

Time-Restricted feeding combined with sucrose access induces pancreatic islet remodeling without compromising glycemic homeostasis in mice

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Abstract

Purpose

Time-restricted feeding (TRF) has emerged as a nutritional strategy capable of modulating energy balance and glucose metabolism; however, its effects may depend on the nutritional composition of the diet consumed during the feeding window. This study investigated whether short-term severe TRF combined with sucrose access affects glycemic homeostasis and pancreatic endocrine adaptations in metabolically healthy mice.

Methods

Male C57BL/6J mice were assigned to a Control group with ad libitum access to standard chow or a TRF group subjected to 20 h of fasting followed by a 4-h feeding window with access to standard chow and a 17% sucrose solution for 10 days. Energy intake, body weight, glucose tolerance, insulin sensitivity, plasma insulin, glucose-regulated insulin and glucagon secretion, and pancreatic islet morphometry were evaluated.

Results

TRF markedly altered the temporal pattern of energy intake, with progressive increases in sucrose consumption and total energy intake, accompanied by an initial reduction in body weight followed by partial recovery. Despite these changes, glucose tolerance and insulin sensitivity were preserved. Plasma insulin concentrations and glucose-stimulated insulin and glucagon secretion were also unchanged. Morphometric analysis revealed a significant increase in the number of pancreatic islets in TRF mice, without changes in total or mean islet area.

Conclusion

Short-term severe TRF combined with sucrose access preserved glycemic homeostasis and pancreatic endocrine function while inducing pancreatic islet remodeling. These findings suggest that structural adaptations of the endocrine pancreas may occur before detectable alterations in systemic glucose homeostasis or endocrine function.

1. INTRODUCTION

Intermittent fasting has emerged in recent years as a widely adopted dietary strategy for weight loss and improvement of metabolic health, both in clinical settings and among the general population. Different intermittent fasting regimens, including alternate-day fasting, time-restricted feeding (TRF), and

prolonged fasting protocols, have been associated with reductions in body weight, an effect largely attributed to decreased total energy intake [1–3].

Among the various intermittent fasting approaches, time-restricted feeding (TRF) consists of limiting food intake to specific daily feeding windows, while maintaining prolonged fasting periods with unrestricted access to water or other non-caloric beverages. Protocols using feeding windows of 4–6 hours have been shown to promote modest reductions in body weight and improvements in cardiometabolic parameters, including lipid profile. However, their effects on glucose metabolism remain heterogeneous and are not yet fully understood [4, 5].

The effects of intermittent fasting on glucose homeostasis appear to depend strongly on the experimental design and feeding protocol employed [4]. Clinical studies have demonstrated that fasting blood glucose may remain unchanged despite weight loss [6, 7], whereas reductions in 24-hour mean glucose levels and improvements in insulin sensitivity have been more consistently observed when TRF protocols employ shorter feeding windows, particularly those aligned with the early hours of the day [6, 8, 9].

In contrast, individuals practicing intermittent fasting frequently concentrate their food intake during the later hours of the day. This pattern may result either from the intrinsic characteristics of the fasting protocol or from the voluntary choice of the feeding window [6–8, 10]. Late-day food intake has been associated with reduced insulin sensitivity and impaired glycemic control compared with early TRF, even in the absence of differences in total energy intake or body weight [6, 8, 9]. Under real-world conditions, this temporal eating pattern is primarily influenced by social, cultural, and occupational factors rather than by metabolic optimization [11].

In addition to meal timing, diet composition is also a major determinant of the metabolic effects of time-restricted feeding [12, 13]. Because TRF protocols generally do not impose restrictions on dietary composition, individuals may continue consuming foods and beverages rich in sugars during the feeding window [14]. Therefore, experimental models combining temporal food restriction with access to sugar-containing solutions are particularly relevant for investigating how this dietary context influences the glycemic and pancreatic adaptations induced by intermittent fasting.

Although the effects of intermittent fasting on systemic metabolic outcomes, including body weight, glucose tolerance, and insulin sensitivity have been extensively investigated [12, 15–17], considerably less is known about the morphofunctional adaptations of the endocrine pancreas, particularly regarding pancreatic islet morphology and endocrine cell secretory function. Understanding these adaptations is essential to determine whether structural changes in the pancreas represent a physiological adaptive response or reflect impairment of endocrine function.

Therefore, the present study aimed to investigate the effects of a severe time-restricted feeding protocol (20 h fasting/4 h feeding) combined with access to a sucrose solution on glycemic homeostasis and endocrine pancreatic adaptations in male C57BL/6J mice. We hypothesized that, in metabolically

healthy mice, this short-term intervention would not impair glucose homeostasis or pancreatic endocrine function and would not induce detectable changes in pancreatic islet morphology. To test this hypothesis, we evaluated feeding behavior, body weight dynamics, glucose tolerance, insulin sensitivity, circulating insulin levels, pancreatic islet morphometry, and glucose-regulated insulin and glucagon secretion. By integrating systemic metabolic measurements with functional and morphological assessments of pancreatic islets, this study aimed to determine whether a short-term severe time-restricted feeding protocol combined with sucrose access is sufficient to induce early metabolic or pancreatic adaptations in otherwise metabolically healthy mice.

