Long-term preoperative glycemic control restored the perioperative neutrophilic phagocytosis in diabetic mice.

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Abstract

BACKGROUND: The risk of surgical site infection has been reported to be higher in patients with poorly controlled diabetes. Since chronic hyperglycemia impairs neutrophil functions, preoperative glycemic control may restore neutrophil function. However, long-term insulin therapy may lead to a delay in surgery, which may be a problem, especially in cancer surgery. It is therefore unfortunate that there have been few studies in which the optimal duration of perioperative glycemic control for diabetes with chronic hyperglycemia was investigated. Therefore, we investigated the effects of preoperative long-term insulin therapy and short-term insulin therapy on perioperative neutrophil functions in diabetic mice with chronic hyperglycemia.

METHODS: Five-week-old male C57BL/6J mice were divided into four groups (Untreated (Diabetes Mellitus: DM), Short-term (DM), Long-term (DM), and Non-diabetic groups). Diabetes was established by administrating repeated low-dose streptozotocin. The Short-term (DM)group received insulin therapy for 6 hours before the operation and the Long-term (DM)group received insulin therapy for 5 days before the operation. The Untreated (DM) group and the Non-diabetic group did not receive insulin therapy. At 14 weeks of age, abdominal surgery with intestinal manipulation was performed in all four groups. We carried out a phagocytosis assay with fluorescent microspheres and a reactive oxygen species (ROS) production assay with DCFH-DA (2',7'-dichlorodihydrofluorescein diacetate) before and 24 hours after the operation using FACSVerse™ with BD FACSuite™ software.

RESULTS: Blood glucose was lowered by insulin therapy in the Short-term (DM) and Long-term (DM) groups before the operation. Neutrophilic phagocytosis activities before and after the operation were significantly restored in the Long-term (DM) group compared with those in the Untreated (DM) group (before: $p = 0.0008$, after: $p = 0.0005$). However, they were not significantly restored in the Short-term (DM) group. Neutrophilic ROS production activities before and after the operation were not restored in either the Short-term (DM) group or Long-term (DM) group.

CONCLUSIONS: Preoperative and postoperative phagocytosis activities are restored by insulin therapy for 5 days before the operation but not by insulin therapy for 6 hours before the operation.

Background

It has been reported that about 10% of patients undergoing elective surgery have diabetes mellitus (DM) [1, 2] and that about 20% of those patients have poor glycemic control preoperatively [2, 3]. The risk of postoperative infectious complications was shown to be significantly higher in patients with diabetes than in patients without diabetes [1]. Chronic hyperglycemia seen in poorly controlled diabetes would inhibit the phosphoinositide 3-kinase (PI3K)-Akt signaling pathway [4], which may be associated with an increase of insulin resistance, and also impairment of neutrophil functions including phagocytosis [5, 6]. Indeed, poorly controlled diabetes was reported to be associated with an additional higher risk of surgical site infection (SSI) [7-9].

Insulin therapy may be beneficial for restoring such an impairment of the PI3K-Akt pathway [10]. Therefore, preoperative glycemic control would improve neutrophil functions in patients with DM, which may result in a reduction in the incidence of SSI. The Centers for Disease Control and Prevention Guideline recommends lowering perioperative blood glucose levels [11], although there have been few studies in which the effect of preoperative insulin therapy in patients with diabetes, especially in those with chronic hyperglycemia, was investigated.

In the ADVANCE study, it was shown that long-term insulin control is beneficial for patients with DM [12]. However, preoperative long-term insulin therapy may lead to a delay in surgery, which may be a problem for some types of surgery, especially cancer surgery. As there have been few studies in which the effects of preoperative short-term insulin therapy and long-term insulin therapy were compared, the optimal duration of preoperative glycemic control in patients with diabetes is still unclear. Considering ethical issues related to a delay of surgery, it might be difficult to perform a clinical study to compare the effects of different durations of preoperative insulin therapy in patients with DM without any clear rationale from basic research.

We therefore conducted a study to assess the effects of preoperative short-term insulin therapy and long-term insulin therapy on perioperative neutrophil functions in diabetic mice with chronic hyperglycemia. Our null hypothesis is that preoperative short-term insulin therapy and long-term insulin therapy have no effect on perioperative neutrophil functions in compared to neutrophil functions with no insulin therapy.

Methods

This study was approved by the Kobe University Animal Experiment Committee (approved on October 23, 2017, No. P151004). Male C57BL/6J mice (4 weeks old; body weight, 16-18 g) were purchased from Japan SLC (Shizuoka, Japan). The animals were maintained in a temperature- and humidity-controlled room (22-25℃, 50-60%) on a 12-hour light-dark cycle. They had free access to normal water and normal chow diet (CLEA Rodent Diet CE-2: CLEA Japan, Inc.). The study flow is summarized in Figure 1.

Mice with streptozotocin-induced chronic diabetes

To assess the impact of preoperative glucose control in mice with chronic hyperglycemia and to avoid the contribution of an effect related to obesity or leptin abnormality on immune function, we induced DM using repeated injections of low-dose streptozotocin (STZ: Wako Pure Chemical Industries, Osaka, Japan). Mice was intraperitoneally administrated streptozotocin (STZ: Wako Pure Chemical Industries, Osaka, Japan) (50 mg/kg body weight) for 5 consecutive days to induce diabetes at the age of 5 weeks according to prior studies. [13, 14] (Figure 1). For non-diabetic controls, mice were administered only a vehicle at the age of 5 weeks.

