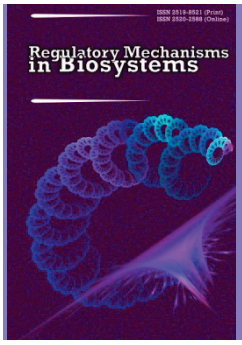

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The prerequisites for the development of type 2 diabetes or prediabetes in rats fed a high-fat diet

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It is known that the pathogenesis of type 2 diabetes in humans is based on two main factors – insulin resistance and inappropriate secretory activity of β-cells of the pancreas. In animals, the role of these mechanisms has not been clearly characterized, and the differences in the manifestations of experimental diabetes under the same conditions are not sufficiently substantiated. In order to study the prerequisites and mechanisms of the development of experimental type 2 diabetes or prediabetes under lipid overload, 6-month-old male Wistar rats were fed a high-fat diet for 4 weeks; after 2 weeks of the experiment, 20 or 25 mg/kg of streptozotocin was administrated. The development of insulin resistance was assessed using the insulin tolerance test. We evaluated the dynamics of glycemia in animals, subcellular signs of liver steatosis, and determined expression of the precursor and mature protein SREBP-1 by immunoblotting. It was found that in rats fed with a high-fat diet during the 2–4th weeks of the experiment, regardless of the administration of streptozotocin, stable insulin resistance and symptoms of prediabetes were detected. The severity of carbohydrate metabolism lesion, which appeared as type 2 diabetes or prediabetes after streptozotocin administration, depended on the level of hepatosteatosis due to high-fat diet, whereas the dose of streptozotocin influenced severity of type 2 diabetes. The use of a high-fat diet led to increased processing and activation of SREBP-1, which was clearly inhibited in type 2 diabetes. Therefore, the level of lipid infiltration of the liver and deregulation of the transcription factor SREBP-1 are risk factors defining development of type 2 diabetes or prediabetes in experimental rats with lipid overloading. Changes in the maturation of SREBP-1 with the use of a high-fat diet confirm that insulin resistance in rats revealed β-cell dysfunction, which closely approximates the mechanisms of experimental type 2 diabetes to main pathways in humans. At the same time, the predisposition to β-cell dysfunction can be a prerequisite that determines compensatory reserves for maintaining carbohydrate and lipid homeostasis under the influence of lipid load in both humans and laboratory animals.

Keywords: lipid overload; streptozotocin dosage; insulin resistance; liver steatosis; SREBP-1 expression.

Introduction

The epidemic growth of metabolic syndrome and type 2 diabetes (T2D) in developed countries has set the task of studying the pathogenesis and risk factors for the development of these diseases (Fan, 2017; Rett & Gottwald-Hostalek, 2019; Younossi et al., 2019; Tanase et al., 2020; Mitchell, 2021), which requires intensive experimental research. The known ways of reproduction of T2D in laboratory animals may be divided into two groups: induced and genetically determined (Kolesnyk et al., 2016; Kwak & Park, 2016; Mierziak et al., 2021). The latter are aimed at studying individual molecular links in the development of T2D, while the first group is closer to the pathogenesis of this disease in humans.

It is known that the pathogenesis of T2D is based on two main factors – insulin resistance and inappropriate secretory activity of β-cells of the pancreas, which in humans are caused by two different types of genetic defects (Leahy et al., 2008; Herder & Roden, 2011; Krentz & Gloyn, 2020; Gołacki et al., 2022). Over the past decade, more than 400 genetic loci have been identified that mediate a person's predisposition to the development of T2D, while the predominant pathogenetic significance of gene polymorphisms responsible for β-cell function has been revealed (Herder & Roden, 2011; Krentz & Gloyn, 2020). At the same time, many observations indicate that environmental factors can play an equally important role as the genetic defects in the development of insulin resistance (Kwak & Park, 2016; Mierziak et al., 2021; Gołacki et al., 2022; Lieshchova & Brygadyrenko, 2022, 2023).

According to these basic mechanisms, animal models with the reproduction of insulin resistance by means of dietary load and dysfunction of β-cells of the pancreas because of their experimental damage are of interest. For this purpose, the use of a high-fat diet (HFD) of various composition (40–58% lipids by caloric content) and administration of streptozotocin (15–60 mg/kg of body weight) has shown its effectiveness in reproducing experimental diabetes (Reed et al., 2000; Srinivasan et al., 2005; Wang et al., 2011; Mansor et al., 2013). However, from the point of view of pathogenetic relevance, the administration of streptozotocin in doses of more than 30–35 mg/kg of body weight for the reproduction of T2D is questionable (Reed et al., 2000). Such model may actually combine type 1 and type 2 diabetes (Mansor et al., 2013; Kolesnyk et al., 2016) and does not allow isolating the mechanisms of development of these diseases. Therefore, the substantiation of the pathogenetic correspondence of experimental models to the mechanisms of development of T2D in humans remains relevant.

The experimental reproduction of type 2 prediabetes (PD), which represents an important clinical issue, is less studied (Nunes et al., 2013; Rett & Gottwald-Hostalek, 2019). Known models of prediabetes are ba-

sed on the use of carbohydrate load (Nunes et al., 2013), which does not allow their results to be compared with the given models of T2D. Therefore, the justification of the experimental reproduction of HFD-induced PD is of considerable interest.