2. MATERIALS AND METHODS

2.1 Animals

All experimental procedures were approved by the Animal Ethics Committee (CEUA; protocol no. 23422.013429/2023-04) of the Federal University for Latin American Integration (UNILA) and follow the National Research Council Guide for the Care and Use of Laboratory Animals. Forty male C57BL/6J mice (*Mus musculus*) with 40 days of age, were used in this study. Animals were housed in a temperature-controlled animal facility ($21 \pm 2^{\circ}\text{C}$, 60% relative humidity) under a reversed 12-h light/dark cycle (lights on at 12:00 h) and maintained under the experimental conditions described below.

2.2 Experimental Design

Following a 7-day acclimation period, animals were randomly assigned to one of two experimental groups: Control (C) and Time-Restricted Feeding (TRF). The Control group had ad libitum access to standard chow (22% protein, 4% fat, and 48% carbohydrate – Table 1) and water throughout the experiment. The TRF group was subjected to an intermittent fasting protocol consisting of 20 h of fasting per day for 10 consecutive days, during which only water was available 24 hours a day, freely [18]. Following the fasting period, TRF mice were allowed access to standard chow and a 17% sucrose solution during a 4-h feeding window (09:00am–01:00pm h). During this period, animals were housed individually to allow quantification of both standard chow, water and sucrose intake. At the end of each feeding session, animals were returned to their home cages.

Table 1
Nutritional composition of the Puro Lab 22PB
standard laboratory chow

Nutrient	g/kg diet	%
Crude protein (min.)	220	22.0
Ether extract (min.)	40	4.0
Crude fiber (max.)	70	7.0
Mineral matter (max.)	80	8.0
Calcium (min.)	10	1.0
Calcium (max.)	12	1.2
Total phosphorus (min.)	8	0.8
Sodium (min.)	4	0.4
Moisture (max.)	120	12.0
Choline (min.)	2	0.2
Magnesium (min.)	1	0.1

To assess fluid intake, each individual cage was provided with two drinking bottles containing water and a 17% sucrose solution, respectively. Bottle positions were alternated daily to minimize side preference. The experimental protocol was adapted from the model described by Yasoshima and Shimura [18].

Body weight was recorded daily throughout the experimental protocol immediately before the beginning of the feeding window. Chow and sucrose intake were determined by calculating the difference between the amount offered and the amount remaining after each feeding period. Intake values recorded in grams were subsequently converted to energy intake (kcal) using the caloric values of the commercial chow (22% protein, 4% fat, and 48% carbohydrate) and the 17% sucrose solution, allowing the determination of energy intake derived from chow, sucrose, and total energy intake.

2.3 Glucose tolerance test (GTT) and insulin tolerance test (ITT)

For the glucose tolerance test (GTT), animals were fasted for 12 h and weighed before blood collection. Blood samples were obtained from the tail vein to determine fasting blood glucose (time 0) using an Accu-Chek Advantage II glucometer (Roche, Switzerland). Animals then received an intraperitoneal (i.p.) injection of glucose (1 g/kg body weight). Blood glucose concentrations were measured at 15, 30, 60, and 120 min after glucose administration [19].

For the insulin tolerance test (ITT), animals were fasted for 2 h. Following determination of baseline blood glucose (time 0), insulin was administered intraperitoneally at a dose of 0.75 U/kg body weight (Human regular insulin - Humulin® R; Eli Lilly and Company, Indianapolis, IN, USA). Blood glucose was measured at 3, 6, 9, 12, 15, 18, and 21 min after insulin administration, as previously described by Akinmokun et al. [20].

2.4 Static insulin and glucagon secretion from isolated islets

Following euthanasia, pancreas was rapidly excised, and pancreatic islets were isolated by collagenase digestion (0.8 mg/mL; Sigma-Aldrich, USA), followed by manual collection under a stereomicroscope [21].

For insulin secretion, isolated islets were preincubated for 30 min at 37°C in Krebs-Ringer bicarbonate (KRB) buffer containing 115 mM NaCl, 5 mM KCl, 10 mM NaHCO₃, 1 mM MgCl₂, 2.56 mM CaCl₂, 15 mM HEPES, supplemented with 5.6 mM glucose and 0.3% bovine serum albumin (BSA), under an atmosphere of 95% O₂ and 5% CO₂ (pH 7.4). After preincubation, groups of five islets were incubated for 1 h in KRB buffer containing either low (2.8 mM) or high (11.1 mM) glucose concentrations to evaluate insulin secretion [22].

For glucagon secretion, groups of 20 islets were preincubated with KRB with 5.6mM glucose or 1 hour, and after that incubated in KRB buffer containing either 0.5 or 11.1 mM glucose [23]. At the end of the incubation period, supernatants were collected and stored at – 20°C. Insulin (E-EL-M1382, Elabscience, USA) and glucagon (E-EL-M0555, Elabscience, USA) concentrations were determined using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions.

To determine total protein content, the remaining islets were lysed in an acid-ethanol solution under continuous agitation, and protein concentration was measured using the Bradford assay. Hormone secretion values were normalized to total protein content.

2.5 Pancreatic islet morphometry

For morphometric analysis, pancreas was weighed and fixed in formalin solution for 72 h. Tissues were subsequently dehydrated through graded ethanol solutions, embedded in paraffin, and sectioned. Two serial sections from each animal were mounted onto slides. The sections were 5-µm thick and 250 µm apart from each other (the 1st and the 50th sections). Sections were stained with hematoxylin and eosin (H&E) as previously described by Carvalho et al. [24]. All islets from slide 1 and 50 were photographed and islet area evaluated with ImageJ software (National Institutes of Health; Bethesda, MD).

