ВЛИЯНИЕ КОНТРОЛИРУЕМОГО ПЕРЕХОДА ОТ ГИПЕРГЛИКЕМИИ ДО ГИПОГЛИКЕМИИ НА АГРЕГАЦИЮ ТРОМБОЦИТОВ И АКТИВНОСТЬ ФИЗИОЛОГИЧЕСКИХ АНТИКОАГУЛЯНТОВ И ФАКТОРА ВИЛЛЕБРАНДА У БОЛЬНЫХ САХАРНЫМ ДИАБЕТОМ 1 ТИПА

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ОБОСНОВАНИЕ. Гипогликемия у больных сахарным диабетом 1 типа (СД1) вследствие коррекции гипергликемии может являться фактором риска развития сердечно-сосудистых и цереброваскулярных осложнений. Одной из причин этих осложнений может быть активация тромбоцитарного и плазменного звена гемостаза при недостаточности физиологических антикоагулянтов.

ЦЕЛЬ. Оценка влияния контролируемого перехода от гипергликемии к эугликемии и затем к гипогликемии на индуцированную агрегацию тромбоцитов, активность физиологических антикоагулянтов и фактора Виллебранда у пациентов с СД1 без макро- и микрососудистых осложнений.

МЕТОДЫ. Обследовано 11 пациентов с СД1: 6 мужчин и 5 женщин (возраст 23,7±5,6 лет, длительность СД 11,7±2,2 года; уровень HbA_{1c} 9,12±2,19%). Показатели индуцированной агрегации тромбоцитов, физиологические антикоагулянты (протеин S, протеин С, АТ III) и фактор Виллебранда (фВб) были изучены в ходе гиперинсулинемического (1 мЕд/кг/мин) гипогликемического клэмпа. Статистическая обработка данных проводилась с использованием пакета программы SPSS 22.0.

РЕЗУЛЬТАТЫ. В период гипогликемии агрегация тромбоцитов на индукторы повышалась по сравнению с показателями на фоне гипергликемии и эугликемии, при этом на этапе эугликемии достоверной активации тромбоцитов не отмечалось, а повышение агрегации наблюдалось только при гипогликемии. Повышение агрегационной активности на фоне гипогликемии от исходной гипергликемии составило 23,9% для тромбина, 30,6% и 30,9% – для АДФ и арахидоновой кислоты и 69,4% и 70,8% – для коллагена и ристоцетина. При этом агрегация на коллаген, АДФ и арахидоновую кислоту оставалась в пределах верхних границ нормы, агрегация на тромбин превышала верхние границы нормы, а агрегация на ристоцетин оставалась достоверно ниже нижней границы нормы. Активность протеина S была выше в условиях гипогликемии по сравнению с эугликемией (p=0,046) и гипергликемией (p=0,046). Концентрация АТ-III на фоне гипергликемии была значительно выше нормы, затем достоверно снижалась при достижении эугликемии и сохранялась на этом уровне при гипогликемии (достоверно выше верхней границы нормы). Активность протеина С и фВб не менялись достоверно при переходе от гипергликемии к эугликемии и к гипогликемии.

ЗАКЛЮЧЕНИЕ. У пациентов с СД1 контролируемый переход от гипергликемии к эугликемии и затем к гипогликемии сопровождается достоверным повышением агрегации тромбоцитов и увеличением активности протеина S. Основное значение в повышении активности тромбоцитов имело быстрое развитие гипогликемии, а не собственно процесс снижения уровня глюкозы. Повышение активности свободного протеина S является компенсаторной реакцией, нивелирующей повышенную агрегацию тромбоцитов.

КЛЮЧЕВЫЕ СЛОВА: сахарный диабет 1 типа; гемостаз; агрегация тромбоцитов; физиологические антикоагулянты; фактор Виллебранда; гиперинсулинемический гипогликемический клэмп

INFLUENCE OF HYPERINSULINEMIC – HYPOGLYCEMIC CLAMP ON INDUCED PLATELET AGGREGATION, ACTIVITY OF PHYSIOLOGICAL ANTICOAGULANTS AND VON WILLEBRAND FACTOR IN PATIENTS WITH TYPE I DIABETES

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BACKGROUND. Intensive glycaemic control in patients with type 1 diabetes may lead to hypoglycaemia and thus increase the risk of cardiovascular and cerebrovascular events. Platelet activation and/or decreased activity of physiological anticoagulants during hypoglycaemia may play a role in the development of cardiovascular or cerebrovascular complications.

