3 and 8 weeks (Figure 1B & Supplement ID). TG content in the liver was decreased (Figure 1C & Supplement IE), which was likely due to increased β-oxidation as indicated by increased gene expression of the rate limiting enzyme CPT1α (Figure 1B & Supplement ID). Thus, hypertriglyceridemia in insulin-deficient mice is not caused by increased *de novo* lipogenesis and hepatic TG secretion.

Hyperglycemia does not drive hypertriglyceridemia

Excess glucose in diabetes can be a substrate for hepatic *de novo* lipogenesis, thereby contributing to diabetic hypertriglyceridemia[14]. To study if hyperglycemia drives hypertriglyceridemia we treated control and STZ-diabetic mice with the glucose-lowering agent dapagliflozin for 3 weeks. Dapagliflozin selectively inhibits the sodium glucose co-transporter 2 in the kidney, thereby increasing urinary glucose excretion and decreasing plasma glucose levels without affecting insulin levels in STZ-diabetic mice as shown before15 and in our study (Figure 2D). Insulin levels in dapagliflozin treated control mice are reduced likely due to the reduction in circulating glucose levels. Despite a significant reduction in plasma glucose levels in STZ-diabetic mice neither plasma TG levels (Figure

2A & 2B) nor TG secretion (Figure 2C) decreased. Similar to the pharmacological approach, an antisense oligonucleotide against SGLT2 did not decrease plasma TG levels in both control and STZ-diabetic mice over the course of four weeks (Supplement II). These studies suggest that diabetic hypertriglyceridemia is not driven by excess glucose.

Insulin deficiency leads to increased postprandial lipemia

As hepatic *de novo* lipogenesis and TG secretion did not appear to contribute substantially to hypertriglyceridemia, we explored whether defective peripheral lipolysis is the culprit for increased plasma TG levels. After an olive oil gavage, STZ-diabetic mice displayed markedly higher plasma TG levels followed by delayed clearance of plasma TG compared to that of controls (Figure 3A). LpL mRNA levels were significantly reduced in skeletal muscle, WAT and heart after 3 weeks of diabetes (Figure 3B). At 8 weeks skeletal muscle LpL was reduced and tended to decrease in the WAT and heart of these mice (Supplement IIIA). However, mRNA levels of LpL do not always reflect LpL activity as LpL is regulated at transcriptional, translational and posttranslational levels[15, 16]. Heparin-releasable LpL activity in the heart, BAT and skeletal muscle was significantly reduced in STZ-diabetic mice (Figure 3C); there was no change in heparin-releasable activity of hepatic lipase and hepatic lipase mRNA in the liver (Supplement IIIB & IV). Hepatic mRNA expression of the LpL regulating proteins angiopoietin-like 3, 4 and 8 (Angptl 3, 4, and 8) and apolipoproteins apoA-V, ApoB and ApoC-III were not significantly altered at both 3 and 8 weeks. We also did not detect significant changes of Angptl4, lipase maturation factor 1 (LMF1) and glycosylphosphatidylinositol-anchored high-density binding protein 1 (GPIHBP1) in the skeletal muscle (Supplement Figure IV). However, we did find an increase in apoC-III in the plasma of STZ-diabetic mice (Figure 3D). LpL activity in postheparin plasma did not correlate with the changes in mRNA levels or heparin-released muscle LpL activity (Supplement IIIC). Taken together, insulin-deficient diabetes caused a marked reduction in postprandial TG removal associated with reduced expression and activity of skeletal muscle LpL.

LpL deficiency is associated with decreased expression of PPARα and PPARδ

To explore how insulin deficiency affects LpL expression and activity we studied LpL regulating factors. LpL expression in several tissues has been linked to peroxisome proliferator-activated receptor (PPAR) expression and activation[17–19]. In our study, reduced LpL mRNA levels were associated with markedly lower PPARα mRNA in the skeletal muscle of diabetic mice after 3 weeks (Figure 3E). PPARδ mRNA was also decreased, while PPARγ was unchanged. We detected similar expression patterns of PPARs in the skeletal muscle after 8 weeks of STZ-diabetes (Supplement V).

