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Title: Metabolic surgery in zucker rats influenced miRNA, caveolin-1 expression and lipid metabolism

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Metabolic surgery in Zucker rats influenced miRNA, caveolin-1 expression and lipid metabolism

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Abstract: Aims: A transposition of the long segment of distal ileum in obese Zucker rats improved glucose tolerance 6 months after IT. It was undertaken to compare the gene expression of miRNA-103, -107 and caveolin-1 in the liver of euglycemic groups of IT relative to SHAM operated rats.

Main methods: Obese, male Zucker rats underwent either transposition of 50% distal ileum or sham surgery. For determining the gene expression, the Real-Time PCR for caveolin-1 and miRNA-103, -107 was performed. Plasma concentrations of LDL, HDL, TG and total cholesterol were measured with enzymatic colorimetric assays after optimization procedure.

Key findings: The Cav-1 expression in liver tissue after ileal transposition was 1.22 times higher compared to the SHAM group (SHAM median 63.58, min 41.3, max 82.4; IT median 77.35, min 60.8, max 95.41, $p < 0.001$). miRNA-107 expression was significantly downregulated by 0.6-fold in the IT group compared to the SHAM group (SHAM median 507.51, min 236.42, max 721.29; IT median 355.2, min 278.15,

max 478.15, $p < 0.015$). The level of TG was significantly higher after IT surgery (SHAM median 115, min 96, max 143; IT median 153, min 115, max 162, $p = 0.001$). The total cholesterol plasma levels decreased after IT (SHAM median 178, min 161, max 183; IT median 128, min 103, max 114, $p < 0.000001$). The LDL plasma level in IT was two-fold lower than in the SHAM (SHAM median 117, min 68, max 151; IT median 58, min 45, max 61, $p < 0.000001$).

Significance: The transposition of 50% of the distal ileum lead to an increase in caveolin-1 and reduction in miR-107 expression compared to those of SHAM group. Endogenous miR-107 is more involved in regulation of the functions of insulin-target liver tissue than miRNA-103. Reduced LDL and cholesterol plasma levels suggest positive effects on lipid metabolism in long-term observations. The present study is the first to show a lack of IT effect regarding triglycerides six months after surgery.

Keywords: miRNA-103, miRNA-107, caveolin-1, ileal transposition, type 2 diabetes mellitus, obesity surgery, metabolic surgery, diabetes surgery, Zucker rats.

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1 Introduction

Increasing evidence of obesity and its comorbidities, such as type 2 diabetes mellitus (T2DM), have tremendous impact on the state of health of modern societies. One of the applied solutions in obesity treatment is the bariatric procedure. Bariatric surgery has been shown to stimulate weight loss and improve glucose homeostasis, thus it became a popular and effective means to treat obesity and type 2 diabetes mellitus. Ileal transposition (IT) was initially performed as weight loss surgery in Sprague-Dawley rats after having observed notably reduced food intake after a jejunoileal bypass [1]. Fast delivery of food to the terminal ileum is thought to be pathophysiologically responsible for type 2 diabetes remission after obesity surgery [2]. In our previous study, we demonstrated that a transposition of 50% of distal ileum in obese Zucker rats improved

glucose tolerance at 1, 3 and 6 months post operatively [2]. This phenomenon suggests a direct effect of the surgical intervention on changes in the regulation of glucose metabolism [4-5]. In animal models, IT leads to a decrease in plasma levels of total cholesterol, free fatty acids and triglycerides [6]. Still, more research is strongly needed in the area of bariatric surgery, with an emphasis on efforts to advance pathophysiological understanding [7].

The caveolae, small invaginations of the plasma membrane, are an important element for lipid uptake and transport, protein trafficking, intracellular signaling and glucose homeostasis [8]. Caveolin (CAV) proteins bind cholesterol and have the ability to move between cellular compartments to help control intracellular cholesterol fluxes [9]. Caveolin-1 is the primary structural protein in caveolae and appears to have principal functions in lipid transport, membrane traffic, and cell signaling [10]. It was also identified as a direct target gene of miR-103/107 and critical regulator of insulin receptors [11]. Contemporary biology has undergone a small revolution with the discovery of microRNAs (miRNAs), a class of 22 nucleotide regulatory RNAs. miRNAs are endogenously expressed, non-coding RNAs that inhibit the translation of target mRNAs by binding to cognate sites of imperfect complementarities found in 3' untranslated regions [12]. To date, thousands of miRNA genes have been identified by a combination of cloning, direct sequencing and bioinformatic techniques. It has been proposed that these molecules influence insulin secretion [13], beta-cell differentiation [14], pancreatic islet development [15], and indirectly control glucose and lipid metabolism [16]. miR103/107 are highly expressed in diabetes. Silencing of miR-103/107 leads to the improvement of glucose homeostasis and insulin sensitivity.