Diabetic mice (n=68) and non-diabetic mice (n=22) were fed for 8 weeks. We measured blood glucose levels in tail vein blood using a glucometer (Glutest Neo alfa®, Sanwa Kagaku Kenkyusho, Japan) every 2 weeks (Figure 1). We defined the development of diabetes as random blood glucose level ≧ 300 mg/dL (17 mM) [15] at the age of 14 weeks.

Insulin therapy

We divided the diabetic mice at 14 weeks of age into three groups: Untreated (DM) (n=23), Short-term (DM) (n=24) and Long-term (DM) (n=21) groups (Figure 1). In the Untreated (DM) group, no insulin was administered preoperatively. In the Short-term (DM) and Long-term (DM) groups, neutral protamine hagedorn (NPH) insulin (Humulin N; Eli Lilly, Indiana, USA) was injected subcutaneously to maintain the blood glucose level below 200 mg/dL using an insulin sliding scale according to 12-hourly blood glucose measurements (supplemental file 1). In the Short-term (DM) group, insulin was injected at 6 hours before the operation, and in the Long-term (DM) group, insulin was injected every 12 hours for 5 days before the operation.

During the study period, mice had free access to food and water. We did not use insulin after the operation in this study, because many of the mice in an experimental pilot study suffered from severe hypoglycemia due to the use of insulin after the operation.

Daily insulin sensitivity factor (ISF) as a surrogate of insulin sensitivity [16], which is the drop in blood glucose caused by 1 unit of insulin, was calculated by using the following formula [17]: ISF ($mg \cdot dL^{-1} \cdot U^{-1}$) = change in blood glucose (mg/dL) / amount of insulin (U).

Surgical procedure

We performed intestinal manipulation in all 4 groups, Untreated (DM) group (n=23), Short-term (DM) group (n=24), Long-term (DM) group (n=21) and Non-diabetic group (n=22), at 14 weeks of age under general anesthesia with 3.5% sevoflurane and air, as shown Figure 2. Each mouse was placed in the supine position on a heating pad (37°C) during the procedure and shaved its hair was shaved (Figure 2; a). After injection of 1% lidocaine (Maruishi Pharmaceutical, Osaka, Japan), a vertical incision of 0.5 cm in length was made in the middle of the abdomen (Figure 2; b-1, b-2). The small bowel luminal contents from the pylorus to the cecum were removed by using two moist and sterile cotton sticks [18] (Figure 2; c). The surgical wound was closed with 5-0 nylon (Natume Seisakusho Co., Tokyo, Japan) (Figure 2; d-1, d-2). After the surgical procedure, EMLA® cream including 2.5% lidocaine and 2.5% prilocaine (Sato Pharmaceutical Co, Tokyo, Japan) was applied to the surgical site for analgesia. Each animal was placed under a heating lamp until recovery from anesthesia. After completing the experiment, mice were euthanized by cervical dislocation.

Analysis of neutrophil function

Neutrophil functions were examined before and 24 hours after the operation by using two assays. The time point of 24 hours after the operation was selected according to results of prior studies showing that the peak of alternation of neutrophil function occurs at 24 hours after inducing infection in mice [19] and 24 hours after surgery in humans [20]. A neutrophil phagocytosis assay was carried out by using fluorescently labeled microspheres. Briefly, neutrophils were isolated from peripheral blood after lysis of red blood cells (RBC). The cells were resuspended with RPMI1640 (Gibco® Carisbad, CA, USA) and incubated with Fluoresbrite® Polychromatic Red Microspheres (particles of 2.0 μm in diameter, 2.0 x 10^7 particles/mL, Polysciences, Inc. Warrington, PA, USA) in RPMI1640 for 2 hours in 5% CO_2 at 37 ℃. The cells that phagocytosed microspheres were re-suspended with FACS buffer (PBS (-) including 100 U/ml penicillin, 100 μg/ml streptomycin, 2% FBS, and 2 mM Na_2EDTA). After incubation with a purified anti-CD16/CD32 antibody (Biolegend®, SanDiego, CA, USA) for blocking Fc receptors, the cells were incubated with Pacific Blue™ anti-mouse Ly-6G antibody (Biolegend®, SanDiego, CA, USA) on ice in the dark. We analyzed the Ly-6G-positive cells with fluorescence of microspheres by using FACSVerse™ with BD FACSuite™ software (BD Bioscience, San Jose, CA, USA). Neutrophil phagocytosis rate was calculated by using following formula: Phagocytosis rate (%) = number of neutrophils with positive fluorescence of microspheres / total number of neutrophils × 100.