LpL expression in the skeletal muscle modulates diabetic dyslipidemia

Fasted wild type mice have a rapid turnover of plasma TG. To study a more human-like model with less robust TG lipolysis, we induced diabetes in heterozygous LpL knockout mice (*Lpl*$^{+/-}$). Our laboratory has previously reported that *Lpl*$^{+/-}$ mice on the ApoB overexpressing transgenic background have marked hypertriglyceridemia when treated with STZ[20]. STZ-treatment of *Lpl*$^{+/-}$ mice led to markedly increased plasma TG (2.45 ± 0.26

mmol/l) compared to non-diabetic *Lpl*$^{+/-}$ mice (1.389 ± 0.316 mmol/l) (Figure 4A & B). Similar to wild type mice, TG secretion was not altered in STZ-diabetic *Lpl*$^{+/-}$ mice after P407 treatment and hepatic TG content was reduced (Figure 4C & D). Again, after olive oil gavage STZ-diabetic *Lpl*$^{+/-}$ mice displayed a significant impairment of TG clearance (Figure 4E). The highest postprandial TG levels in STZ-treated mice were identical to those of diabetic wild type mice, but the rate of reduction between 4–6 hours was reduced compared to diabetic wild type mice (4.2 mmol/l/hour versus 7.7 mmol/l/hour).

These data suggest that impaired TG clearance is the major cause for hypertriglyceridemia in STZ-diabetic mice. To test whether LpL overexpression would alter these diabetes-induced TG changes, we studied postprandial lipemia in mice expressing human LpL primarily in skeletal muscle; the transgene is denoted MCK-LpL. We note that the MCK-LpL transgene also leads to a small amount of LpL expression in the heart[21]. STZ-diabetic MCK-LpL/*Lpl*$^{+/-}$ mice had significantly lower fasting TG levels compared to STZ-diabetic *Lpl*$^{+/-}$ mice (Figure 5A). In addition, TG clearance after olive oil gavage was significantly improved in STZ-diabetic MCK-LpL/*Lpl*$^{+/-}$ mice compared to STZ-diabetic *Lpl*$^{+/-}$ mice (Figure 5B). Therefore, LpL overexpression corrects diabetic dyslipidemia in insulin deficient mice.

Discussion

In this report, we show that hypertriglyceridemia in insulin deficient diabetic mice is primarily due to changes in peripheral lipolysis and not due to changes in hepatic insulin signalling and hepatic TG secretion. Similar to humans with either T1DM or T2DM, STZ-induced diabetes in C57BL/6 mice led to a significant increase in plasma TG levels. This observation was similar to that described by others[22, 23]. The primary lipoprotein abnormality in human diabetes is an increase in VLDL, which was reproduced in our STZ-treated mice. There are two possible causes of diabetes-associated increase in TGs: elevated secretion of TGs from the liver and/or decreased clearance of TGs[24, 25]. 1) Hepatic overproduction of TGs has been attributed to compromised ApoB degradation due to the loss of insulin action, which eventually leads to increased VLDL assembly and secretion[26, 27.] Loss of insulin action also increases FFA flux to the liver and increases hepatic lipogenesis[10]. In addition, it has been suggested that hyperinsulinemia can drive *de novo* lipogenesis in the presence of increased plasma glucose levels and hepatic insulin resistance[5]. It should be noted that most human kinetic studies on TG production are carried out by injection of glycerol which cannot separate *de novo* lipogenesis from substrate driven TG production[28–31]. Therefore these studies cannot determine the contribution of hepatic *de novo* lipogenesis and substrate driven TG production to hypertriglyceridemia. 2) Diabetic humans exhibit a marked defect in the clearance of postprandial lipemia[32, 33]. These findings are in accordance with reports of decreased LpL activity in postheparin plasma[34] and in skeletal muscle biopsies of diabetic patients[35, 36]. Accordingly, Taskinen *et al*. have shown that >20% of the hypertriglyceridemia in obese men is associated with increases in $VLDL_1$ secretion but almost 50% is related to impaired fractional catabolic rate[28]. It is not clear, however, why only some patients develop hypertriglyceridemia with diabetes. For instance, in the ACCORD trial the average triglycerides level in all diabetic patients was 1.829 mmol/l (162 mg/dl), a level below the clinical threshold of hypertriglyceridemia

(2.258 mmol/l (200 mg/dl))[37]. Therefore, it is likely that the minority of patients who develop severe hypertriglyceridemia have a defect in lipolysis.