In contrast, increased miR103/107 function in liver or adipose tissue is sufficient to induce impaired glucose homeostasis and insulin resistance. miR-103/107 seems to regulate multiple mRNA targets in biochemical pathways of lipid levels. In adipose tissue, miRNA 103 was upregulated during adipogenesis and tended to be downregulated in the obese state [17]. Elevated expression of miR103/107 is also proven to lead to insulin resistance by down regulating of Cav-1 [11].

This paper is a continuation of a research project examining the long term effects of ileal transposition on physiologic background and long-term adaption in Crl:ZUC-Lepr^{fa} Zucker rats. In our previous studies, we demonstrated that long segment ileal transposition, 50% of distal ileum, improves glucose tolerance and GLP-1 stimulation six months after IT surgery in comparison to 25% distal and 50% proximal [2,18]. The principal

goal of this study was to investigate the impact of ileal transposition on separate, selected parameters of glucose and lipid administrations, such as: LDL, HDL, TG and total cholesterol. Relations between miRNA-103/miRNA-107 and caveolin-1 expression during in vivo experiments on the Crl:ZUC-Lepr^{fa} rat's liver six months after IT surgery was also investigated. In pursuit of this goal, the following questions were addressed and experimental approaches adopted: What is the level of endogenous miRNA-103 and miRNA-107 gene expression in liver of IT relative to SHAM animals six months after IT surgery? Do levels of plasma LDL, HDL, TG and total cholesterol persist six months after ileal transposition?

2 Materials and Methods

This work is part of a wider experiment which focused on ileal transposition treatment of obesity and insulin resistance in the animal model of Crl:ZUC-Lepr^{fa} Zucker rats [2,18]. All previously obtained data, surgical and experimental procedures were presented by Grüneberger 2013, 2014 [2,18]. In this paper we briefly present only the crucial points [2,18]. The animal experimental protocols were approved by the Ethics Committee of the University of Freiburg [2,18].

2.1 Surgery

Based on our own data, we expected that six months after surgery, investigation of the essential parameters of energy homeostasis would provide important information about the long term metabolic adaptations post IT surgery. After 7–8 days of acclimatization period, the rats underwent ileal transposition surgery involving 50% of the distal ileum [2]. The animals were fasted for 12 hours before surgery. Anesthesia was induced and maintained using Isoflurane 2% (Abbott GmbH & Co. KG, Wiesbaden, Germany) and oxygen flow at 2 L/min under spontaneous breathing. After a midline incision of 4–5 cm to gain abdominal access, Bauhin's valve was identified. We had determined the total small bowel length of Zucker rats to be approximately 85 cm. Transections of 50% distal segment (25 cm) were conducted in relation to Bauhin's valve.

2.2 Ileal Transposition

Ten male Zucker rats underwent IT surgery. For IT, the ligament of Treitz was identified and the jejunum divided 5 cm aborally. The ileal loop was then interpositioned

in an isoperistaltic fashion forming two end-to-end anastomoses. Mesenterial openings were closed with Prolene 6/0 (Ethicon). Fascia and skin closure were performed as a continuous suture using Monocryl 4/0 and Vicryl 4/0. Postoperative analgesia was ensured via subcutaneous Carprofen (Rimadyl, Pfizer, Switzerland) injection (4 mg/kg) at the beginning of the operation. Animals were fasted on day 1 after the operation with free access to tap water, and oral food was continuously increased, leading up to free access by day 6 [18].

2.3 SHAM

Ten male Zucker rats were selected for SHAM surgery. For SHAM surgery, a division of small intestine with subsequent anastomoses was performed at the 3 corresponding positions without IT. Pre and postoperative treatment was identical for the IT group [18].

2.4 Blood and tissue collection

Six months after surgery, anesthesia was induced and maintained using isoflurane 2% and oxygen flow at 2 L/min under spontaneous breathing. 26-gauge cannula was placed in the tail vein for blood collection. We drew 400 μ L of whole blood *via* the cannula at 0 and 20 minutes after oral glucose (1.5 mg/kg) was given via tubes containing 10 mL EDTA (Sigma-Aldrich, St. Louis, Mo). After centrifugation at 4000 rpm for 10 minutes at 4°C, plasma samples were collected and snap frozen in liquid nitrogen and stored at -80°C until analysis. After blood sampling, tissues were harvested and the animals were euthanized. Liver tissue was explanted and snap frozen in liquid nitrogen and stored at -80°C until further analysis.

2.5 Real time PCR

For the gene expression and mRNA quantification, Real-Time PCR was performed for rat caveolin-1 gene and miRNA-103, -107. The method routinely used for transcript level estimations is relative quantification to a reference gene, described by Livak [19]. Expression of the cav-1 gene was calculated to the reference GAPDH gene in both groups of animals, and results were presented as an expression ratio. Similar calculations were performed for miRNA-103 and -107 using U6 snRNA as an internal reference for miRNAs expression. The sets of primers and TaqMan probes for caveolin-1 (cat. No. Mm00483057_m1), GAPDH (cat. No. Mm99999915_g1), miRNA-103 (cat. No.