An assay of reactive oxygen species (ROS) generated by neutrophils was carried out according to a previous study [21]. Peripheral blood was incubated for 30 minutes at 37 ℃ with 5 μM 2', 7'-dichlorofluorescein-diacetate (DCFH-DA, Merck KGaA, Darmstadt, Germany). Blood samples were incubated with phorbol myristate acetate (PMA: 25 μg/ml, Wako Pure Chemical Industries, Osaka, Japan) for 30 minutes at 37 ℃ to stimulate neutrophils. Then samples were placed on ice to stop the reactions. After lysis of RBC, neutrophils were isolated from blood samples and were incubated with purified anti-CD16/CD32 antibody for Fc block and with Pacific Blue™ anti-mouse Ly-6G antibody on ice to stain neutrophils. Green fluorescence intensity of 2', 7'-dichlorodihydrofluorescein (DCF) in Ly-6G-positive cells was measured using a FACSVerse™. The results are shown as mean fluorescence intensity (MFI).

Statistical analysis

Data are shown as median values with interquartile range (IQR). Comparisons between two groups were performed using the Mann-Whitney U test for unpaired data and Wilcoxon's signed-rank test for paired data with Prism 8 (GraphPad Software, San Diego, USA).

As our null hypothesis was that preoperative short-term insulin therapy and long-term insulin therapy have no effect on perioperative neutrophil functions in compared to neutrophil functions with no insulin therapy, we considered the Untreated (DM) group as a reference for comparisons among the four groups.

Considering the bias of multiple comparisons (3 times), a p-value < 0.0167 was considered to indicate statistical significance for the analysis of blood glucose levels and results of neutrophil function tests. For comparisons of insulin demand and ISF value, we considered the value on the first day in the Long-term (DM) group as a reference. For this analysis, a p-value < 0.01 was considered to indicate statistical significance for adjusting the bias of multiple comparisons (5 times).

To calculate the sample size for the current study, we considered an absolute difference of 10% in the phagocytosis rate and 500 in ROS to be meaningful. Assuming standard deviations of 8% for phagocytosis rate and 250 for ROS, an α level of 0.0167 and a power of 0.80, approximately 15 mice and 6 mice were required in each cohort.

Results

Perioperative blood glucose levels

Figure 3 shows perioperative blood glucose levels. In the Non-diabetic group (green), blood glucose levels were within the normal range throughout the perioperative period. In the Untreated (DM) group (red), blood glucose level was around 600 mg/dL throughout the perioperative period. In the Short-term (DM) group (orange), blood glucose level was around 600 mg/dL for 5 preoperative days and then decreased before and after the operation. In the Long-term (DM) group (blue), blood glucose level was around 600 mg/dL before insulin therapy and then decreased after commencement of insulin therapy. The blood glucose levels before and after the operation in the Untreated (DM) group were significantly higher than the levels in the other three groups. The blood glucose levels before and after the operation in the Short-term (DM) group were not significantly different from those in the Long-term (DM) group.

Insulin sensitivity factors during the insulin therapy period

On the first day, the median insulin dose in the Long-term (DM) group was 7 U (IQR: 4.5-10), which was not significantly different from the median dose of 6 U (IQR: 5.25-10) in the Short-term (DM) group (p = 1.00). In the Long-term (DM) group, the insulin dose required to control glucose levels gradually decreased, and the doses were significantly lower on the 3rd, 4th and 5th days of insulin therapy than on the first day (3rd, 4th, and 5th days vs. first day: p = 0.0001, < 0.0001, and < 0.0001, respectively).

Figure 4 shows the ISFs in the Long-term (DM) and Short-term (DM) groups during the insulin therapy period. The median ISF on the first day in the Long-term (DM) group was 51.3 mg□dL^{-1}□U^{-1} (IQR: 43.1-76.1), which was not significantly different from the median ISF of 67 mg□dL^{-1}□U^{-1} (IQR: 46.7-83.3) in the Short-term (DM) group (p = 0.47). In the Long-term (DM) group, ISF gradually increased and was significantly higher on the 3rd, 4th and 5th days of insulin therapy than on the first day (3rd, 4th, and 5th days vs. first day: p = 0.0061, 0.0004, and < 0.0001, respectively).

Phagocytosis activities before and after the operation

Figure 5 (left side) shows the phagocytosis rates before the operation in the four groups. In the Short-term (DM) group, the median preoperative phagocytosis rate was 16.2% (IQR: 13.6-25.6), which was not significantly different from the median rate of 19.0% (IQR: 16.8-21.0) in the Untreated (DM) group (p = 0.87). In the Long-term (DM) group, the median phagocytosis rate was 25.1% (IQR: 21.5-30.1), which was significantly higher than that in the Untreated (DM) group (p = 0.0008). The median phagocytosis rate in the Long-term (DM) group was comparable to the median rate of 27.9% (IQR: 23.3- 34.2) in the Non-diabetic group (p = 0.63). Figure 5 (right side) shows the phagocytosis rates 24 hours after the operation in the four groups. The trends were similar to those before the operation. In the Short-term (DM) group, the median postoperative phagocytosis rate was 11.7% (IQR: 9.8-20.6), which was not significantly different from the median rate of 12.1% (IQR: 8.8-15.1) in the Untreated (DM) group (p = 0.41). In the Long-term (DM) group, the median postoperative phagocytosis rate was 22.2% (IQR: 18.3-32.0), which was significantly higher than that in the Untreated (DM) group (p = 0.0005). The median phagocytosis rate in the Long-term (DM) group was comparable to the median rate of 17.3% (IQR: 11.7- 25.1) in the Non-diabetic group (p = 0.14).