Our objective was specifically to determine whether the effects of insulin signalling on hepatic triglyceride production found with genetic modifications were evident in mice with insulin deficiency. In our mouse model of STZ-induced diabetes, insulin deficiency did not lead to a significant reduction in hepatic TG secretion nor did the lack of insulin actions reduce expression of genes involved in *de novo* lipogenesis. As reported by others[13, 23] hepatic TG content was significantly reduced and plasma FFA levels were increased in STZ-diabetic mice. The reduced hepatic TG content could be partially due to increased fatty acid oxidation as mRNA levels of the rate limiting enzyme of β-oxidation CPT1-α were increased. Jourdan *et al.* have also noted an increase in CPT1-α and reported increased β-oxidation of palmitate in the livers of STZ-treated mice [23]. It is possible that without this increase in oxidation, more FFA would be converted to TG leading to greater hepatic TG secretion. Taken together, our data do not support the hypothesis that insulin deficiency reduces TG production and suggests that the hypertriglyceridemia in STZ-diabetic mice is primarily due to a catabolic defect.

No model of diabetes is perfect but the STZ model used in our studies is the one recommended by the NIH Animal Models of Diabetic Complications Consortium (AMDCC)[38]. The low dose STZ leads to partial insulin deficiency that causes hyperglycemia but allows sufficient insulin to prevent ketoacidosis and early death. This model does not lead to obesity and peripheral insulin resistance as would be seen in T2DM. In fact, STZ-treated mice have reduced adipose stores and the effects of insulin deficiency in obese animals are likely to be different. Specifically, greater adipose would be expected to lead to greater FFA release and high plasma FFA levels that are likely to drive greater liver production of TG. Nonetheless, we believe that STZ-treatment is more likely to reflect human disease than models that lead to non-physiologic modification of gene expression.

Independent of insulin action on hypertriglyceridemia, glucose also generates a signal that may modulate TG production and TG clearance[39, 40]. Glucose controls the expression of key genes involved in energy metabolism through the carbohydrate response element binding protein (CHREBP). CHREBP binds to the promoter region of glycolysis- (e.g. pyruvate kinase) and lipogenesis-associated genes[40] and may drive diabetic hypertriglyceridemia by increasing transcription of *de novo* lipogenesis genes[41]. Glucose may also exert direct effects on TG clearance. Hyperglycemic conditions downregulate LpL activity in humans[42, 43] and in cultured human adipocytes[44]. This suggests that hyperglycemia per se contributes to the decreased LpL activity and subsequent hypertriglyceridemia in poorly controlled diabetes. Until recently it has been difficult to separate the effects of hyperglycemia versus defective insulin actions on diabetic hypertriglyceridemia because glucose-reducing interventions were achieved by either insulin administration or by improved insulin sensitivity. By inhibiting SGLT2 in the kidney, which leads to increase glucose output in the urine, we were able to reduce plasma glucose levels without affecting insulin levels in STZ-diabetic mice, although we cannot rule out changes in insulin signalling[45]. Both the pharmacological inhibition by dapagliflozin and antisense oligonucleotide mediated inhibition of SGLT2 significantly reduced plasma glucose levels

in STZ-diabetic mice, but did not change plasma TG levels. More importantly, hepatic TG secretion was similar in STZ-diabetic mice with normalized glucose levels making it unlikely that increased glucose levels contribute substantially to hypertriglyceridemia in STZ-diabetic mice.