2.6 Plasma insulin determination

Whole fasting blood samples were collected at euthanasia and centrifuged at 5,000 rpm for 15 min at 4°C to obtain plasma. Plasma insulin concentrations were measured using a commercial ELISA kit (E-EL-M1382, Elabscience, USA) according to the manufacturer's instructions.

2.7 Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 10. Data distribution was initially assessed using the Shapiro–Wilk normality test. Variables measured repeatedly over time were analyzed using two-way repeated-measures ANOVA or one-way repeated-measures ANOVA, as appropriate, followed by Šídák's multiple comparisons test when applicable.

Comparisons between two independent groups were performed using the unpaired Student's *t*-test. Insulin and glucagon secretion data were analyzed by two-way ANOVA considering experimental group and glucose concentration as independent factors, followed by Šídák's multiple comparisons test.

For the glucose tolerance test (GTT) and insulin tolerance test (ITT), the area under the curve (AUC) was also calculated and compared between groups using the unpaired Student's *t*-test. Data are presented as mean ± SEM, and statistical significance was accepted at $p < 0.05$.

3. RESULTS

3.1 Food Intake and Body Weight

Figure 1A shows chow-derived energy intake during the 4-h feeding window throughout the 10-day experimental protocol. Significant effects of group ($F(1,29) = 243.3; p < 0.0001$), day ($F(2.848, 82.61) = 13.82; p < 0.0001$), and the group × day interaction ($F(9,261) = 15.37; p < 0.0001$) were observed, indicating that TRF mice consumed more chow-derived energy during the feeding window and exhibited a distinct temporal pattern of food intake compared with the Control group.

Figure 1B shows energy intake derived from the 17% sucrose solution during the 4-h feeding window throughout the experimental period. A significant effect of day was observed ($F(1.552, 31.04) = 17.16; p < 0.0001$), indicating a progressive increase in sucrose-derived energy intake during the protocol. Multiple comparisons revealed significantly greater sucrose consumption on the last experimental day compared with the first day ($p = 0.0036$).

Total energy intake during the 4-h feeding window is shown in Fig. 1C. Significant effects of group ($F(1,29) = 560.7; p < 0.0001$), day ($F(2.567, 74.45) = 20.22; p < 0.0001$), and the group × day interaction ($F(9,261) = 21.31; p < 0.0001$) were detected, demonstrating that inclusion of sucrose-derived calories further accentuated the difference in energy intake between groups. Multiple comparisons revealed that total energy intake during the feeding window was significantly greater in the TRF group throughout the entire experimental protocol ($p < 0.0001$).

Total daily energy intake over the 10-day experimental period is presented in Fig. 1D. Significant effects of day ($F(2.838, 82.31) = 18.77; p < 0.0001$) and the group × day interaction ($F(9,261) = 15.88; p < 0.0001$) were observed, whereas the main effect of group was not significant ($F(1,29) = 3.352; p = 0.0774$). Multiple comparisons showed that total daily energy intake was significantly lower in the TRF group on

days 2 ($p = 0.0051$) and 4 ($p = 0.0101$), whereas significantly higher intake was observed on days 7 ($p = 0.0002$), 8 ($p = 0.0040$), 9 ($p < 0.0001$), and 10 ($p = 0.0002$) compared with the Control group.

Body weight dynamics throughout the experimental protocol are shown in Fig. 1E. Significant effects of group ($F(1,29) = 4.982$; $p = 0.0335$), time ($F(1.231, 35.70) = 9.856$; $p = 0.0020$), and the group $\times$ time interaction ($F(9,261) = 5.145$; $p < 0.0001$) were observed. Multiple comparisons demonstrated that TRF mice exhibited lower relative body weight on days 2, 3, and 4 compared with Control animals. However, these differences were no longer observed during the remaining experimental period, indicating progressive recovery of body weight.

3.2 Glucose and insulin tolerance tests

The glycemic response during the glucose tolerance test (GTT) is shown in Fig. 2A. Significant effects of time ($F = 19.91$; $p = 0.0005$) and the group $\times$ time interaction ($F = 5.60$; $p = 0.0009$) were observed, whereas no significant effect of group was detected ($F = 2.11$; $p = 0.1969$). Despite the significant interaction, Šídák's multiple comparisons test did not identify differences between groups at any individual time point. Likewise, the area under the curve (AUC) (Fig. 2B) did not differ significantly between groups ($t = 1.857$; $p = 0.1368$).

Insulin sensitivity was evaluated using the insulin tolerance test (ITT) (Fig. 2C). No significant effects of time ($F = 3.24$; $p = 0.1318$), group ($F = 0.92$; $p = 0.3918$), or the group $\times$ time interaction ($F = 0.29$; $p = 0.9524$) were observed. Similarly, the AUC (Fig. 2D) did not differ significantly between groups ($t = 1.490$; $p = 0.2104$).