463628_mat), miRNA107 (cat. No. 465082_mat) and U6 snRNA (cat. No. 4427975) for rats were custom designed and ordered at Applied Biosystems, USA.

Transcript levels for caveolin-1 were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and miRNA levels were measured using TaqMan microRNA Assays (Applied Biosystems; cat. No. 4440886) and were normalized to U6 snRNA levels; for real-time PCR TaqMan 2x Universal PCR Master Mix was used.

The total set of RNA and miRNAs was isolated from manually homogenized rat liver, with RNase free and low temperature appropriate conditions. Before freezing, all extracted tissues were stored in RNeasy lysis buffer (Qiagen; cat. No. AM7020) to avoid nucleic acid degradation, and all other reagents and sets were obtained from Applied Biosystems. For both mRNA and miRNAs isolation, mirVana™miRNA Isolation Kit and protocol were used (cat. No. AM1560), making it possible to obtain miRNAs or mRNA from the same samples at different steps of isolation. In the next step, reverse transcription was performed using a High Capacity Reverse Transcription Kit (for caveolin mRNA; cat. No. 4368814) and TaqMan®MicroRNA Reverse Transcription Kit (for miRNA-103, -107; cat. No. 4366596), where 100ng of cDNA was obtained for each studied gene or miRNAs tagged probes. The Real-Time PCR with a Chromo4 thermocycler (Biorad) was routinely assigned, using TaqMan®Universal PCR Master Mix, No AmpErase®UNG (cat. No. 4324018), and conventional reagents in a standard thermal profile reaction for TaqMan probes. The thermal reactions profile was as follows: initiation in 50°C for 2 min; enzyme activation in 95°C for 10 min; 40 cycles with denaturation in 95°C for 15 s and anneal/extend in 60°C for 60 s; hold in 4°C permanently whilst in storage. The miRNAs quantification was done using specific primers for reverse transcription reactions, and then TaqMan MicroRNA assays where primer and probe sets were designed to detect and quantify mature miRNAs using Applied Biosystems Real-time PCR protocol. The assays can detect and quantify small RNA in 1 to 10 ng of total RNA with a dynamic range of greater than six logs. When used for microRNA analysis, the assays can discriminate mature miRNA sequences from their precursors. These assays are ideal for targeted quantification, screening, and validation of miRNA profiling results.

2.6 Serum cholesterol assessment

Plasma total cholesterol, LDL, HDL and TG were measured with the use of enzymatic colorimetric assays (PZ CORMAY S.A., Poland).

2.7 Real-time data analysis

For Real-time PCR data analysis and sample comparison, we used the comparative Ct ($\Delta\Delta C_t$) method for calculating relative quantification of gene and miRNA expression [19]. Normalization to the simple gene (GAPDH) and miRNA (U6 snRNA) was done, without a common calibrator for each sample usage. The method is useful for endogenous level of gene expression estimation, using relative expression compared to the internal reference gene in each sample. Levels of *cav-1* expression and its relationship to the regulatory miRNAs for each animal from IT and SHAM groups were analyzed. Results were analyzed using $2(-\Delta\Delta C_t)$ method [19].

2.8 Statistics

Statistical analysis was conducted using Statistica package, (StatSoft, Inc. (2010). STATISTICA (data analysis software system), version 10.0. www.statsoft.com). For group comparison, Mann-Whitney U *post hoc* test was used and $p < 0.05$ was considered significant. Spearman's rank correlation coefficient was calculated for $p < 0.05$. For estimation of the relationships among the analyzed variables, F-test was applied, $p < 0.005$.

3 Results

The aim of our project was to determine the potential long term effect of IT on glucose and lipid homeostasis in obese Zucker rats. The group of rats which underwent 50% distal ileum transposition and showed improved glucose tolerance six months after surgery were chosen for further investigation. The general characteristics concerning body mass, oral glucose tolerance test (OGTT), incretin hormones and insulin serum levels for both IT operated rats and the SHAM group were presented in our previous publication [18].

3.1 Liver miRNA-103/107 expression

In this experiment we sought to compare endogenous levels of selected gene expression between the SHAM and IT groups. We observed that caveolin-1 and miRNA-103 were differentially expressed between the IT and SHAM groups. The caveolin-1 expression in liver tissue after ileal transposition was 1.22 times higher than that of the SHAM group (SHAM median 63.58, min 41.3, max