Postprandial lipemia is markedly increased in humans with diabetes[46–48]. Accordingly, in our study STZ-diabetic mice displayed increased plasma TG levels and delayed clearance after gavage with olive oil. LpL is the rate-limiting enzyme for the hydrolysis of TGs in circulating chylomicrons and VLDL. LpL-mediated hydrolysis products, fatty acids and monoacylglycerol, are taken up by the tissues and stored as neutral lipids in WAT, or oxidized in skeletal and cardiac muscle[15, 16]. LpL is regulated at the transcriptional, post-transcriptional, and post-translational level in a tissue-specific manner[49]. Food-intake decreases LpL activity in skeletal muscle and increases it in WAT. Fasting increases LpL activity in the skeletal muscle and heart and decreases LpL in the WAT. We detected a significant decrease of LpL gene expression in the skeletal muscle, heart and WAT after 3 and 8 weeks of STZ-diabetes. Accordingly, heparin-releasable LpL activity in the skeletal muscle, heart and BAT of STZ-diabetic mice was significantly reduced; all these tissues are major sites for LpL synthesis[16, 50]. We were unable to study heparin-releasable LpL activity in WAT since STZ-diabetic mice were lipodystrophic, but the reduction in total adipose LpL, as we have shown before, likely contributes to the defective lipolysis[51]. Therefore these results show that decreased LpL activity in BAT, skeletal muscle and heart correlates with hypertriglyceridemia in STZ-diabetic mice. Increased apoC-III that we detected in plasma might also affect lipolysis or the changes in lipoproteins associated with LpL deficiency and diabetes might have caused a secondary increase in apoC-III. Perhaps this ApoC-III increase is also responsible for the defect in LDL uptake previously reported in diabetic LDL receptor knockout mice[52].

LpL activity has been studied previously in diabetic humans and rodents. In patients with T2DM LpL mRNA, protein expression and activity were significantly decreased in skeletal muscle[35, 36]. Conflicting results have been reported in studies of LpL activity in the heart and the skeletal muscles in STZ-treated rodents: Both decreased[53] and increased[54] activity levels have been observed. The reported differences might relate to the length as well as severity of diabetes and methodological differences, e.g. measurement of LpL activity in homogenized tissue versus heparin-releasable LpL activity and data obtained from mice versus rats. In fact, although we found reduced heparin-releasable LpL activity in the muscles of diabetic mice, LpL activity measurements of tissue homogenates were not significantly different (data not shown).

In contrast to the decreased LpL activity in the skeletal muscle, postheparin lipase activity in the plasma did not differ between our STZ-diabetic and control mice (2.4 ± 0.16 μm FFA/ml/h versus 2.41 ± 0.10 μm FFA/ml/h respectively). Similarly, Pollare *et al.* did not detect differences in postheparin LpL activity in the plasma of T2DM patients but reported decreased LpL activity in the skeletal muscle of these patients[36]. It is questionable whether total postheparin plasma LpL activity accurately reflects changes in total lipolytic activity.

In order to explain the reduced levels of LpL we tested gene expression levels of two well-known transcriptional regulators of LpL, PPARα and PPARδ. Schoonjans *et al.* showed that the expression of LpL is regulated by PPARα (and PPARγ), which interact with a response element in the LpL promoter[17]. PPARδ agonist treatment increases LpL mRNA and protein levels in skeletal muscle cells[18]; PPARδ also increases expression of the LpL inhibitor Angplt4[55], which was not significantly decreased in our studies. A number of clinical studies suggested that the TG-lowering action of PPARα agonists is associated with an increase in LpL activity[56–58]. Others have shown that PPARα and PPARγ agonists enhance LpL expression and activity in human macrophages[19]. Considering these studies, we hypothesized that decreased LpL expression in the skeletal muscle of STZ-diabetic mice was due to a decreased transcription of PPARα. Insulin enhances the transcriptional activity of PPARα *in vitro*[59]. We found reduced expression of PPARα and PPARδ with insulin deficiency in skeletal muscle of STZ-diabetic mice. We hypothesize that the lack of insulin leads to a decrease primarily of PPARα followed by a decrease of LpL expression in the skeletal muscle. Surprisingly, we noted reduced CD36 expression in skeletal muscle of diabetic mice. While one might expect that myocytes would shift to greater fatty acid oxidation with insulin deficiency, we suspect that muscle metabolism in our animals was heavily dependent on glucose, but with glucose uptake primarily via non-insulin- and non-GLUT4-dependent pathways.