3.3 Insulin and glucagon secretion

Insulin secretion increased significantly in response to stimulation with 11.1 mM glucose compared with the basal concentration of 2.8 mM (main effect of glucose concentration: $F(1,14) = 27.58$; $p = 0.0001$). Multiple comparisons did not detect significant differences between the Control and TRF groups at either 2.8 mM ($p = 0.4853$) or 11.1 mM glucose ($p = 0.3361$).

Glucagon secretion was significantly reduced following stimulation with 11.1 mM glucose compared with 0.5 mM glucose ($F(1,6) = 34.00$; $p = 0.0011$). Likewise, multiple comparisons revealed no significant differences between the Control and TRF groups at either 0.5 mM ($p = 0.9971$) or 11.1 mM glucose ($p = 0.9573$).

Plasma insulin concentrations did not differ between the Control and TRF groups ($t = 0.9253$, $df = 22$, $p = 0.3649$) (Fig. 3C), indicating that the time-restricted feeding protocol combined with sucrose access did not alter basal circulating insulin levels.

3.4 Pancreatic islet morphometry

Pancreatic islet morphometric analysis is presented in Fig. 4. No significant differences were observed between groups in total islet area ($t = 1.505$, $df = 6$, $p = 0.1832$) or mean islet area ($t = 0.5444$, $df = 6$, $p = 0.6058$). In contrast, the TRF group exhibited a significantly greater number of pancreatic islets than the Control group ($t = 4.340$, $df = 6$, $p = 0.0049$).

4. DISCUSSION

The present study demonstrates that a severe time-restricted feeding protocol (20 h fasting/4 h feeding) combined with access to a sucrose solution promotes marked adaptations in energy intake patterns and pancreatic morphology without compromising glycemic homeostasis. Although animals progressively increased their energy intake during the feeding window and exhibited a greater number of pancreatic islets, no changes were observed in glucose tolerance, insulin sensitivity, insulin or glucagon secretion, or circulating insulin levels. Thus, the structural changes observed in the endocrine pancreas occurred without detectable functional impairment.

The marked increase in food intake during the feeding window is consistent with the original experimental model described by Yasoshima and Shimura [18]. In that study, mice exhibited a significant increase in chow intake during the 4-h feeding window, followed by stabilization from approximately the fifth day of the protocol. Similarly, sucrose intake progressively increased and stabilized around the eighth day. A comparable temporal pattern was observed here, with chow intake reaching a plateau from the sixth day onward and sucrose intake stabilizing after the eighth day.

Across different experimental models of time-restricted feeding, redistribution of energy intake toward the available feeding window has been consistently reported, regardless of diet composition [8, 9, 12]. This feeding pattern likely represents a physiological adaptation to prolonged fasting, allowing animals to consume a substantial proportion of their daily energy intake within a limited time frame without necessarily reducing total caloric intake throughout the experimental protocol.

The initial reduction in body weight closely paralleled the lower total energy intake observed during the first days of the protocol. As energy intake progressively increased, body weight partially recovered, suggesting behavioral and metabolic adaptation to the time-restricted feeding regimen. This temporal profile resembles that reported in the original model, in which an early decrease in body weight was followed by partial recovery after approximately five to six days of treatment [18]. Similar findings have also been described by Paim et al. [25] using the same experimental paradigm, as well as in other time-restricted feeding models with different dietary compositions [26, 27]. This pattern suggests that animals progressively adapt to restricted food availability by reorganizing feeding behavior and energy balance, thereby attenuating the initial weight loss.

Despite the temporal redistribution of energy intake and transient changes in body weight, the TRF protocol did not impair glucose tolerance or insulin sensitivity. Although a significant group $\times$ time interaction was detected during the GTT, neither individual time points nor the AUC differed between groups. Interestingly, the glycemic curve tended toward a lower excursion in TRF mice, although this

pattern did not reach statistical significance. The absence of glucose intolerance may be related to the short duration of the protocol and the lack of sustained energy overconsumption, once energy intake initially reduces and increases above control levels only during the final protocol days.

Time-restricted feeding can preserve or improve glucose regulation even in the absence of caloric restriction or under metabolically challenging dietary conditions [12, 28]. Moreover, improvements in glucose tolerance have been reported when the feeding window is appropriately aligned with the circadian cycle [29]. Therefore, the 10-day duration of the present protocol may have been insufficient to induce detectable glucose impairments front of sucrose and energy intake, particularly because diet-induced metabolic disturbances in C57BL/6 mice are commonly investigated after longer dietary exposures [30]. Moreover, given the methodological and physiological factors that influence metabolic tolerance tests [31], the preserved ITT response further supports the absence of detectable impairment in systemic glucose regulation during this short-term intervention.

Morphometric analysis revealed structural remodeling of the endocrine pancreas, characterized by an increased number of pancreatic islets without significant changes in total or mean islet area. Pancreatic responses to fasting appear to depend strongly on the duration and pattern of dietary restriction. Acute fasting for 24–48 h did not alter islet area or number in mice, despite reducing glucose-stimulated insulin secretion [32]. More recently, repeated cycles of a fasting-mimicking diet were shown to induce pancreatic islet plasticity, including changes in endocrine cell phenotypes and β -cell ultrastructure [33]. The increased islet number observed here may therefore represent a distinct morphological response to repeated 20-h fasting/4-h feeding cycles, occurring without detectable impairment of pancreatic endocrine function.