At the same time, the definitions of prediabetes and type 2 diabetes and their mechanisms in animals are still under discussion. Animal studies have shown no evidence that insulin resistance induces β-cell dysfunction resulting in T2D, as is known in humans (Gilor et al., 2016). On the other hand, all known experimental animal models demonstrate a wide range of individual responses: from severe T2D to the absence of a response to the administration of streptozotocin (or another damaging factor), as well as the diversity of the course of the pathological process. The prerequisites for such differences in the response to the same pathological factors, namely, the development of a pathological process of varying severity, which is observed both in the experiment and in the clinic, are currently not sufficiently understood.

The aim of the study was to determine the time-dependent effects of lipid overload on development of metabolic disorders in rats, and to determine prerequisites and mechanisms of development of type 2 diabetes or prediabetes in the experiment.

Materials and methods

Experimental animals and experimental design. The animal studies were performed in accordance with the ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines 2.0 (Percie du Sert et al., 2020) and were approved by The Local Ethics Committee at Bogomoletz Institute of Physiology (Kyiv, Ukraine) (protocol No. 11, 10.04.2020), as the investigations conducted according to requirements of European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986), and the current legislation of Ukraine on the protection of experimental animals.

Two separate experiments were performed on 80 male Wistar rats aged 6 months. The animals were randomized into the following groups: 1) control rats (n = 16), which were fed a standard chow diet for 4 weeks; 2) rats with high-fat diet for 4 weeks (HFD4, n = 10); 3) rats with HFD for 4 weeks, and administration of streptozotocin at a dose of 20 or 25 mg/kg (n = 24 and n = 30, respectively). From the animals of the last group, subgroups with T2D (n = 34) and PD (n = 16) were selected according to research results.

The HFD was based on a standard vivarium chow (dry combined feed for rodents) mixed with lard up to 58% fat in the total caloric content. Streptozotocin (Sigma-Aldrich, Merck KGaA, Germany) dissolved in 0.1 M citrate buffer (pH 4.5) was injected intraperitoneally after 2 weeks of HFD, the effects of the development of hyperglycemia were determined on second day and then in the dynamics of the experiment. The animals were kept in standard vivarium conditions during the 4 weeks of the experiment with access to water ad libitum. At the end of the experiment, the animals were anesthetized with urethane (1.5 g/kg), and tissue samples were removed for further examination.

Assessment of carbohydrate metabolism disorders and insulin resistance. Glucose concentration was measured using an automatic glucometer OneTouch Ultra (LifeScan, USA, 2018) in peripheral blood obtained from the tip of the animal's tail. The concepts of "diabetes" and "prediabetes" were defined according to the modern criteria of the American Diabetes Association (2020).

The development of insulin resistance was determined using the insulin tolerance test (ITT) after 2 weeks of HFD (before the administration of streptozotocin), and after 4 weeks of the experiment. ITT was performed according to the modified method (Furuya et al., 2020). The rats deprived of food for about 2 h were tested using intraperitoneal injection of 0.5 IU/kg short-acting recombinant human insulin (Humodar, Ukraine). The response was evaluated by measuring the glucose content in peripheral blood obtained from the tip of the animal's tail prior to (0 minutes) and 30, and 60 minutes after insulin administration.

Morphological examination. The preparations for electron microscopic studies were made by a widely used technique. Tissue samples removed from anesthetized animals were immediately fixed in a 2.5% solution of glutaraldehyde, and postfixed in Caulfield's reagent (based on 2% OsO_4, pH = 7.3). Dehydration of the material was carried out in alcohols of increasing concentration, absolute alcohol and acetone, and then specimens were embedded into Epon. Ultrathin sections 40–60 nm thick were contrasted with 1% uranyl acetate solution and lead citrate solution (Reynolds, 1963). All reagents were from Sigma-Aldrich, Merck KGaA, Germany. The preparations were examined using the electron microscope PEM-125K (Ukraine, 2006).

Determination of protein expression. Protein expression was determined by immunoblotting with current protocols and equipment from BioRad Laboratories (Bio-Rad Laboratories, Inc., USA, 2012). The tissue samples removed from anesthetized animals were immediately frozen in liquid nitrogen and kept at –40 °C. For protein extraction, the frozen tissue samples were homogenized on ice using an ultrasonic homogenizer, lysed for 30 min in buffer containing Tris-HCl (5 mmol/L, pH 7.5), glycerol (10%), EDTA and EGTA (0.5 mmol/L each), dithiothreitol (2 mmol/L), phenylmethylsulfonyl fluoride (0.2 mmol/L), protease inhibitor cocktail (1%) and Triton X-100 (0.1%), and then centrifuged for 20 min at 12,000 g at 4 °C (all reagents from Sigma-Aldrich, Merck KGaA, Germany). The supernatants were collected, and protein concentration was assayed using the Pierce bicinchoninic method (BCA protein assay kit, Thermo Fisher Scientific Inc., USA). The protein samples were denatured in Laemmli buffer (95 °C for 7 min), and then were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis in volumes calculated for 100 mg of protein, using 7.5% and 5.0% acrylamide for the separating and stacking gels, respectively. The proteins were transferred onto a polyvinylidene difluoride membrane (Sigma-Aldrich, Merck KGaA, Germany). Blots were saturated for 30 min with Pierce Clear Milk Blocking Buffer (Thermo Fisher Scientific Inc., USA) and incubated overnight with rabbit polyclonal anti-SREBP-1 specific antibodies (sc-366, Santa Cruz Biotechnology, USA) in working dilution 1:500. After the washing with PBST buffer (Thermo Fisher Scientific Inc., USA), blots were treated for 1 h with peroxidase conjugated anti-rabbit IgG (A0545, Sigma-Aldrich, Merck KGaA, Germany) in working dilution 1:2000. After the washing with PBST buffer, positive bands were revealed using tetramethylbenzidine substrate with Pierce TMB-Blotting 1-Step Solution (Thermo Fisher Scientific Inc., USA). The intensity of the protein signal was determined using densitometric analysis with ImageJ (NIH Image, USA, 2022) and normalized to the parameters of the mature form of the protein in the control group.