82.4; IT median 77.35, min 60.8, max 95.41, $p < 0.001$, Fig.1). The miRNA-103 expression was estimated to be 1.95 times higher in animals that underwent IT surgery relative to the SHAM group (SHAM median 21.92, min 17.03, max 73.14; IT median 42.70, min 28.98, max 71.18, $p < 0.025$; Fig.2). Conversely, expressions of miRNA-107 were downregulated significantly by 0.6-fold in the IT group relative to the SHAM group. What this might suggest is that miRNA is more involved in the regulation of insulin-target liver tissue function for the IT group (SHAM median 507.51, min 236.42, max 721.29; IT median 355.2, min 278.15, max 478.15, $p < 0.015$; Fig.2). Observed upregulation of caveolin-1 gene expression by miR-107 was 1.7-fold for the IT group (SHAM median 0.19, min 0.07, max 0.2; IT median 0.19, min 0.12, max 0.29, $p = 0.0009$, Fig.3), whereas miR-103 expression was significantly downregulated in comparison with the SHAM group (SHAM median 2.66, min 0.49, max 4.5; IT median 1.56, min 1.0, max 3.05, $p = 0.001$; Fig.3). The ratio of miR-107/miR-103 fold-change was significantly lower in the IT group (median 7.75, min 4.01, max 13.67) relative to the SHAM group (median 15.39, min 5.01, max 42.35, $p < 0.001$; Fig.1). Six months after IT surgery, the relative expression between miR107 and miR103 was two times lower than in the SHAM group, which might relate to the change of insulin resistance after IT. Spearman correlations showed significant relationships between Cav-1/miR-103 and glucose in the SHAM group. This tendency is not present in the IT group. Interestingly, the relationship between miRNAs fold-change and Cav-1 were common before and after IT surgery (Table 1). The results of HOMA-IR and glucose were previously presented by Gruneberger [18]. IT surgery changed the relationship between HOMA-IR and Cav-1, miR-103 and glucose, respectively (Table 1).

3.2 Plasma lipids concentrations

Obesity is associated with an increase in total cholesterol, triglycerides, fasting glucose and insulin levels, increased HOMA-IR, and reduced insulin sensitivity index. Some of those parameters were previously presented [18]. Regarding metabolic variables, the plasma lipids concentration patterns in the obese Zucker rats six months after ileal transposition were diverse. Surprisingly, the level of triglycerides (TG) was significantly higher after IT surgery relative to the SHAM group (SHAM median 115, min 96, max 143; IT median 153, min 115, max 162, $p = 0.001$), and total cholesterol plasma level decreased after IT surgery in the long term (SHAM median 178, min 161, max 183; IT median 128, min 103, max 114, $p < 0.000001$,

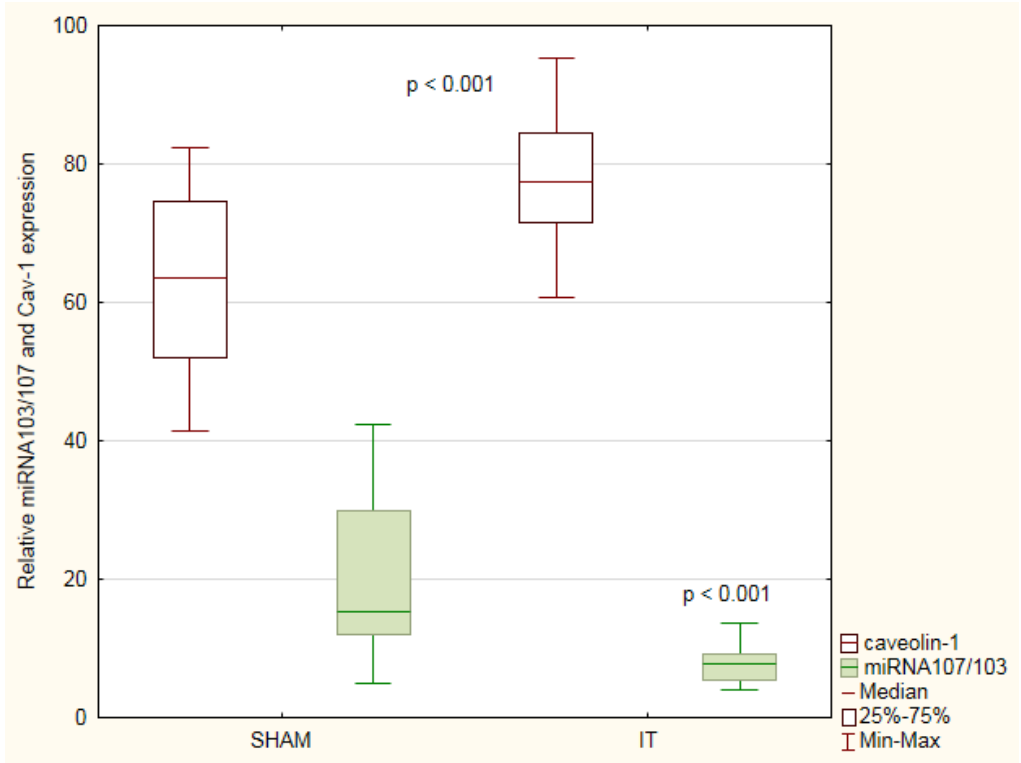


Figure 1. Comparison of caveolin-1-expression levels and the ratio of miR-103/miR-107 fold-change in obese Zucker rat liver of SHAM and IT groups. Mann-Whitney U test, $p < 0.05$.