AIMS. To investigate induced platelet activity, the activity of physiological anticoagulants, and the von Wil-lebrand factor in patients with type 1 diabetes with the hyperinsulinaemic–hypoglycaemic clamp.

MATERIALS AND METHODS. We examined 11 patients with type 1 diabetes without macro- and micro-vascular complications (6 males, 5 females, mean age 23.7 ± 5.6 years, A1C 9.7 ± 2.3%). Induced platelet aggregation, physiological anticoagulants (Protein S, Protein C, AT III) and the von Willebrand factor were studied at hyperglycaemic, euglycaemic, and hypoglycaemic stages during use of a hyperinsulinaemic (1 mU/kg/min) hypoglycaemic clamp.

RESULTS. Platelet aggregation to all agonists increased significantly during the hypoglycaemic stage, compared with the euglycaemic or hyperglycaemic stages. There was no difference in platelet aggregation between the euglycaemic and hyperglycaemic stages. Platelet aggregation to all agonists increased during the hypoglycaemic stage compared with the hyperglycaemic period: thrombin–23.9%, ADP–30.6%, arachidonic acid–30.9%, collagen–69.4% and ristocetin–70.8%. During hypoglycaemia aggregation to ADP, arachidonic acid and collagen remained within normal limits (upper quartile); aggregation to thrombin was significantly above normal limits and aggregation to ristocetin remained significantly below lower limits. Protein S activity was significantly increased during hypoglycaemia compared with euglycaemia ($p = 0.046$) and hyperglycaemia ($p = 0.046$). Antithrombin-III activity decreased significantly at the euglycaemic and hypoglycaemic stages, compared with the hyperglycaemic period, but still remained significantly elevated above the upper threshold. Protein C and vWf activity did not change significantly.

CONCLUSIONS. In patients with type 1 diabetes platelet aggregation and protein S activity increases significantly at the hypoglycaemic stage of the hyperinsulinaemic–hypoglycaemic clamp. Platelet activation is directly caused by hypoglycaemia and not by decreasing glucose levels. Increased protein S activity is a compensatory response to platelet activation.

KEYWORDS: type 1 diabetes; hemostasis; induced platelet aggregation; physiological anticoagulants; von Willebrand factor; hyperinsulinemic hypoglycemic clamp

For patients with type 1 diabetes mellitus (DM1), the development of vascular disorders begins with hemostasis and endothelial dysfunction [1, 2]. These patients have complicated, sometimes multidirectional, challenges representing the imbalance of platelet and plasma hemostasis, as well as physiological anticoagulants [3, 4, 5]. Glucose levels and drug induced carbohydrate metabolism can also influence hemostasis [6, 7]. A recent investigation into the dynamics of endothelial dysfunction markers, local inflammatory processes, and plasma hemostasis has demonstrated a direct relationship between the duration diabetes and the number of disease related events [8]. In DM1 patients, changes in the content of physiological coagulants, primarily protein S and protein C, are noted to correlate with the duration of the disease and the presence and severity of complications [9, 10]. Changes in platelet hemostasis are also typical [11]. In general, a procoagulative shift is observed [5], manifested by the presence of activated platelet microparticles (membrane fragments) [12, 13] in addition to an increase in mean volume dispersion, platelet heterogeneity and the number of large-sized platelets [14].

Episodes of hypoglycaemia may accompany intensified insulin therapy in DM1 patients. Repeated episodes of hypoglycaemia can lead to a worsening of endothelium-dependent vasodilation and to even more rapid changes within the intima-media complex. Observing the changes within the intima-media complex enables investigation into recurrent hypoglycaemia as a possible factor related to accelerated development of an atherosclerotic lesion in the vascular bed [15]. Single and repeated episodes of hypoglycaemia, both in DM1 patients and in healthy subjects, lead to an increase in the concentration of vascular and intercellular adhesion molecules, inhibitors of the plasminogen activator and vascular endothelial growth factor in the blood [16, 17]. Hypoglycaemia for healthy individuals and DM1 patients has a diverse effect on thrombocyte homeostasis. Thus, in healthy individuals, a decrease in blood glucose levels is accompanied by an increase in intra-platelet calcium, the destruction of the mitochondria [18] and platelet aggregation [19]. Acute hypoglycaemia (1.7–2.9 mmol/L in healthy subjects) is accompanied by an increased sensitivity of platelets to proaggregants and the release response [20]. Hypoglycaemia also increases the sensitivity of platelets to the pro-apoptotic molecule BH3I-20, which triggers the signal pathway of apoptosis in the mitochondria and causes cell death. Cell death events increase intravascular coagulation and damage to the vascular wall [18]. The increase in platelet aggregation in hypoglycaemia has been shown to be associated with the reaction to stress, activation of the sympathetic-adrenal system and release of catecholamines [21]. When this happens, platelet α-adrenoreceptors, under the influence of catecholamines, sensitise other platelet receptors and prepare them for active interaction with the corresponding agonists [22].