To study if changes in LpL expression would actually modify diabetes-induced hypertriglyceridemia we studied mice with a heterozygous deletion of the LpL gene (as homozygous LpL knockout mice are not viable). $Lpl^{+/-}$ mice exhibited marked hypertriglyceridemia after induction of STZ-diabetes. More importantly, TG secretion was again not significantly different in STZ-diabetic $Lpl^{+/-}$ mice but TG clearance was delayed after gavage with olive oil. Similar to our diabetic $Lpl^{+/-}$ mice, human diabetic carriers of dysfunctional LpL alleles are at risk for severe lipemia[60]. We hypothesized that increasing LpL expression would ameliorate diabetic hypertriglyceridemia in our animal model. Constitutive skeletal muscle-specific expression of human LpL eliminated hypertriglyceridemia in the STZ-diabetic $Lpl^{+/-}$ mice and TG clearance after olive oil gavage was reduced to almost normal non-diabetic levels. We presume that the constitutive expression of LpL increased lipolysis and VLDL remnant removal. Similarly, Shimada *et al.*[61] reported that diabetes-mediated increases in TG were eliminated by overexpression of LpL in STZ-diabetic C57BL/6 mice. Thus, a major regulator of circulating TG in diabetes is LpL action regardless of changes in liver TG production.

In conclusion, hypertriglyceridemia in insulin deficient mice is primarily caused by a defect in peripheral lipolysis and not by an increase in hepatic TG secretion as suggested by mouse models of impaired hepatic insulin signalling. signalling. Most humans with diabetes do not have marked hypertriglyceridemia. However humans, like mice, that have a defect in LpL regulation are most likely to develop severe hypertriglyceridemia with diabetes[60, 62,63.] On the other hand, humans with robust LpL activity are likely to be protected from hypertriglyceridemia regardless of their degree of insulin deficiency or resistance. Therefore, methods to augment peripheral TG lipolysis are likely to be most efficacious in the treatment of diabetes-induced hypertriglyceridemia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Nonstandard Abbreviations

ASO	antisense oligonucleotide
BAT	brown adipose tissue
CHREBP	carbohydrate response element binding protein
CVD	cardiovascular disease
FFA	free fatty acid
LpL	lipoprotein lipase
PPARα/γ/δ	Peroxisome proliferator-activated receptor α/γ/δ
SGLT2	sodium glucose co-transporter 2
SREBP-1c	sterol response element binding protein-1c
STZ	streptozotocin
T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus
TG	Triglycerides
WAT	white adipose tissue

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Significance

In this report, we show that insulin deficiency in mice leads to increased plasma TG levels and defective removal of postprandial TG. Our data are most compatible with that in humans and suggests that significant hypertriglyceridemia in insulin deficient diabetes is primarily due to changes in lipolysis and not changes in hepatic insulin signalling.

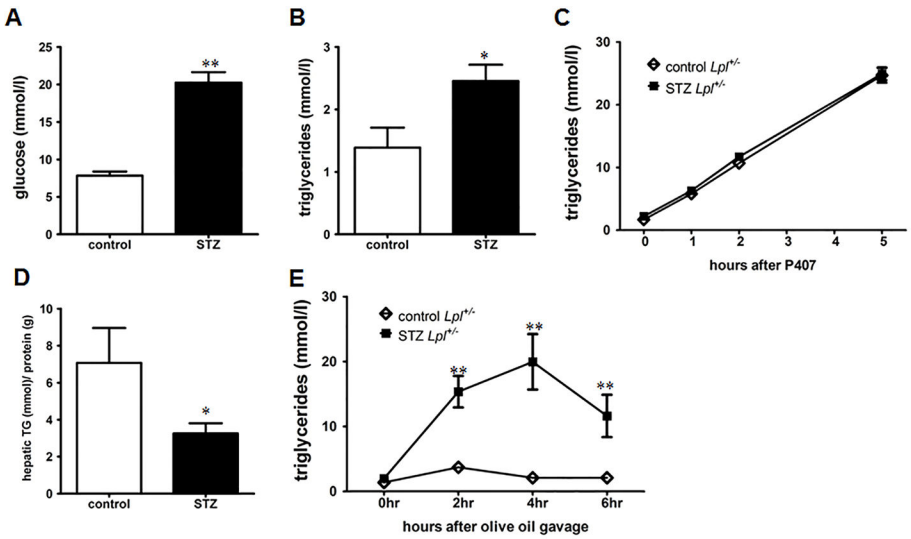

Figure 1. Insulin deficiency does not change hepatic *de novo* lipogenesis and hepatic TG secretion