The preservation of circulating insulin levels and ex vivo insulin and glucagon secretion further indicates that the morphological changes were not accompanied by detectable endocrine dysfunction. Duration appears to be particularly relevant in this regard. Matta et al. [17] reported that short-term intermittent fasting improved glucose homeostasis without affecting islet morphology or function, whereas prolonged intermittent fasting impaired β -cell maturation and glucose-stimulated insulin secretion in young mice. The preserved hormone secretion observed after only 10 days of TRF may therefore reflect an early adaptive stage, preceding functional alterations that could emerge with prolonged exposure.

Pancreatic islets remained responsive to glucose stimulation, with increased insulin secretion at high glucose concentrations and appropriate suppression of glucagon release, supporting the maintenance of both β -cell and α -cell function.. Although most studies of time-restricted feeding have focused primarily on systemic metabolic outcomes, direct ex vivo assessment of pancreatic hormone secretion remains relatively limited [12, 34–36]. Taken together, these findings demonstrate that, under this short-term experimental condition, pancreatic morphological remodeling can occur without detectable alterations in endocrine secretory function, suggesting that structural adaptations may precede measurable functional changes.

5. CONCLUSION

In conclusion, a short-term time-restricted feeding protocol with a 4-h feeding window combined with access to a sucrose solution promoted a marked temporal redistribution of energy intake, accompanied by transient body weight changes and structural remodeling of the endocrine pancreas. Despite these adaptations, glycemic homeostasis, insulin sensitivity, and pancreatic hormone secretion remained preserved throughout the experimental period. These findings demonstrate that pancreatic islet remodeling can occur as an early adaptive response to severe time-restricted feeding without compromising endocrine function or systemic glucose homeostasis, providing new insights into the pancreatic adaptations associated with intermittent fasting.

DECLARATIONS

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics Approval

All experimental procedures involving animals were approved by the Ethics Committee on Animal Use (CEUA) of the Federal University for Latin American Integration (UNILA), protocol no. 23422.013429/2023-04. All procedures were conducted in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals*.

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Author Contribution

FAAL: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Validation, Writing – original draft.

CFC: Data curation, Formal analysis, Methodology, Project administration.

JSCSS: Data curation, Formal analysis, Methodology, Project administration.

YSG: Data curation, Formal analysis, Methodology, Project administration.

PHG: Data curation, Formal analysis, Methodology, Project administration.

HDC: Conceptualization, Data curation, Validation, Writing – original draft.

MCG: Conceptualization, Data curation, Validation, Writing – original draft.

FAF: Data curation, Formal analysis, Methodology, Writing – review & editing.

AMFJ: Data curation, Formal analysis, Methodology, Writing – review & editing.

JFV: Conceptualization, Data curation, Formal analysis, Methodology, Resources, Writing – review & editing.

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Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Figures