Statistical analysis. The results were expressed as means ± standard error (x ± SE). The data were analyzed using GraphPad Prizm 8.0.2.263 (GraphPad Software Inc., USA, 2019). Two-way analysis of variance (ANOVA) with Tukey's post hoc test was used to detect significant differences between the groups in dynamics, one-way ANOVA with Tukey's post hoc test – to determine the significant differences within the same group. $P < 0.05$ was considered as statistically significant difference. Pearson correlation analysis was used to quantify the strength of the linear relationship between two variables, and describe the cause and effect of HFD or streptozotocin on glycemic status.

Results

Disturbances of carbohydrate metabolism in rats. To establish the development of T2D and PD in rats, the glycemic status and manifestations of insulin resistance were evaluated in the dynamics of the experiment. It was shown that after a 2-week HFD in rats, an increase in glycemia was observed ($P = 0.0322$), which remained stably elevated during the next 2 weeks of lipid overloading (Fig. 1). According to current criteria of American Diabetes Association (2020), the obtained indicators (over 6.0 mmol/L) can be considered transitional to the diabetic type (prediabetes). On the basis of results of two-way ANOVA, the HFD factor had the strongest effect on the rise of glycemic indicators ($F = 20.39$; $P = 0.0015$), although the influence of the time factor ($F = 3.868$; $P = 0.0358$) or their combination ($F = 3.409$; $P = 0.0317$) was also significant.

Among the animals with HFD and administration of streptozotocin, subgroups were selected according to the glycemic levels obtained in the dynamics of the experiment. A subgroup in which glycemia was from 10 mmol/L inclusive 2 days after administration of streptozotocin and 10–23 mmol/L after 2 weeks was characterized as experimental T2D (n = 16

and n = 18 with administration of 20 or 25 mg/kg of streptozotocin, respectively). The other subgroup, in which the glycemic levels after the administration of streptozotocin transiently increased to 7.0–9.9 mmol/L and subsequently remained at the level of 6.0–6.9 mmol/L, was assigned to experimental PD (n = 7 and n = 9 with the introduction of 20 or 25 mg/kg streptozotocin, respectively). It is noticed that 7.4% of experimental rats did not develop either PD or T2D in response to the influence of experimental factors.

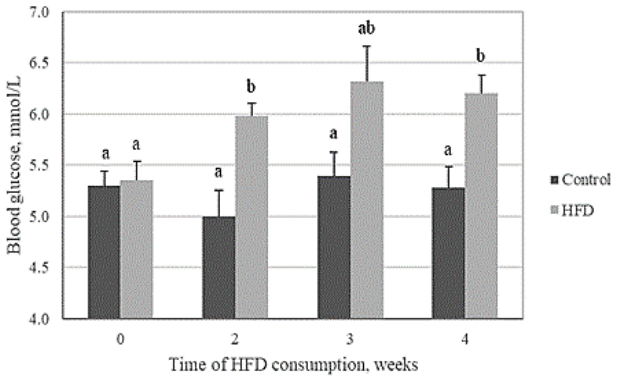

Fig. 1. The effects of high-fat diet on glucose homeostasis of rats in dynamics of the experiments: each graph represents the dynamics of glucose concentration (mmol/L) in blood samples taken from tails of rats fed normal chow (control, n = 10) or high-fat diet (HFD, n = 10) contained 58% fat in the total calories during 4 weeks of experiments; x ± SE; different letters indicate statistical samples that are significantly different from each other according to two-way ANOVA with Tukey's post hoc test results (P < 0.05)

The dose-dependent effect of streptozotocin was studied in order to distinguish pathological manifestations that depend on pancreatic β-cell dysfunction from those that are based on other pathogenetic factors. It was established that the indicated doses of 20 or 25 mg/kg caused the appearance of hyperglycemia in 2 days over 17 mmol/L in 33.3 (n = 8) vs 46.7% (n = 14) of rats of the respective subgroups, while animal mortality was not observed. The analysis of the data showed that with the increase in the dose from 20 to 25 mg/kg, the number of rats with high glycemia (over 17.0 mmol/L) was growing mainly due to a decrease in the number of animals with a glycemia level of 10.0–16.9 mmol/L. At the same time, the number of animals in which glycemia above 9.9 mmol/L was not observed remained constant (29.2–30.0%). These data make it possible to distinguish the last group of animals as relatively streptozotocin-tolerant, in which PD is detected, in contrast to other streptozotocin-sensitive rats, in which T2D manifests, which indicates the corresponding differences in the mechanisms of the development of the pathological process.