(A) TG secretion was measured in STZ-diabetic and non-diabetic C57BL/6 mice after i.p. injection of Poloxamer 407 (P407) (n = 5/group). **(B)** Hepatic gene expression of *Fasn, Dgat2, Scd1, Acc1, Elovl6, Cpt1α and Acc2* was assessed using quantitative real-time PCR. Gene expression is expressed relative to fed non-diabetic control mice (n = 5/group). **(C)** Hepatic TG, FFA and cholesterol content (n = 5/group). *: $p \leq 0.05$, **: $p \leq 0.01$. Results are presented as mean ± SEM.

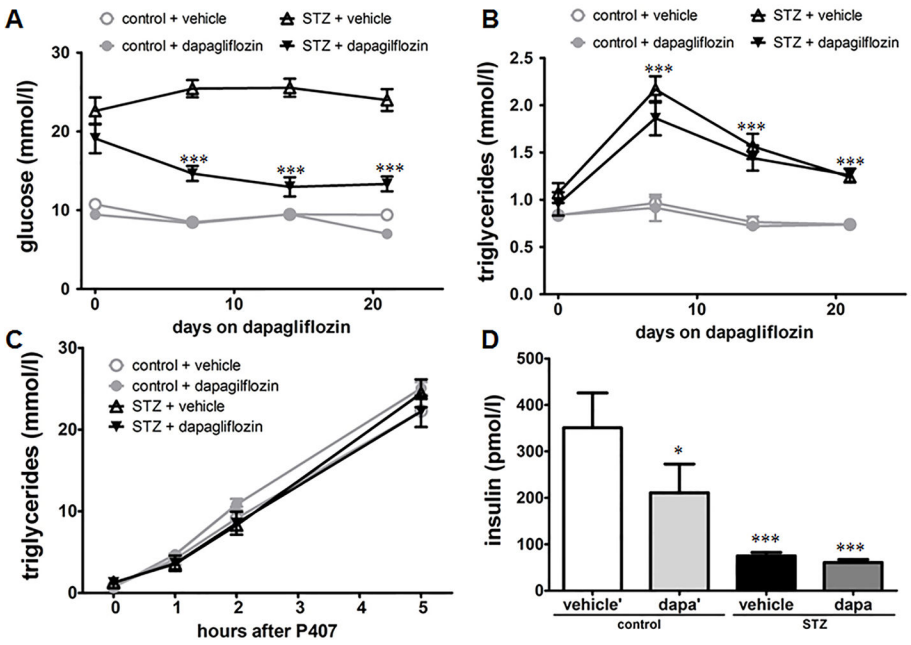

Figure 2. Hyperglycemia does not drive hypertriglyceridemia

(A) STZ-diabetic and control C57BL/6 mice were treated with the SGLT2 inhibitor dapagliflozin or vehicle and plasma glucose was measured at indicated time points (n = 10/group). ***: $p \leq 0.001$ vs. STZ + vehicle. **(B)** 4 hour fasting plasma TG levels over time (n = 10/group). ***: $p \leq 0.001$ vs. non-diabetic controls. **(C)** Hepatic TG secretion was measured by i.p. injection of Poloxamer 407 (P407) (n = 5/group). **(D)** Plasma insulin levels after 4 hour fasting (n = 10/group). *: $p \leq 0.05$, ***: $p \leq 0.001$ vs. control + vehicle. Results are presented as mean ± SEM.