ROS production activities before and after the operation

Figure 6 (left) shows the MFIs as measures of ROS production activity before the operation in the four groups. In the Untreated (DM) group, the median MFI was 1282 (IQR: 920-1623), which was not significantly different from the median value of 1381 in the Short-term (DM) group (IQR: 1043-1565, p = 0.90) or the median value of 935 in the Long-term (DM) group (IQR: 716-974, p = 0.059). The median MFI in the Non-diabetic group was 704 (IQR: 665-823), which was significantly different from that in the Untreated (DM) group (p = 0.0043). Figure 6 (right) shows the MFIs 24 hours after the operation in the four groups. The trends were similar to those before the operation. In the Untreated (DM) group, the median MFI was 1093 (IQR: 918-1574), which was not significantly different from the median value of 1303 in the Short-term (DM) group (IQR: 888-2165, p = 0.78) or the median value of 1201 in the Long-term (DM) group (IQR: 1032-1311, p = 0.64). The median MFI in the Non-diabetic group was 829 (IQR: 655-930), which was not significantly different from that in the Untreated (DM) group (p = 0.026).

Discussion

Key findings

The aim of the present study using mice with induced by repeated injections of low-dose STZ chronic diabetes was to determine the impact of the duration of preoperative insulin therapy on phagocytosis activity and ROS production activity of neutrophils before and after an operation. In our study, chronic

hyperglycemia lasting for about 8 weeks was associated with a 40% reduction in neutrophil phagocytosis activity. Surgical stress induced a further 20-40% suppression of neutrophil phagocytosis activity in all four groups. We found that impaired phagocytosis activity induced by chronic diabetes was restored to a level similar to that in the Non-diabetic group by preoperative insulin therapy for 5 days but not by preoperative insulin therapy for 6 hours. Such a difference between phagocytosis activities with short-term and long-term preoperative insulin therapy was observed without a significant difference in blood glucose levels immediately before and 24 hours after the operation.

Related animal studies

The literature includes two prior relevant studies. Yano et al. conducted a study using 16-week-old diabetic db/db mice and high-fat diet-fed mice [22]. Blood glucose levels in their diabetic mice were approximately between 216 and 252 mg/dL. Blood glucose level gradually decreased with insulin treatment and was >126 mg/dL after 7 days of insulin therapy. Their insulin treatment significantly improved preoperative phagocytosis activity of neutrophils and decreased the maximal diameter of surgical site infection in both types of mice. Insulin treatment significantly increased superoxide production in db/db mice but decreased it in high-fat diet-fed mice. They did not assess neutrophilic function after the operation. We found that long-term preoperative insulin therapy improved preoperative phagocytosis activity of neutrophils, being in agreement with the results of Yano's study. The novelty of our study is that it showed the effect of preoperative insulin therapy on phagocytosis activity of neutrophils that lasted for up to 24 hours after the operation, and such a recovery seen in the Long-term (DM) group was significant compared with that the short-term (DM) group.

Kroin et al. conducted a study using Sprague-Dawley rats with diabetes induced by STZ [23]. Blood glucose levels in their rats were over 250 mg/dL. The long-term insulin therapy group received insulin treatment for 2 weeks and the short-term insulin therapy group received insulin treatment just before the operation. Both groups achieved a normal blood glucose level on day 3 and day 6 after operation, and the bacterial burden in the biceps femoris muscle was reduced compared to that with no glycemic control. Regarding the effect of preoperative short-term insulin therapy, the result of our study is conflict with the results of Kroin's study. The differences in results might come from the following notable points in Kroin's study: blood glucose levels for 6 days after surgery were equally controlled in both the short-term and long-term groups, and the period of chronic hyperglycemia was relatively short (3 weeks) for the long-term group. It is possible that both the preoperative insulin therapy and postoperative insulin therapy contributed to their results.

Interpretation of our findings

Our findings and the results of the above-described relevant studies might translate to human diabetic patients and generate the hypothesis that preoperative glycemic control influences postoperative outcomes in diabetic patients with chronic hyperglycemia. Our results obtained for the Long-term (DM) group are in agreement with the results of the above-described studies [22, 23]. However, short-term insulin therapy did not significantly improve neutrophil phagocytosis activity. There are several possible explanations for this finding.

First, inhibition of the phosphoinositide 3-kinase (PI3K)-Akt signaling pathway by chronic hyperglycemia has been reported to contribute to the suppression of neutrophil phagocytosis activity [4, 10]. Such an impairment of the PI3K-Akt pathway is also associated with deterioration of insulin resistance, which may be restored by insulin therapy [10]. In the current study, ISF gradually improved and the improvement reached statistical significance after insulin therapy for 3 or more days. These results suggest that restoration of insulin sensitivity though various mechanisms including the PI3K-Akt pathway may require a certain duration of insulin therapy rather than single insulin administration. Since the PI3K-Akt pathway contributes to both insulin resistance and neutrophil phagocytosis, our diabetic model may require long-term insulin therapy for a significant improvement of neutrophil phagocytosis activity. Second, in mouse bone marrow, promyelocytes grow into mature neutrophils during a period of 5 days. The mature neutrophils are pooled in bone marrow for 2 days and then released into blood. Finally, neutrophils end their life within 6 hours [24]. Since hyperglycemia would influence the glucose level in bone marrow, insulin therapy for 5 days may improve growth circumstances of mature neutrophils in bone marrow and then may contribute to the improvement of neutrophil phagocytosis.