The correlation analysis confirmed this assumption. Thus, the dose of streptozotocin (0, 20, or 25 mg/kg) had a very slight effect on the level of glycemia in animals with prediabetes among rats given a HFD (Fig. 2, PD). At the same time, in animals that had T2D (Fig. 2, T2D), the dose of streptozotocin more significantly influenced the level of glycemia (r = 0.6328, P < 0.05).

Development of insulin resistance in rats fed HFD. The results of ITT showed that in control rats, glycemic indicators were lower 30 min after insulin administration (F = 23.17; P = 0.0429; Fig. 3, control). In contrast, animals after 2 weeks of HFD showed no insulin-induced decrease in blood glucose during 60 minutes of observation (F = 2.26; P = 0.1183; Fig. 3, HFD), which was a marked reduction in insulin sensitivity. Thus, the use of HFD for 2 weeks is a sufficient experimental period for the manifestations of insulin resistance.

When ITT was performed after 4 weeks of the experiment, it was found that in rats with HFD4, as well as with HFD and injection of streptozotocin, despite the level of glycemia, there was no significant response of blood glucose to insulin administration (P > 0.05), i.e., these animals can be characterized as insulin resistant (Fig. 4, HFD4, T2D, PD). In control rats, glycemic indicators showed the decrease 30 min after insulin administration (F = 8.037; P = 0.0496; Fig. 4, control). This confirms that the reproduced pathological processes may be characterized as insulin-independent (type 2) diabetes and prediabetes.

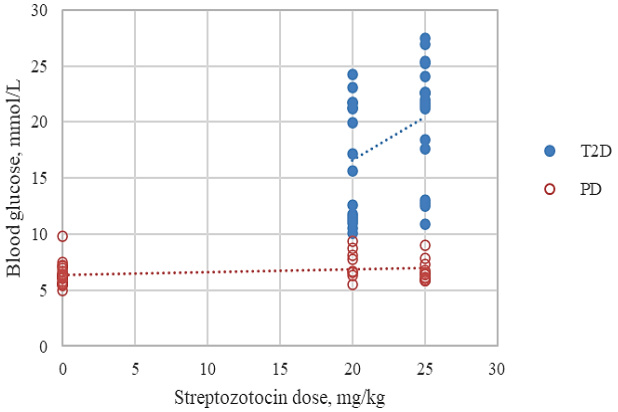

Fig. 2. Relationships between dose of streptozotocin (mg/kg) and blood glucose concentration (mmol/L) in rats fed a high-fat diet (HFD), which manifested type 2 diabetes (T2D, n = 34) or prediabetes (PD, n = 16): HFD contained 58% fat in the total calories; streptozotocin was injected intraperitoneally after 2 weeks of HFD at a dose of 20 mg/kg (n = 24) or 25 mg/kg (n = 30), and one group of rats fed HFD (n = 10) did not receive streptozotocin (0 mg/kg); the glucose concentration (mmol/L) was determined in peripheral blood on second day after streptozotocin administration; some rats (n = 4) did not develop T2D or PD after streptozotocin administration, and were excluded; the data from two separated experiments were analyzed by Pierson correlation analysis

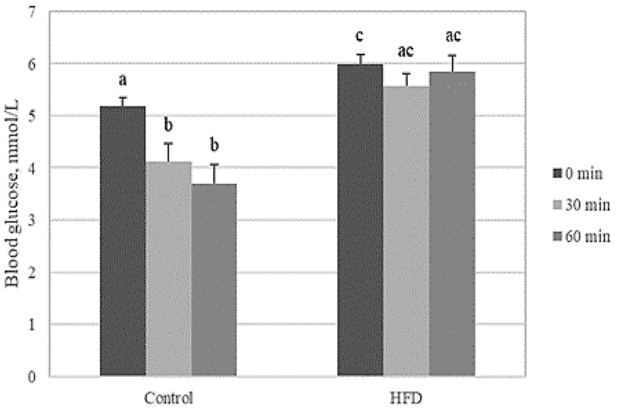

Fig. 3. The effects of high-fat diet for 2 weeks on insulin tolerance in rats: insulin tolerance test was performed on rats fed standard chow (control) or high-fat diet (HFD) containing 58% fat in the total calories; each bar chart group represents glucose concentration (mmol/L) in peripheral blood of 6 rats in each experimental group prior to (0 minutes) and 30 and 60 minutes after 0.5 IU/kg short-acting insulin administration; x ± SE; different letters indicate statistical samples that are significantly different from each other according to two-way ANOVA with Tukey's post hoc test results (P < 0.05)

Taken together, these results indicate that exposure to applied experimental factors reproduces the development of T2D and PD in rats as measured by glycemic profile and insulin resistance. At the same time, these pathological manifestations were caused by various mechanisms. The development of PD in animals was associated with lipid overload by HFD and manifestations of insulin resistance, while streptozotocin potentiated the development of T2D and dose-dependently affected its severity, but apparently only in animals prone to pancreatic β-cell dysfunction (60.0–66.6% of rats in corresponding groups).