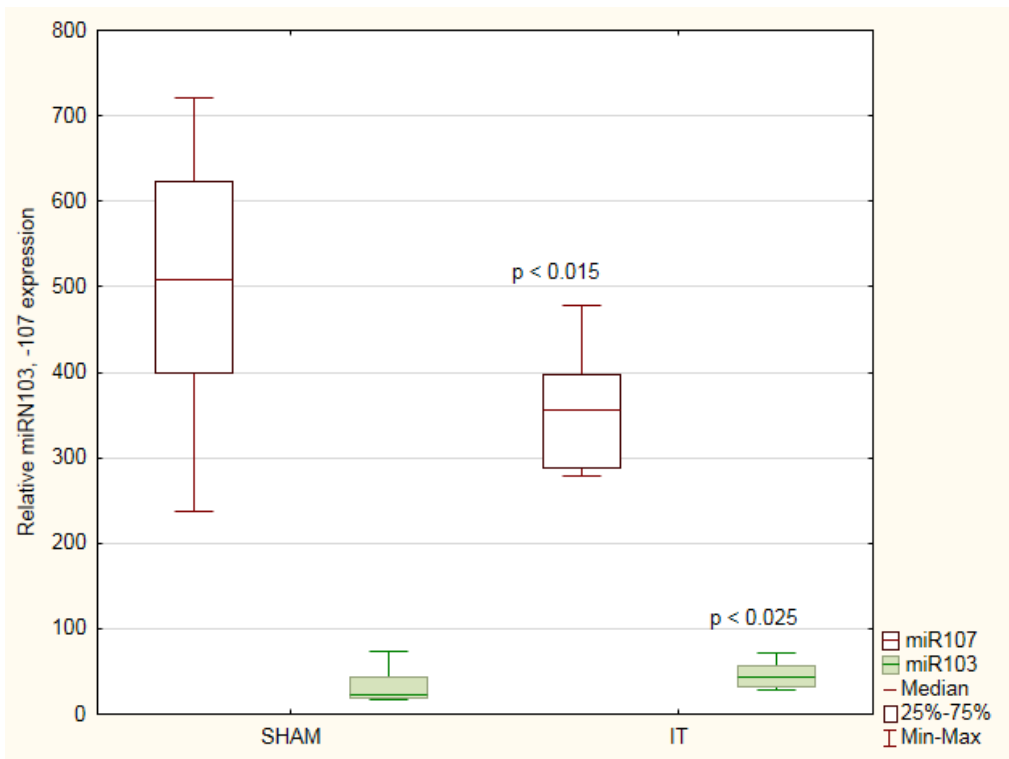


Figure 2. Comparison of miR-103, miR-107 expression levels in obese Zucker rat liver for SHAM and IT groups. Mann-Whitney U test, $p < 0.05$.

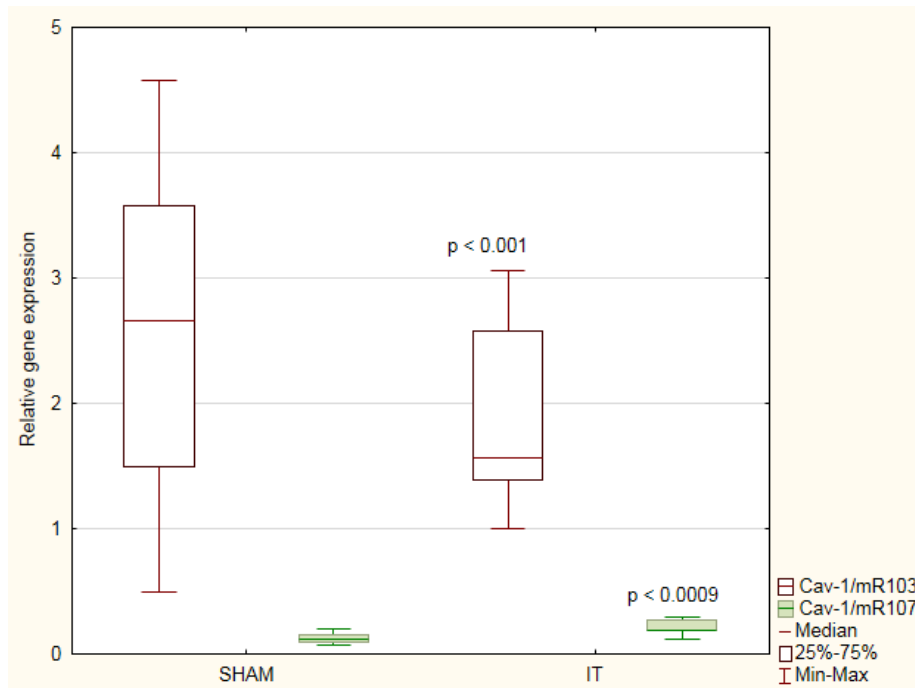


Figure 3. Comparison of caveolin-1 to miR-103 and miR-107 fold-change expression levels in SHAM and IT groups. Mann-Whitney U, $p < 0.05$.