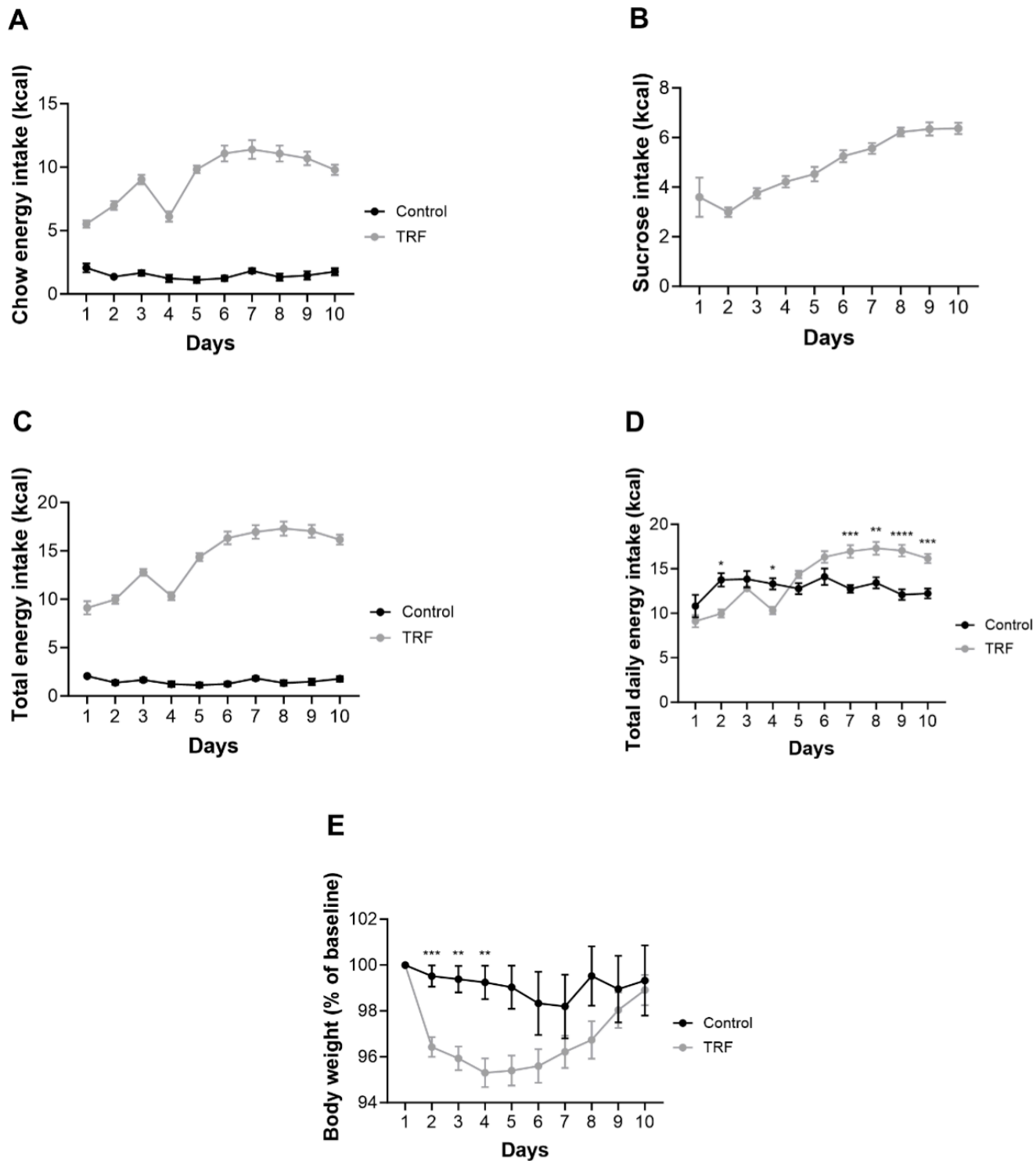


Figure 1

Effects of time-restricted feeding combined with sucrose access on energy intake and body weight. (A) Chow-derived energy intake during the 4-h feeding window throughout the 10-day experimental protocol. (B) Energy intake derived from the 17% sucrose solution during the 4-h feeding window in the time-restricted feeding (TRF) group. (C) Total energy intake during the 4-h feeding window. (D) Total daily energy intake (24 h). (E) Body weight expressed as a percentage of baseline body weight. Data are

presented as mean $\pm$ SEM. Panels A, C, D, and E were analyzed using two-way repeated-measures ANOVA, whereas panel B was analyzed using one-way repeated-measures ANOVA followed by Šídák's multiple comparisons test. Asterisks indicate significant differences between groups at each experimental time point ($p < 0.05$, $p < 0.01$, $p < 0.001$, $p < 0.0001$). $n = 21$ (TRF) and $n = 10$ (Control).