Morphological manifestations of the development of experimental PD and T2D. In order to establish the mechanisms that can be associated with the ambiguous response of animals to the influence of experimental factors, a study of the development of tissue steatosis due to lipid loading was conducted. In the liver, significant differences were found in the sub-

groups of rats with PD and T2D. In rats with PD, fat droplets were often found in hepatocytes, the number of which indicated the initial stage of fatty degeneration of the liver (Fig. 5a). Areas of swelling and destruction in hepatocytes were also observed. In the liver of rats with T2D, the development of massive fatty degeneration with the formation of significant areas of tissue filled with lipid conglomerates was revealed (Fig. 5b). Mitochondria were often tightly attached to the fat droplets, which can probably be considered as an adaptation mechanism for the replenishment of high-energy substrate and plastic material in T2D. The obtained data indicate that the severity of carbohydrate metabolism disorder, which was manifested as PD or T2D after administration of streptozotocin, depended on the degree of hepatosteatosis when using HFD.

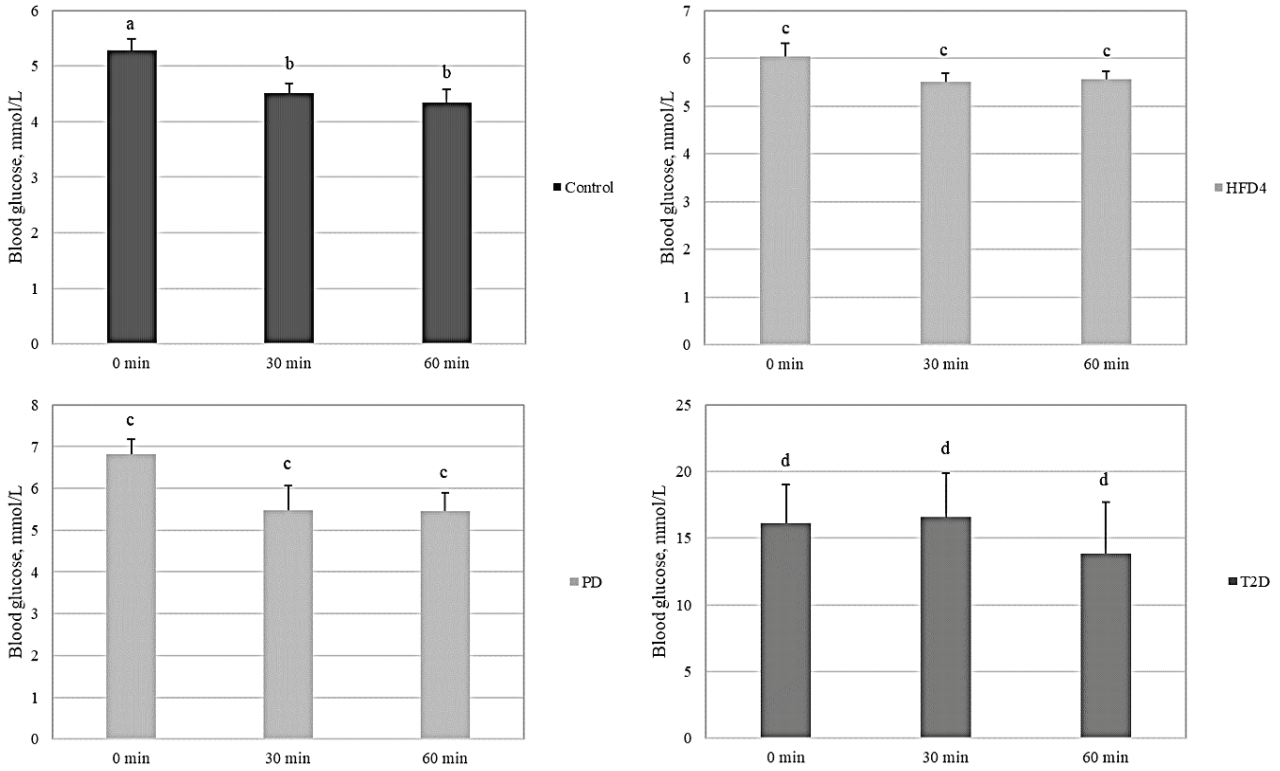

Fig. 4. Changes in insulin tolerance test indicators in rats after 4 weeks of high-fat diet with or without streptozotocin administration; the high-fat diet (HFD) contained 58% fat in the total calories, streptozotocin was injected intraperitoneally after 2 weeks of HFD: insulin tolerance test was performed in rats fed standard chow (control) or high-fat diet for 4 weeks (HFD4), and in rats with high-fat diet and streptozotocin administration, which developed type 2 diabetes (T2D) or prediabetes (PD); each bar chart group represents glucose concentration (mmol/L) in peripheral blood of 6 rats in each experimental group before and 30 and 60 min after 0.5 IU/kg short-acting insulin administration; x ± SE; different letters indicate statistical samples that are significantly different from each other according to one-way ANOVA with Tukey's post hoc test results ($P < 0.05$)