In prior clinical studies, intensive insulin therapy for 2 or 3 weeks was shown to improve insulin sensitivity [25, 26]. Results of radionuclide studies suggested that about 11-12 days are necessary for the transition from myeloblasts to mature neutrophils in bone marrow [27]. If the above mechanisms contribute to the difference in restoration of neutrophil phagocytosis between long-term and short-term preoperative insulin therapy, one may speculate that approximately 2 or 3 weeks is the duration of good glycemic control needed in humans. Since no clinical study has been carried out to assess this concept, it is definitely necessary to conduct future studies to refute or confirm this hypothesis.

Perioperative ROS production activity of neutrophils in diabetic mice and impact of preoperative insulin therapy

Hyperglycemia has been reported to induce activation of the protein kinase C pathway [10] and advanced glycation end-products pathway [28], which may result in an increase in the production of ROS in neutrophils [29, 30]. As was observed in *db/db* mice in Yano's study [22], the Untreated group (DM) in our study had significantly greater production of ROS than that in the Non-diabetic group before surgery.

Although there was a trend for improvement in the production of ROS before surgery in the Long-term (DM) group, short-term and long-term insulin treatment had no significant effect on perioperative

neutrophilic ROS production in our study. The effect of insulin therapy on perioperative production of ROS may be influenced by various factors including the cause of diabetes [22]. Further examination is needed to clarify the relationship between duration of insulin therapy and changes in production of ROS by neutrophils.

Limitations

Several limitations of our study need to be considered. First, we did not perform additional insulin therapy after the operation, which may have contributed to our results. Second, we used mice with DM that was induced by using repeated low-dose STZ injections to avoid the contribution of an effect related to obesity or leptin abnormality on immune function. However, considering the high prevalence of type II DM, a future study should be conducted to assess the generalizability of our findings into a type II DM model. Third, we selected 24 hours after the operation as the time point for evaluating postoperative neutrophil function according to prior studies [19, 20]. Since the time trend of neutrophil function should be relevant, a future study with observations at multiple time points during the postoperative period should be conducted. Fourth, we did not conduct a study in a group with sham surgery (surgery but not bowel manipulation) or a group with just general anesthesia. Further study including these groups is needed to investigate the effects of bowel manipulation, surgical incision and general anesthesia. Finally, we did not assess the effects of hyperglycemia and insulin therapy on intracellular signaling. The detailed signaling pathways involved in the restoration of neutrophil phagocytosis with different durations of insulin therapy remain to be identified.

Conclusion

In our model in which chronic hyperglycemia was sustained for 8 weeks, preoperative and postoperative phagocytosis activities of neutrophils were restored by insulin therapy for 5 days before the operation but not by insulin therapy for 6 hours before the operation.

Abbreviations

DM: diabetes mellitus

ADVANCE: Action in Diabetes and Vascular disease: PreterAx and DiamicroN Controlled Evaluation

STZ: streptozotocin

NPH: neutral protamine Hagedorn

ISF: insulin sensitivity factor

RBC: red blood cells

FACS: fluorescence-activated cell sorting

PBS: phosphate buffered saline

ROS: reactive oxygen species

DCFH-DA: 2’, 7’-dichlorofluorescein-diacetate

PMA: phorbol myristate acetate

DCF: 2’, 7’-dichlorodihydrofluorescein

MFI: mean fluorescence intensity

IQR: interquartile range

PI3K: phosphoinositide 3-kinase

PKC: protein kinase C

AGEs: advanced glycation end-products

Declarations

Ethics approval

This study was approved by the Kobe University Animal Experiment Committee (approved on October 23, 2017, No. P151004).

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

DF, YN, ME and SM designed experiments. DF and YN carried out the experiments. DF, YN, and ME analyzed data and drafted the manuscript. All authors confirmed the original data and reviewed the findings. All authors read and approved the final manuscript and were responsible for archiving the study files.

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Figures

Figure 1: Study flow.