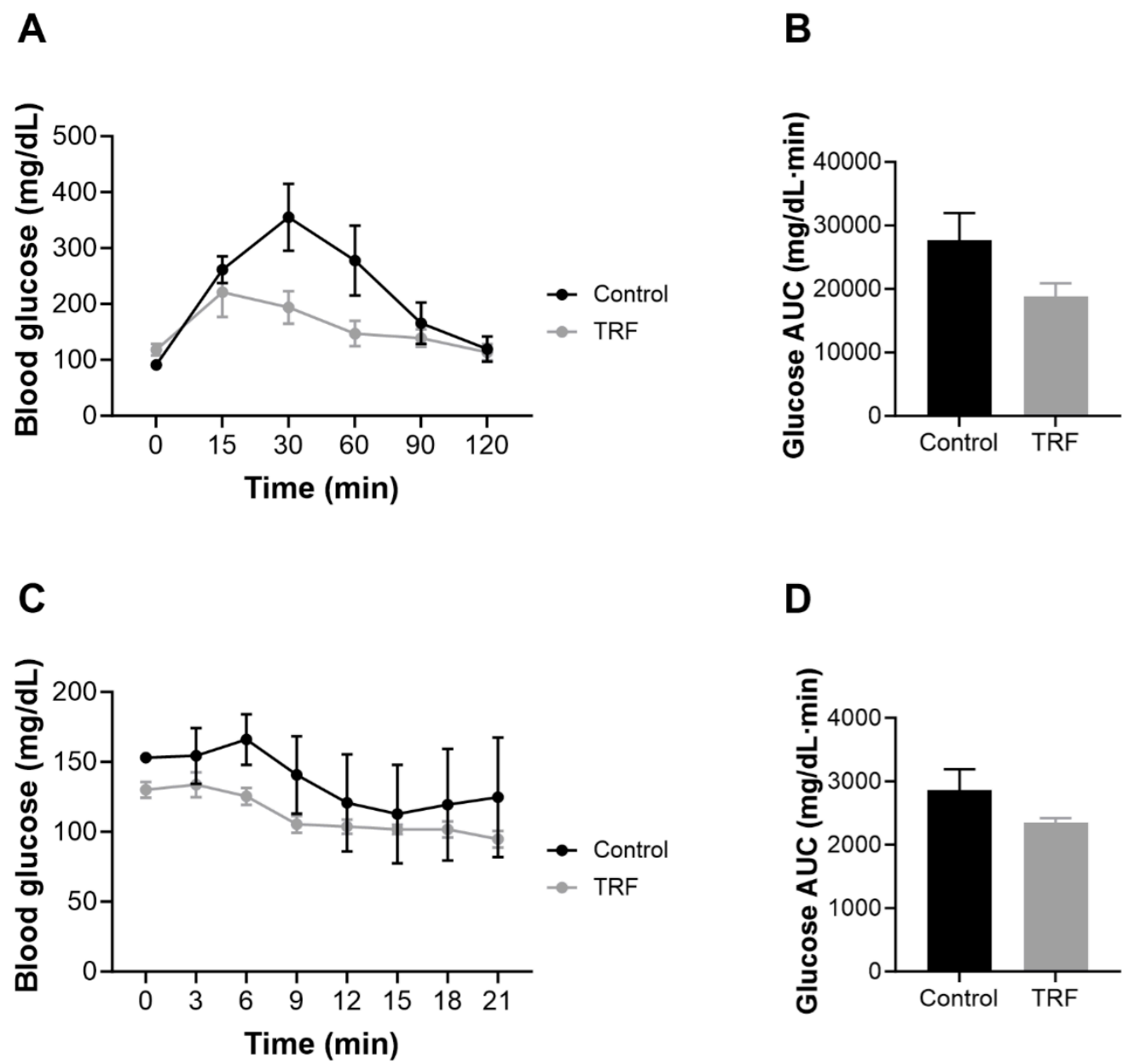


Figure 2

Effects of time-restricted feeding combined with sucrose access on glucose metabolism. (A) Glycemic curve during the glucose tolerance test (GTT). (B) Area under the curve (AUC) of the GTT. (C) Glycemic curve during the insulin tolerance test (ITT). (D) Area under the curve (AUC) of the ITT. Data are presented as mean $\pm$ SEM. Panels A and C were analyzed using two-way repeated-measures ANOVA followed by Šídák's multiple comparisons test. Panels B and D were analyzed using the unpaired

Student's t-test. No significant differences were observed between groups. $n = 4$ per group for the GTT and $n = 3$ per group for the ITT.

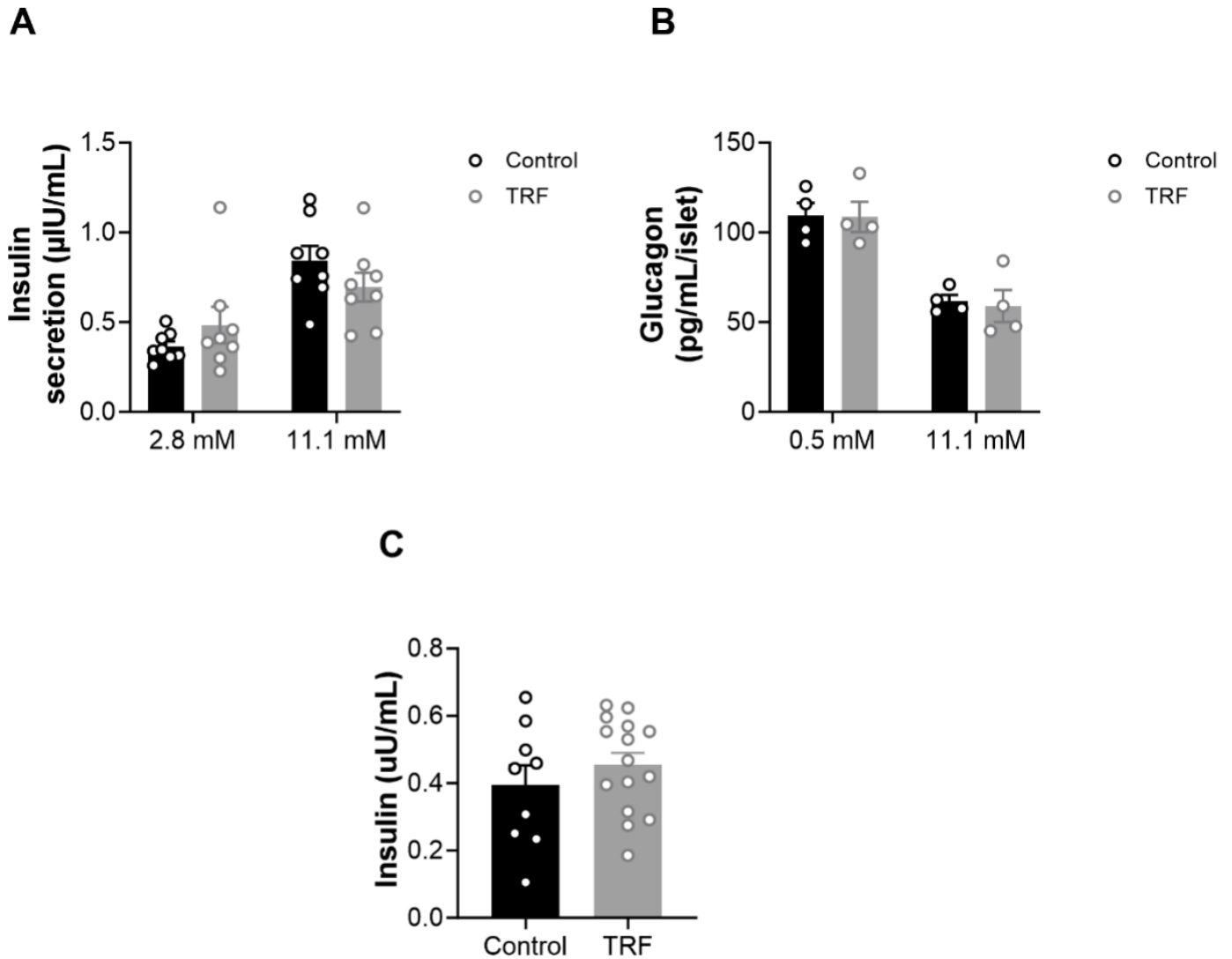


Figure 3

Effects of time-restricted feeding combined with sucrose access on plasmatic insulin levels and endocrine pancreatic islet function. (A) Insulin secretion from pancreatic islets incubated in low (2.8 mM) and high (11.1 mM) glucose concentrations. (B) Glucagon secretion from pancreatic islets incubated in low (0.5 mM) and high (11.1 mM) glucose concentrations. (C) Plasma insulin concentration. Data are presented as mean $\pm$ SEM. Panels A and B were analyzed using two-way ANOVA followed by Šídák's multiple comparisons test. Panel C was analyzed using the unpaired Student's t-test. No significant differences were observed between the Control and TRF groups. $n = 8$ per group for panels A and B and $n = 9$ (Control) and $n = 15$ (TRF) for panel C.