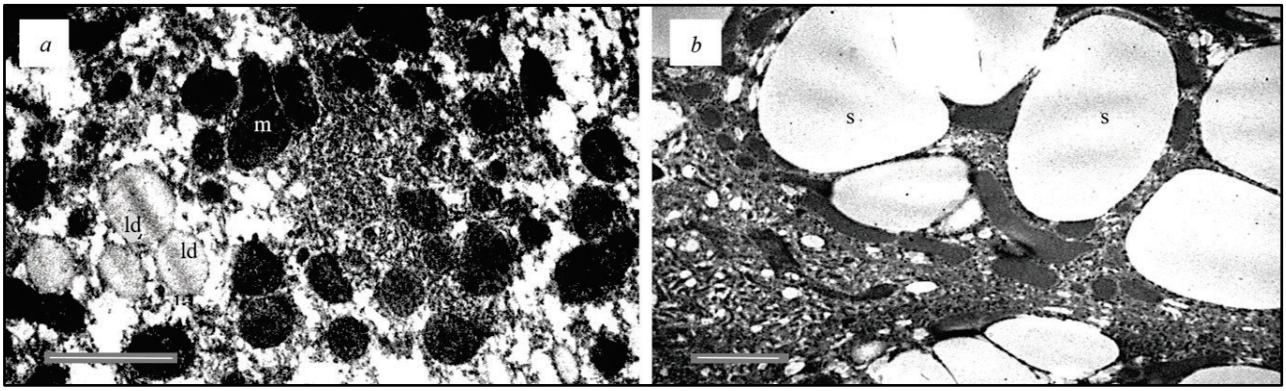

Fig. 5. Subcellular signs of liver steatosis in rats with prediabetes (*a*) and type 2 diabetes (*b*): the high-fat diet (HFD) contained 58% fat in the total calories, streptozotocin was injected intraperitoneally after 2 weeks of HFD; the liver tissue samples removed from anesthetized rats fed a high-fat diet with streptozotocin administration, which developed type 2 diabetes or prediabetes, were examined by electron microscopy after 4 weeks of experiments; scale bar – 1 μm; *ld* – lipid droplets; *m* – mitochondria; *s* – steatosis

Mechanisms underlying development of experimental PD and T2D. Changes in the expression of the transcription factor SREBP-1 were considered as a mechanism that supports the accumulation of lipids in tissues. It was found that the use of HFD4 led to increased processing of this protein and raised expression of its mature nuclear fraction of 68 kDa compared to control rats (Fig. 6, HFD4, control). In rats with T2D, the maturation of the SREBP-1 protein was clearly inhibited compared to the group of HFD4, at the same time in animals with PD there was only a tendency to decrease (Fig. 6, T2D, PD).

Discussion

Metabolic effects of lipid overload in human and animals. Results obtained in this research indicate that 1) dietary lipid overload for 2 weeks is sufficient for the development of insulin resistance and manifestations of prediabetes in rats; 2) the proportion of rats with PD or T2D in the experimental groups did not depend on the dose of streptozotocin, but the latter dose-dependently increased the severity of T2D; 3) the development of T2D in rats was associated with more pronounced lipid infiltration of the

liver than in the group with PD, and was accompanied by differences in the maturation of the SREBP-1 protein.

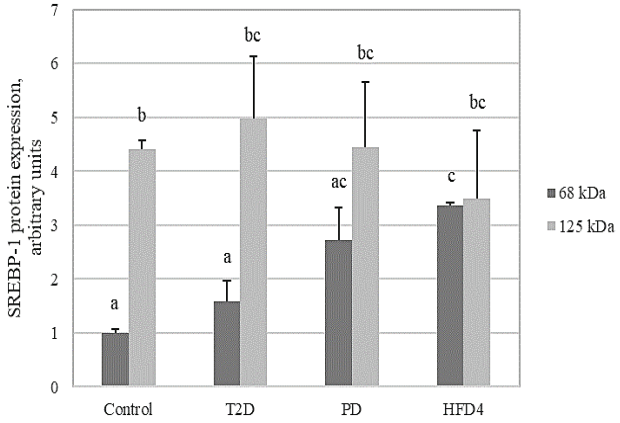

Fig. 6. Changes in the expression of precursor (125 kDa) and mature form (68 kDa) of SREBP-1 protein in liver of rats after 4 weeks of high-fat diet with or without streptozotocin administration: the high-fat diet (HFD) contained 58% fat in the total calories, streptozotocin was injected intraperitoneally after 2 weeks of HFD; the SREBP-1 protein expression was assayed by immunoblotting in liver tissue samples removed from anesthetized rats fed normal chow (control) or high-fat diet for 4 weeks (HFD4), and from rats exposed to high-fat diet and streptozotocin administration, which developed type 2 diabetes (T2D) or prediabetes (PD); the densitometric values of expression of the precursor and the mature form of the SREBP-1 protein have been normalized to the values of mature form of the protein in control group; x ± SE; different letters indicate statistical samples that are significantly different from each other according to two-way ANOVA with Tukey's post hoc test results ($P < 0.05$)