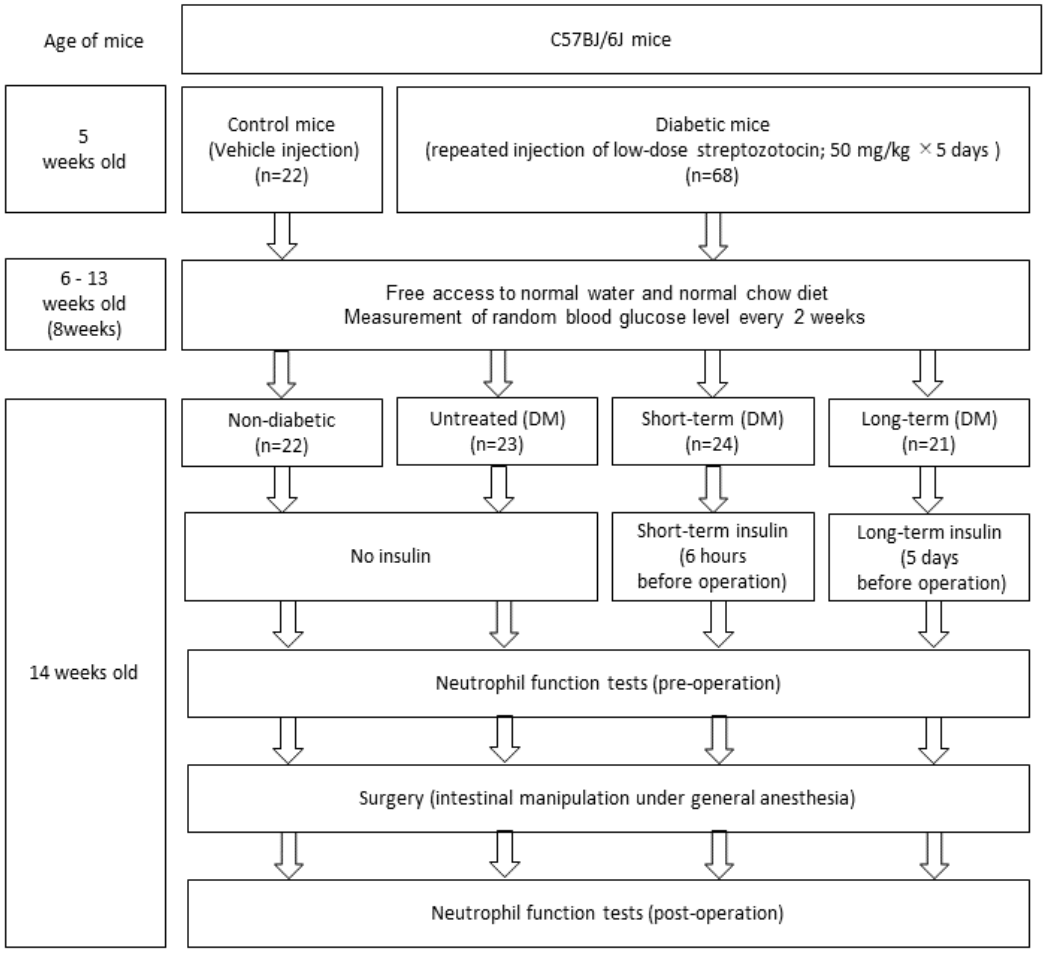

Figure 1

Study flow.

Figure 2: Surgical procedures.

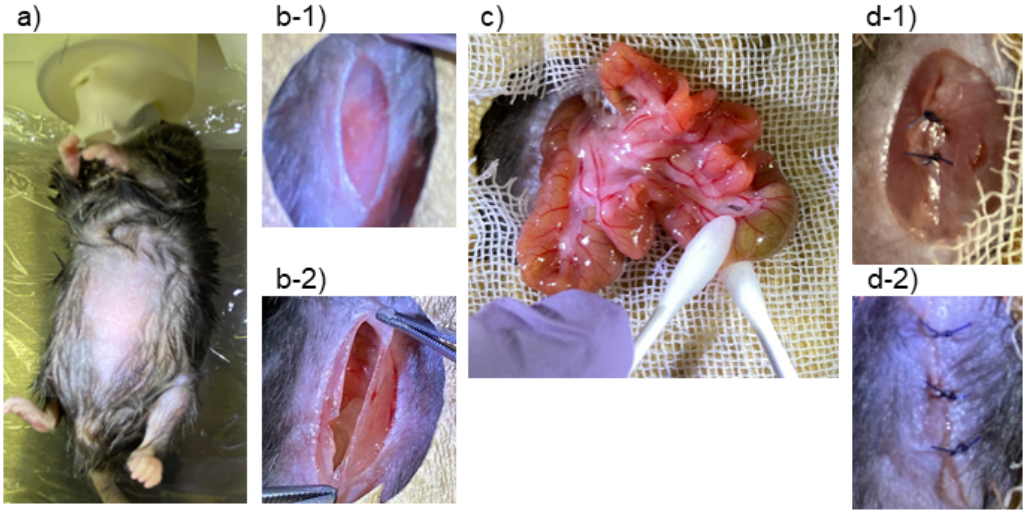

Figure 2

Surgical procedures. a) A mouse was placed in the supine position on a heating pad (37°C) and its hair was shaved after induction of general anesthesia using sevoflurane and air. b-1, b-2) After injection of 1% lidocaine, a vertical incision of 0.5 cm in length was made in the middle of the abdomen. c) The small bowel luminal contents from the pylorus to the cecum were removed by using two moist and sterile cotton sticks. d-1, d-2) The surgical wound was closed with 5-0 nylon.

Figure 3: Perioperative blood glucose levels in the four groups.