Table 1. Significant correlations between analyzed parameters in obese Zucker rat liver of SHAM and IT groups, Spearman correlations, $p < 0.05$.

SHAM group	$r(X,Y)$	r^2	p
Cav-1 vs. miR-103	-0.531	0.282	0.005
Cav-1 vs. Glucose	-0.547	0.299	0.003
miR-107 vs. Cav-1/miR-107	-0.822	0.676	0.0000002
miR-107 vs. miR-107/miR103	0.658	0.433	0.0002
miR-103 vs. TG	-0.471	0.222	0.01
miR-103 vs. Cav-1/miR-103	-0.828	0.686	0.0000001
miR-107 vs. Cav-1/miR-107	-0.822	0.676	0.0000002
miR-103/miR-107 vs. Cav-1	0.420	0.176	0.032
Glucose vs. HOMA-IR	-0.406	0.164	0.039
Glucose vs. TG	0.441	0.194	0.024
TG vs. CHOL	0.5811	0.337	0.0018
LDL vs. HDL	-0.793	0.629	0.000001
IT group	$r(X,Y)$	r^2	p
miR-103/miR-107 vs. miR-107	0.687	0.472	0.00007
miR-107.miR-103 vs. miR-103	-0.494	0.244	0.008
miR-103/miR-107 vs. Cav-1/miR-103	0.480	0.230	0.011
miR-107/miR-103 vs. Cav-1/miR-107	-0.459	0.211	0.015
HOMA-IR vs. Cav-1	-0.457	0.209	0.016
HOMA-IR vs. miR-103	-0.462	0.214	0.015
HOMA-IR vs. Glucose	0.554	0.307	0.002
TG vs. LDL	-0.454	0.206	0.017
CHOL vs. Glucose	0.397	0.157	0.040

Fig.4). Low density lipoprotein (LDL) plasma level in the IT group was two-fold lower than in the SHAM group (SHAM median 117, min 68, max 151; IT median 58, min 45, max 61, $p < 0.000001$, Fig.5). HDL levels did not differ between the analyzed groups. Among analyzed plasma lipids in the SHAM animals, LDL showed significant correlation with HDL (Table 1.).

3.3 F-test results

The results of F-test analysis showed that there were significant effects of both group affiliation and miRNA-103 on caveolin-1 expression ($\beta = -0.00353$; +95% CI = -0.00515 ; -95% CI = -0.0019 ; $p = 0.00002$). TG levels were significantly related to SHAM group ($\beta = -0.104$; +95% CI = -0.1597 ; -95% CI = -0.0483 ; $p = 0.000253$).

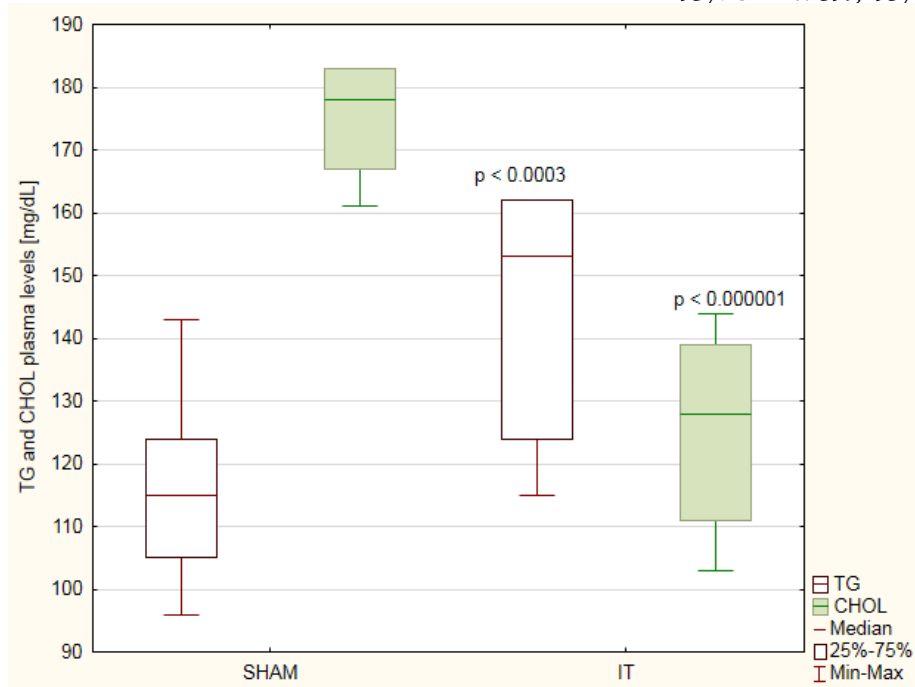


Figure 4. Comparison of triglycerides (TG) and cholesterol (Chol) plasma levels [mg/dL] in SHAM and IT groups. Mann-Whitney U test, $p < 0.05$.