Dietary lipid overload can significantly affect energy metabolism and cell structure. Along with this, dietary influences can regulate gene expression through activation of transcription factors by signaling cascades or direct ligand binding to nuclear receptors, epigenetic mechanisms, and non-coding RNA (Bravo-Ruiz et al., 2021; Mierziak et al., 2021). Due to this combined effect, the development of insulin resistance may occur in rats with lipid overloading, which various researchers have shown after 2 or 4 weeks of HFD consumption (Wang et al., 2011; Mansor et al., 2013). This is consistent with our results, but there is no evidence of earlier symptoms of insulin resistance. It should be noted that longer use of HFD is also accompanied by the development of obesity (Krasova et al., 2019). Despite the fact that in clinical medicine, obesity is considered the main cause of the development of insulin resistance, the interaction of these pathogenetic components of the metabolic syndrome currently appears to be more complex and is still the subject of discussion (Tanase et al., 2020; Gołacki et al., 2022). Experimental results obtained by us and other researchers (Mansor et al., 2013; Tanase et al., 2020) indicate that insulin insensitivity can develop as a separate symptom of the metabolic syndrome and earlier than obesity. Since clinical observations usually do not cover the early, initial period of disease development, these experimental data provide new insight into the pathogenesis of the metabolic syndrome, in particular, in relation to the development of insulin resistance. At the same time, the presence of obesity certainly potentiates and maintains the state of insulin insensitivity (Tanase et al., 2020; Gołacki et al., 2022). Thus, lipid overload reduces insulin sensitivity, which in humans reveals a genetic predisposition to T2D in the presence of relevant gene polymorphisms that are responsible for the regulation of insulin synthesis and secretion (Kwak & Park, 2016; Krentz & Gloyn, 2020).

There is no such information regarding laboratory, farm and domestic animals. In experimental rats, the development of insensitivity to insulin was accompanied by a moderate increase in fasting blood glucose (by approximately 1 mmol/L in our experiment). This manifestation of insulin resistance and prediabetes, confirmed by our results and the data of other researchers (Reed et al., 2000; Srinivasan et al., 2005; Wang et al., 2011; Mansor et al., 2013; Gilor et al., 2016), may be primarily associated with lipid overload in both rodents and larger mammals. The development of insulin resistance before the appearance of significant obesity has also been shown in other models, in particular, in guinea pigs with the administration of protamine sulfate under the conditions of a high-carbohydrate diet (Rushchak et al., 2012). In view of these and other evidences, it may be considered that insulin resistance can be an early manifestation of the metabolic syndrome, which can be supported by the subsequent development of obesity with the formation of a "vicious circle" (Tanase et al., 2020; Gołacki et al., 2022).

Predisposition to type 2 diabetes or prediabetes, and steatosis. According to accumulated data, two groups of genetic polymorphisms are responsible for the development of T2D in humans, one of which is associated with a predisposition to insulin resistance, and the other – with a susceptibility to pancreatic β-cell dysfunction (Herder & Roden, 2011; Kwak & Park, 2016; Krentz & Gloyn, 2020). We found that experimental pancreatic dysfunction due to its moderate damage by streptozotocin dose-dependently increases the severity of experimental T2D. These data are also comparable with the results of other researchers (Wang et al., 2011; Mansor et al., 2013). Therefore, the parameters of lipid overload and the dose of streptozotocin can independently determine the effect of modeling T2D, which confirms the existence of separate pathogenic mechanisms or groups of mechanisms that mediate the development of this pathology in both humans and animals.

However, the question of the difference in the response to these influencing factors – the development of T2D or only PD – has not yet been resolved in any way. Our experimental results indicate that, under the influence of the same pathogenic factors, the development of T2D was associated with a greater individual susceptibility to lipid infiltration of the liver, while the development of PD was associated with a lower severity of this process. At the same time, at the initial stages of the development of T2D in the experiment, there was a reverse inhibitory regulation of SREBP-1 activity, which, however, was not effective enough to compensate pathological structural disorders (see Fig. 6). This corresponds to the data that in cultured hepatocytes and in the liver of rats with experimental T2D, steatosis was correlated with a compensatory decrease in SREBP-1c and PPARα gene expression (Ziamajidi et al., 2013).

In long-term type 2 diabetes, this regulatory relationship is disrupted, as clinical observations have shown (Pérez-Belmonte et al., 2017). Therewith, the lack of inhibition of SREBP-1 processing in rats with PD apparently indicates an initial, compensated stage of the metabolic disorder in contrast to the decompensated stage in patients with long-term T2D.

In patients with T2D, steatosis was shown to be associated with insulin resistance (Schmid et al., 2011). Despite the view that T2D is the cause or risk factor for the development of steatosis (Younossi et al., 2019; Tanase et al., 2020), our data indicate that steatosis may develop before the manifestation of carbohydrate metabolism disorders and be a prerequisite for the subsequent development of such disorders. Thus, in HFD, lipid infiltration of tissues can be the primary link that leads to disorders of cellular carbohydrate metabolism, although hereafter these pathways are mutually potentiated.

SREBP-mediated ways of metabolic disturbances. The molecular mechanisms that are responsible for differences in the development of prediabetes or T2D under the influence of the same factors can be multiple. Among them, the pathways associated with changes in the expression of SREBP proteins were of interest to us. The reasons for such changes could be: 1) stimulation of expression/binding of LXR receptors, especially LXRα with dominant expression in the liver, activation of PI3K/AKT/mTORC1/p70S6K signal ways and, as a result, increased expression of the SREBP-1 precursor; 2) enhancement of SREBP-1 maturation due to insulin-induced depletion of INSIG protein expression, and 3) reduction of proteasomal degradation of both precursor and mature SREBP-1c protein due to insulin-induced phosphorylation of serine 418, serine 419, and serine 422 (Yellaturu et al., 2009; Owen et al., 2012; Bravo-Ruiz et al., 2021).