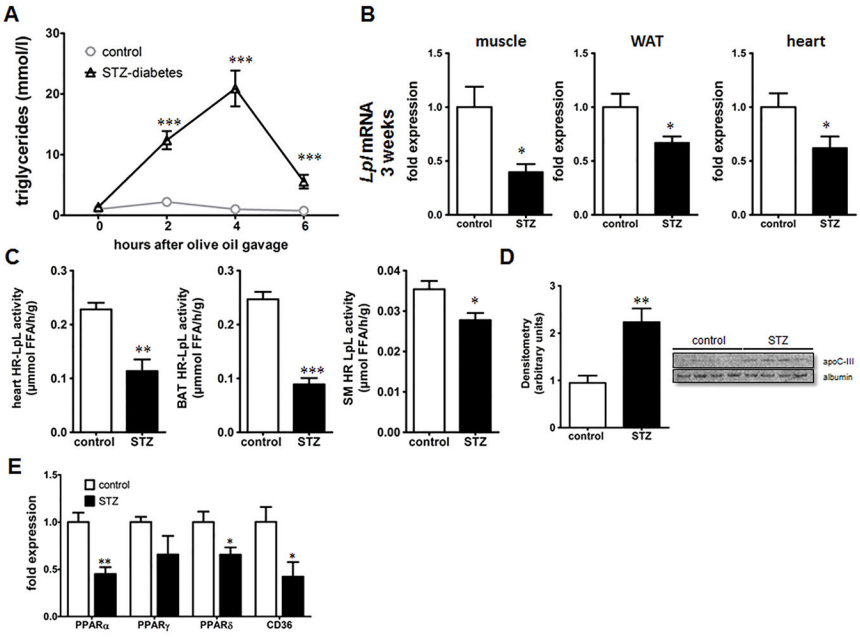

Figure 3. Increased postprandial lipemia with insulin deficiency

(A) TG turnover in mice after gavage of 10 ml/kg BW olive oil. Plasma TG levels were measured after 2,4 & 6 hours. **(B)** *Lpl* mRNA analysis by quantitative PCR in skeletal muscle, WAT and heart at 3 weeks. **(C)** Heparin-releasable (HR) LpL activity from freshly isolated heart, BAT and skeletal muscle (SM) using a radiolabeled triglyceride substrate. **(D)** Immunoblot of plasma for ApoC-III **(E)** Gene expression of *PPARα*, *PPARδ*, *PPARγ* and *CD36* in the skeletal muscle after three weeks of STZ-diabetes using quantitative real-time PCR (n = 5/group). *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$ Results are presented as mean ± SEM.

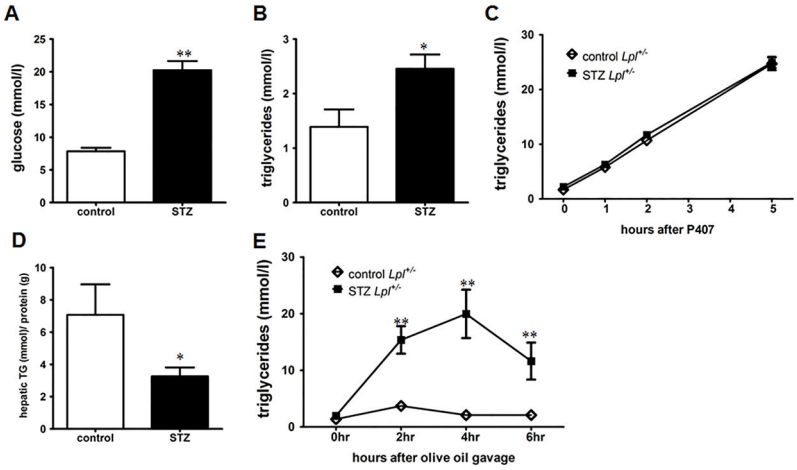

Figure 4. STZ-diabetic heterozygous *Lpl*$^{+/-}$ mice display increased plasma TG and postprandial hypertriglyceridemia