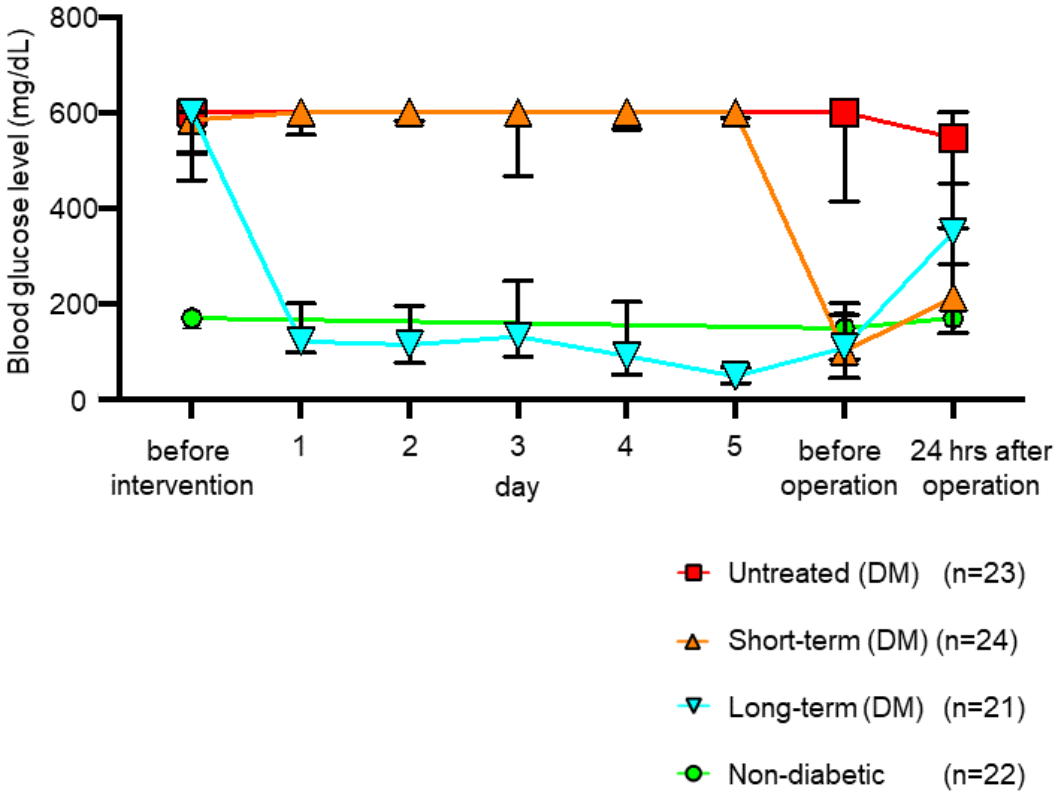

Figure 3

Perioperative blood glucose levels in the four groups. Data are presented as median values and interquartile range. The red boxes and red line indicate mean blood glucose level in the Untreated (DM) group. The orange triangles and orange line indicate mean blood glucose level in the Short-term (DM) group. The blue inversed triangles and blue line indicate mean blood glucose level in the Long-term (DM) group. The green circles and green line indicate mean blood glucose level in the Non-diabetic group.

Figure 4: Change in insulin sensitivity factor during insulin therapy.

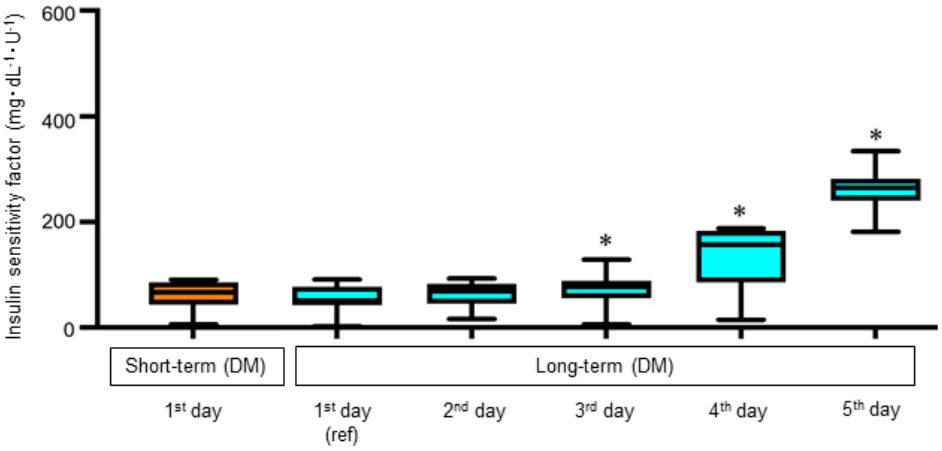

Figure 4

Changes in insulin sensitivity factor during insulin therapy. Orange box plot shows ISF in the Short-term (DM) group. Blue box plots show ISF in the Long-term (DM) group for 5 days. The ISF on each day is compared with the value on the first day in the Long-term (DM) group. *p<0.01 (as five comparisons)

Figure 5: Neutrophil phagocytosis rates before and 24 hours after the operation in the four groups.