In our experiment, a marked increase in the expression of the SREBP-1 precursor was not observed. At the same time, as a result of HFD4, there was a strong increase in the maturation of SREBP-1, therefore, the development of insulin resistance could promote the INSIG-dependent cleavage of the SREBP-1 protein precursor and limit its proteasomal degradation, which led to an increase in lipogenesis and fat infiltra-

tion of the liver. Changes in the maturation of SREBP-1 with the use of HFD confirm that insulin resistance in rats revealed β-cell dysfunction, which closely approximates the mechanisms of experimental T2D to its main pathways in humans (Leahy, 2008; Gilor et al., 2016).

It should be noted that glucose can be an insulin-independent factor in the regulation of lipogenesis, which is important in the early stages of the development of metabolic disorders, when changes in insulinemia are not yet observed (Wang et al., 2011; Mansor et al., 2013). With the increase of glucose transport into the cell through alternative receptors (in particular, EGFR), N-glycosylation of the cleavage-activating protein (SCAP) of the SREBP protein occurs, which may be the cause of increased maturation of SREBP-1 and lipogenesis (Cheng et al., 2015), however, it is not known in detail how this mechanism functions in conditions of insulin resistance. Despite this, changes in the expression and maturation of the SREBP protein seem to be one of the main factors that can mediate the prerequisites for the development of PD, T2D and the progression of this disease against the background of lipid infiltration of tissues.

Further, SREBP-1 maturation was significantly lower in T2D rats, but remained high in PD rats (similar to HFD4, Fig. 6). This may indicate that in the first subgroup a reversible sterol-dependent inhibition of SREBP-1 maturation was observed due to significant lipid infiltration of tissues (Ye & DeBose-Boyd, 2011), whereas in the second one this process was much less rapid and intense. This mechanism, which is insulin- and glucose-dependent (Bravo-Ruiz et al., 2021), may be the basis for differences in the development of PD or T2D in rats under the influence of the same factors, as well as determine the severity of the course of T2D in humans, which is based on the presence genetic predisposition to pancreatic β-cell dysfunction. Interestingly, the total expression of the precursor and mature form of SREBP-1 protein was not significantly different between groups, but tended to increase in rats with HFD4 and especially with PD. This may indicate that the proteasomal degradation of both forms of SREBP-1 was also somewhat limited under HFD intake and PD, but this was not observed in T2D rats.

Why do not all rats or patients with lipid overload get type 2 diabetes? Taken together, the data obtained may indicate that lipid overload causes insulin resistance associated with a lack of insulin receptors leading to changes in SCAP/INSIG-dependent regulation, increased maturation of the SREBP-1 protein, limitation of its proteasomal degradation, and increased lipogenesis. In rats with impaired insulin-dependent regulatory links, this process leads to rapid accumulation of lipids in tissues (steatosis), and additional damage to β-cells by streptozotocin causes more severe T2D. At the initial stages of the disease development, there is a feedback-regulatory inhibition of the maturation of SREBP-1, which limits further lipogenesis; however, this does not lead to compensation of disturbances of carbohydrate metabolism. In other rats, not prone to dysfunction of insulin-dependent regulatory links, insulin resistance causes less intense lipogenesis and lipid infiltration of tissues. Against this background, experimental damage to β-cells does not cause the development of T2D, and hyperglycemia is compensated at the level of prediabetes.

According to clinical observations, long-term T2D may be accompanied by further dysregulation of lipid metabolism with overexpression of SREBP proteins, which are recognized as risk factors for cardiovascular and metabolic complications (Pérez-Belmonte et al., 2017). Therefore, SREBP proteins can be a promising target both for establishing the risk of development and prognosis of T2D in patients with insulin resistance, and for pharmacological influence.

Thus, the data obtained may indicate that the development of T2D in rodents, as well as in humans, is based on two main pathogenetic ways: 1) insulin resistance, which can be caused by the influence of environmental factors (including HFD), leads to an increased load on insulin-dependent mechanisms of lipid metabolism regulation and can reveal pancreatic β-cell dysfunction and manifestations of prediabetes, and 2) genetic predisposition to such β-cell dysfunction, which serves as a risk factor for the development of T2D and potentiates the severity of this disease in both animals and humans. The degree of steatosis and the associated level of expression and maturation of the SREBP-1 protein may determine the development of PD or T2D under the same factors of influence in experimental animals and serve as a prognostic criterion in the clinic.

Conclusion

The consumption of a high-fat diet for 2 weeks is sufficient for the development of insulin resistance and prediabetes in adult rats; these manifestations were effectively supported by lipid overload during the next 2 weeks of the experiment.

The degree of hepatic lipid infiltration and changes in the regulation of the transcription factor SREBP-1 are risk factors associated with the development of either type 2 diabetes mellitus or prediabetes in experimental rats treated with streptozotocin against the background of lipid loading. Alterations in SREBP-1 maturation upon high-fat diet may indicate that insulin resistance in rats reveals β-cell dysfunction, thereby linking the development of experimental type 2 diabetes to major pathways in humans. In turn, for both large and small mammals, the susceptibility to β-cell dysfunction may be a prerequisite that limits compensatory reserves of carbohydrate and lipid homeostasis under lipid (or other metabolic) overload.

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