(A) 4 hour fasting glucose and **(B)** TG levels in STZ-diabetic and non-diabetic *Lpl*$^{+/-}$ mice at 4 weeks (n = 6–10/group). **(C)** Hepatic TG secretion in *Lpl*$^{+/-}$ mice was measured by i.p. injection of Poloxamer 407 (P407) (n = 6–10/group). **(D)** Hepatic TG content in STZ-diabetic and non-diabetic *Lpl*$^{+/-}$ mice (n = 5–7/group). **(E)** TG turnover was quantified in mice after gavage of 10 ml/kg BW olive oil. Plasma TG levels were measured after 2,4 & 6 hours (n = 5–7/group). *: $p \leq 0.05$, **: $p \leq 0.01$ and vs. non-diabetic *Lpl*$^{+/-}$ mice. Results are presented as mean ± SEM.

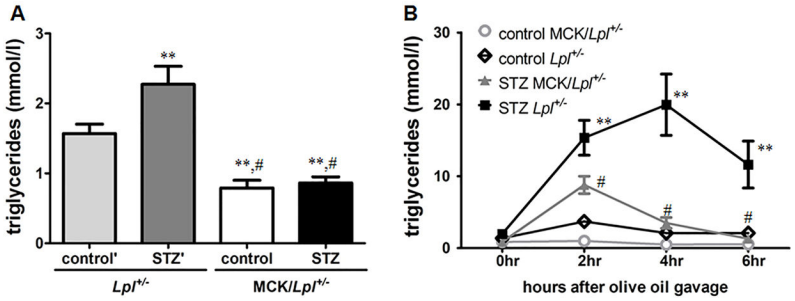

Figure 5. LpL overexpression in the skeletal muscle corrects diabetic dyslipidemia

(A) 4 hour fasting plasma TG levels in STZ-diabetic and non-diabetic $Lpl^{+/-}$ and MCK/$Lpl^{+/-}$ mice (n ≥ 10/group). : p ≤ 0.01 vs. non-diabetic $Lpl^{+/-}$ control mice, #: p ≤ 0.001 vs. STZ-diabetic $Lpl^{+/-}$ mice. **(B)** TG turnover was quantified in mice after gavage of 10 ml/kg BW olive oil. Plasma TG levels were measured after 2,4 & 6 hours (n = 5–7/group). **: p ≤ 0.01 vs. non-diabetic $Lpl^{+/-}$ control mice, #: p ≤ 0.01 vs. STZ-diabetic $Lpl^{+/-}$ mice. Results are presented as mean ± SEM.

Table 1A

Metabolic parameters in control and STZ-administered mice

Parameter	2nd week		6th week	
	non-diabetic control	STZ	non-diabetic control	STZ
Glucose (mmol/l)	6.66 ± 0.5	25.25 ± 0.72*	9.44 ± 0.33	27.58 ± 1.28 *
Triglycerides (mmol/l)	0.82 ± 0.03	1.42 ± 0.09*	0.91 ± 0.06	1.99 ± 0.18†
Cholesterol (mmol/l)	2.56 ± 0.13	2.79 ± 0.1	2.61 ± 0.1	2.69 ± 0.16
FFA (mmol/l)	0.88 ± 0.08	1.31 ± 0.17‡	1.58 ± 0.13	3.1 ± 0.34‡
Body weight (g)	27.5 ± 0.5	23.5 ± 0.5*	27.2 ± 0.5	22.2 ± 0.8*
N	24	22	14	12

* $p < 0.0001$,

† $p < 0.001$,

‡ $p<0.01$ vs. non-diabetic control, Student's t-test

Table 1B

Cholesterol and triglycerides subfractions in control and STZ-administered mice

Parameter	triglycerides		cholesterol	
	non-diabetic control	STZ	non-diabetic control	STZ
VLDL (mmol/l)	0.71 ± 0.02	1.33 ± 0.09*	0.36 ± 0.03	0.65 ± 0.05‡
LDL (mmol/l)	0.26 ± 0.02	0.27 ± 0.03	0.6 ± 0.08	0.62 ± 0.08
HDL (mmol/l)	0.20 ± 0.02	0.20 ± 0.03	1.50 ± 0.08	1.32 ± 0.1

*$p < 0.0001$,

‡$p<0.01$ vs. non-diabetic control, Student's t-test