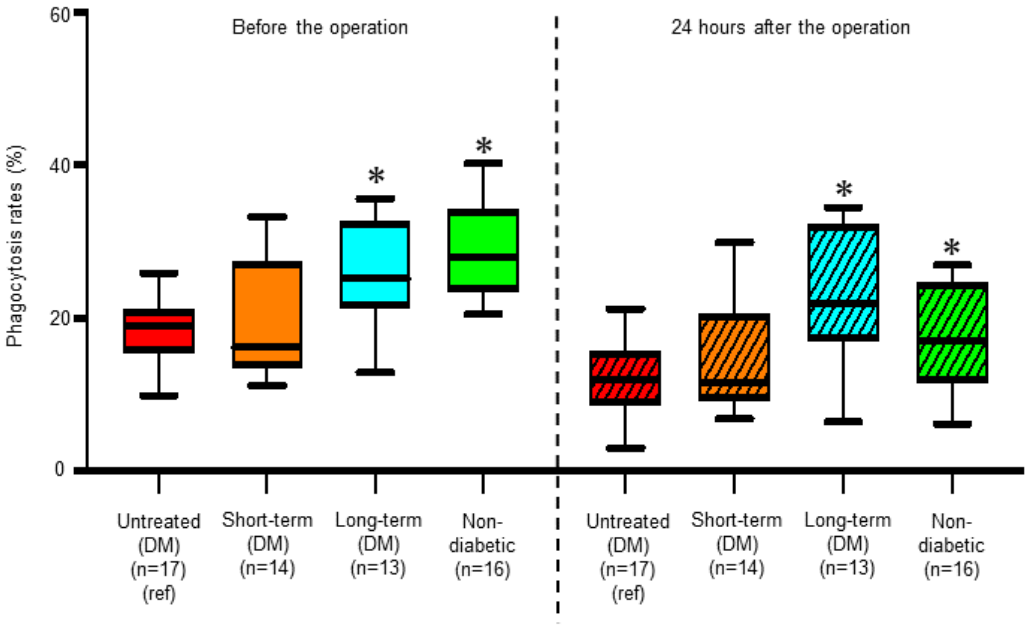

Figure 5

Neutrophil phagocytosis rates before and 24 hours after the operation in the four groups. Left box plots show neutrophil phagocytosis rates before the operation. Right box plots show the rates 24 hours after the operation. We used the neutrophil phagocytosis rate in the Untreated (DM) group (red) as a reference and compared it with the rates in the Short-term (DM) group (Orange), Long-term (DM) group (blue) and

Non-diabetic group (green). *p<0.0167 (as three comparisons) Data are presented as median values and interquartile range.

Figure 6: Neutrophil ROS production before and 24 hours after the operation in the four groups.

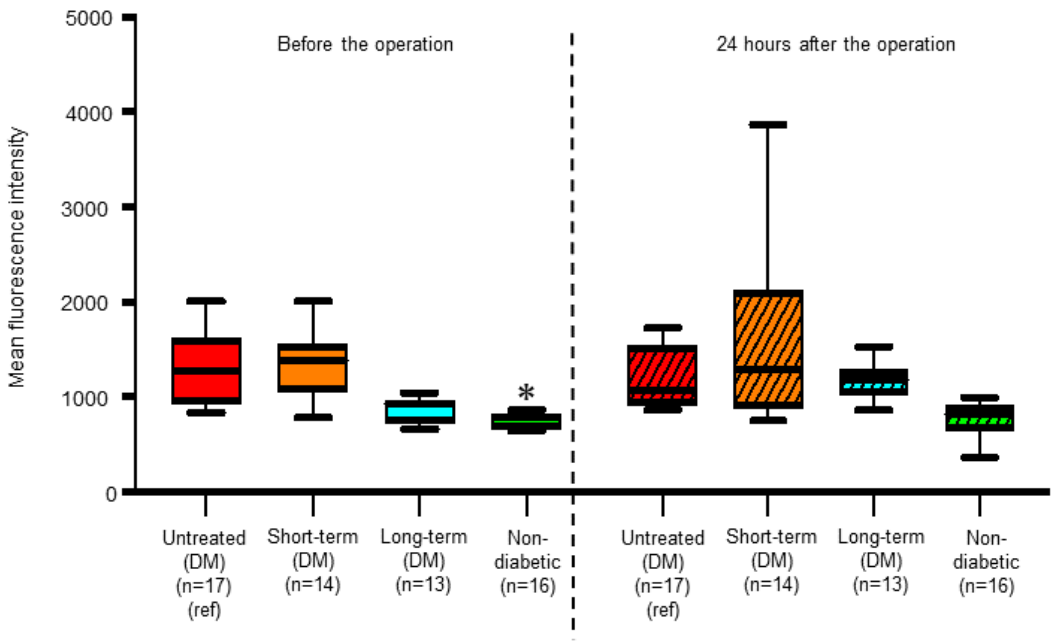

Figure 6

Neutrophil ROS production before and 24 hours after the operation in the four groups. Left box plots show neutrophil ROS production levels before the operation. Right box plots show neutrophil ROS production levels 24 hours after the operation. We used neutrophil ROS production in the Untreated (DM)

group (red) as a reference and compared it with ROS production levels in the Short-term (DM) group (Orange), Long-term (DM) group (blue) and Non-diabetic group (green). *p<0.0167 (as three comparisons) Data are presented as median values and interquartile range.

Supplementary Files

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