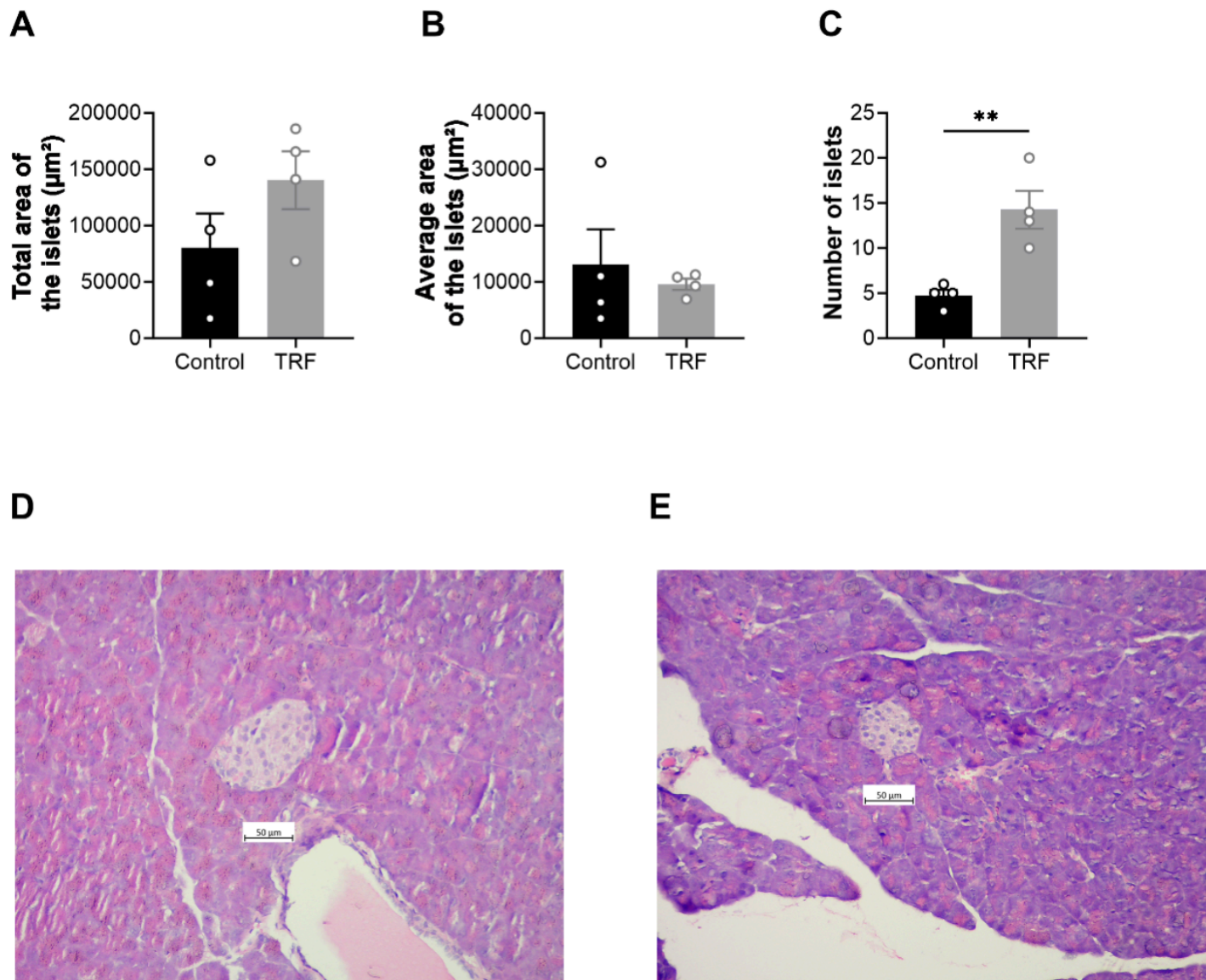


Figure 4

Effects of time-restricted feeding combined with sucrose access on pancreatic islet morphometry. (A) Total pancreatic islet area, calculated as the sum of the areas of all islets analyzed in each animal. (B) Mean pancreatic islet area, calculated from the individual areas of all islets analyzed in each animal. (C) Total number of pancreatic islets identified across the semi-serial pancreatic sections analyzed for each animal. Representative histological images of pancreatic tissue from Control (D) and TRF (E) mice. Scale bar = 50 μm . Data are presented as mean $\pm$ SEM. Each dot represents one animal. Comparisons between groups were performed using unpaired Student's t-test. ** $p < 0.01$. $n = 4$ animals per group.