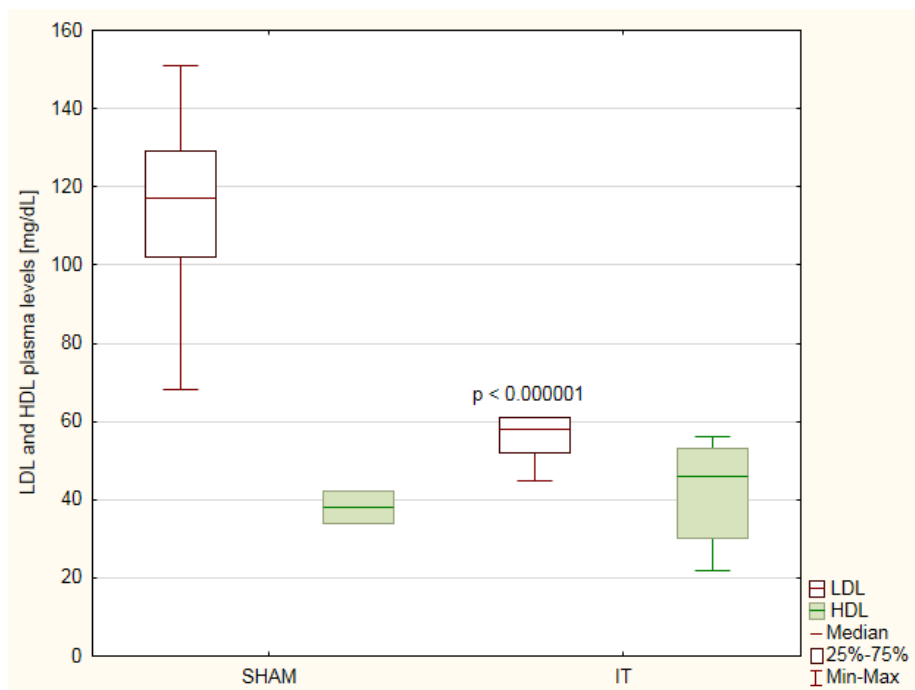


Figure 5. Comparison of LDL and HDL plasma levels [mg/dL] in SHAM and IT groups. Mann-Whitney U, $p < 0.05$.

4 Discussion

Ileal transposition (IT) involves the transfer of an ileal segment behind the ligament of Treitz, which triggers incretin stimulation from endocrine K and L cells and restores insulin sensitivity [20]. This type of surgery has been shown to improve glucose metabolism and beta-cell functions in rodent and human studies, leading to diabetes remission [21]. In our previous study, animals that underwent IT surgery showed improved glucose control 6 months after long segment transposition relative to SHAM animals [2]. In this paper, we have presented the results obtained from the same group of animals 6 months after undergoing 50% distal ileal transposition surgery.

miRNAs-103, -107 act primarily on glucose metabolism, generally favoring hepatic glucose liberation by inhibiting hepatic glucose storage [22]. Meningen and co-workers revealed acute postprandial expression changes of microRNAs 103/107 in the liver of rainbow trout that were related to changes in components of the hepatic insulin signaling pathway and mRNA expression of metabolic genes [22]. Other studies have shown that miRNA-103 and miRNA-107 were upregulated in the liver of diet-induced obese (DIO) C57BL/6J, ob/ob mice and in diabetic Goto-Kakizaki rats [23]. Differential expression of miR-103 and miR-107 were shown during adipogenesis in the rat model of type 2 diabetes and glucose regulated miRNAs from pancreatic β cells [23]. Silencing of miR-103/107 significantly improved insulin signaling due to higher expression of insulin receptor b-subunit (IRb) in adipose tissue of obese mice. Insulin-stimulated levels of phosphorylated Akt1 and IRb (pAkt1 and pIRb) were augmented in the fat and liver of ant-103-treated mice. Nevertheless, the direct regulation of insulin sensitivity by microRNAs *in vivo* has not been yet demonstrated [8, 11]. Caveolin-1, miR-103 upregulations and simultaneous downregulations of miRNA-107 expression in liver tissues of rats were observed in the studied group 6 months after IT surgery compared to the SHAM group. This result is in agreement with other findings showing that overexpression of miR-107 in the liver of mice can induce hepatic insulin resistance [11]. The long term effect of IT on the endogenous level of caveolin-1 showed increased expression when compared to SHAM animals. In the SHAM group, Cav-1 was negatively correlated with miR-103, glucose level and miR-107 ratio. Six months after IT surgery, Cav-1 was again negatively correlated with HOMA-IR and miR-103. Over-expression or antagomir mediated silencing of miR-103/107 in diet-induced obese mice lacking caveolin-1 demonstrated a central role of caveolin-1 in mediating the miR-103/107 effects on

glucose tolerance and insulin sensitivity. Upregulation of caveolin-1 by miR-103/107 inactivation in adipose tissue is connected with stabilization of the insulin receptor, enhanced insulin signaling, decreased adipocyte size and enhanced insulin-stimulated glucose uptake. This finding demonstrates the central importance of miR-103/107 to insulin sensitivity and identifies a new target for the treatment of type 2 diabetes and obesity [11].

24 weeks after IT surgery, plasma triglyceride levels were increased significantly relative to the SHAM group, but LDL and total cholesterol levels were significantly diminished. TG plasma levels were related to LDL in the IT group, whereas TG plasma levels were related to total plasma cholesterol in the non-surgical group. Lipid and carbohydrate administrations were positively correlated in both SHAM and surgical animals. Interestingly, miR-103 in the SHAM group was functionally related to the IT group. Cummings (2010) observed reduced levels of triglycerides and cholesterol in UCD-T2DM rats 2 months after surgery [24]. Also, De Paula (2009), in the prospective randomized controlled trial on diabetes patients 2 years after IT surgery, showed reduced cholesterol and triglyceride levels as one of the metabolic outcomes of ileal transposition [25]. It has been demonstrated that hepatic insulin resistance is the effect of TG liver deposition, and influences increased circulating TG, resulting in greater TG deposition in peripheral tissues [26]. We suggest that increased levels of plasma circulating TG was the effect of greater TG deposition in peripheral tissues and/or blood vessels as the mechanism to compensate and to normalize glucose homeostasis. Caveolin-1 controls both production and degradation of plasma TG [27]. Our findings do not correspond with data showing increased plasma TG levels observed in Cav-1 $^{-/-}$ mice, due to reduced degradation of plasma TG. Caveolin-1 directly regulates hepatic lipid metabolism, and caveolin concentration is associated with fatty acid uptake by adipose tissue and regulates cellular cholesterol homeostasis. Caveolae formation and caveolin-1 expression are strongly related to the availability of cholesterol [27, 28]. It was also reported that a high cholesterol diet upregulates caveolin-1 content in caveolae and influences insulin signaling in the liver [29]. In the current study, lowered levels of cholesterol and LDL fractions may suggest improved lipid metabolism and its relation to carbohydrate administration. Grüneberger et al. observed improved glucose sensitivity but not GLP-1 levels and insulin secretory capacity 24 weeks after IT, thus the reduced level of those lipids cannot be explained by the mechanism of GLP-1 potentiating glucose stimulated insulin secretion or increased insulin synthesis [18, 29]. Dib et al. (2013), using biliopancreatic diversion (BPD) and

gastric sleeve formation according to Scopinaro surgery in Wistar rats, reported an interesting phenomenon: the fasting plasma glucose level was significantly lowered after surgery, while the triglyceride level as well as the total cholesterol content remained unchanged, in spite of the profound changes in the pattern of plasma intestinal hormones levels [30]. The main function of the low density lipoprotein (LDL) receptor is the removal of highly atherogenic LDL particles from the circulation. Up to 75% of LDL turnover is mediated by the LDL receptor pathway in rats, and the liver contains about 70% of the total LDL receptors in the rat body. Ness (2003) observed that in rat liver, the fractions with the highest concentrations of LDL receptor coincided with the location of caveolin-1, and the LDL receptor is mainly located in caveolae [31]. Low-density lipoproteins (LDL) are particulate carriers of cholesterol and have also been found extensively in association with caveolin-1. In endothelial cells, caveolae play a major role in the transcytosis of native and modified LDL, and caveolin-1 is upregulated on LDL exposure [32].

The present study describes for the first time the long-term effect of ileal transposition on caveolin-1, miRNA-103, -107 expression in the liver of Zucker rats and cholesterol plasma levels in comparison with SHAM surgery. In our experiment six months after the transposition of 50% of ileum, increased caveolin-1 and reduced miR-107 expression was demonstrated. LDL and cholesterol plasma levels suggested positive effects on glucose and lipid metabolism in long-term observations. The present study is the first to show a lack of IT effect on triglycerides six months after the surgery.

Conflict of interest: The authors have no conflicts of interest or financial ties to disclose.

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Statement of Animal Rights: Procedures followed were in accordance with the ethical standards of the Ethics Committee of the University of Freiburg, Germany.

Author contributions: DS, TS, JF conducted IT surgery; IK-S, BS-P analyzed the data; BD, MS, MK carried out experiments; DS, KZ-K, WKK conceived the study; DS, JP, ME were involved in writing manuscript. All authors had final approval of the submitted and published version.

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