AIM

This study aimed to assess the effect of a controlled transition from hyperglycaemia to euglycaemia and onto hypoglycaemia by measuring induced platelet aggregation, the activity of physiological anticoagulants and the concentration of von Willebrand factor (vWF)

in DM1 patients without macro- or micro-vascular complications.

METHODS AND MATERIALS

Design of the study

Eleven DM1 patients were examined: 6 men and 5 women aged 23.7 ± 5.6 years. Patients experienced DM for 11.7 ± 2.3 years without macro- or micro-vascular complications (Table 1). All patients were informed about the details of this study and voluntarily consented to participate.

Tacceptance criteria

Patients were excluded from the study, if they had any of the following signs, symptoms, or history:

1. Diabetic retinopathy detected during ophthalmoscopy of the fundus under conditions of mydriasis;
2. Reduced glomerular filtration rate (<60 ml/min/1.73 m2) or the presence of microalbuminuria (>20 mg/l) in the morning's first urine;
3. A history of cardiovascular events including myocardial infarction and acute cerebrovascular disorder, according to anamnesis or medical documentation;
4. Impairment of consciousness during hypoglycaemia, or a previous serious reaction to hypoglycaemia;
5. Craniocerebral injury;
6. Abuse of alcohol or narcotic drugs;
7. Thrombocytopathy and coagulopathy;
8. Diseases of the liver with a history of its dysfunction;
9. Intake of antiaggregants, anticoagulants and oral contraceptives.

Glycated haemoglobin (HbA_{1c}) was detected in capillary blood using high-performance liquid cation chromatography on the 'Dia Stat' analyser (Bio-Rad, Germany). The level of urinary albumin excretion was measured with immunoturbidimetry on the biochemical analyser 'HITACHI 912' (Roche) using Tina-Quant A ALBUMIN diagnostic kits.

Table 1. Clinical characteristics of the patients

Characterisitics	Values (M ± SD)
Number of patients	11
Men/women	6/5
Age, years	23.7±5.6
Body mass index, kg/m^2	26.4±4.4
Duration of DM1, years	11.7±2.3
HbA1c level, %	9.12±2.19
Creatinine level, μmol/l	73.5±5.7
GFR (EPI), ml/min/1.73 m^2	115.7±10.0
ALT, U/L	18.3±9.7
AST, U/L	14.0±6.0
Total cholesterol, mmol/l	4.5±0.74
LDL, mmol/L	2.8±0.54
HDL, mmol/l	1.4±0.3
TG, mmol/L	0.95±0.54
Microalbuminuria, mg/l	13.8±8.6

Protocol of hyperinsulinemic hypoglycaemic clamp

Patients were monitored and controlled during the transition from hyperglycaemia to euglycaemia and onto hypoglycaemia, with blood collections at the end of each stage. Patients were divided into two subgroups depending on insulin therapy type: 1) Patients receiving intensified insulin therapy via the basal-bolus regimen, repeated daily subcutaneous injections using individual pen injectors, or 2) Patients continuously receiving subcutaneous administration of fast-acting insulin via an insulin pump. In the first subgroup, hyperglycaemia was achieved by decreasing the dose of prolonged insulin before bedtime. In the second subgroup, hyperglycaemia was achieved by lowering the basal rate of insulin administration by 50% four h before the procedure.

The study began at 9:00AM with a hyperinsulinemic hypoglycaemic clamp performed on patients fasting for 8–12 h. Controlled glycaemia was achieved by the simultaneous administration of fast-acting genetically engineered human insulin with hyperinsulinemic speed (1 mU/kg/min) and 20% glucose solution. Some patients required a correction of hyperglycaemia; for them, short-acting insulin was administered intravenously with additional bolus dosing (MDC 3-8U). Glycaemia was monitored every 5 min during euglycaemia and every 3–5 min during hypoglycaemia with an assessment tool for the clinical state of patients that included hypoglycaemia tolerability measures.

Blood samples were taken from the ulnar vein of patients and deposited into a microcuvette for the HemoCue 201+ glucose analyser to monitor glucose levels. The first blood sample was taken at initial hyperglycaemia (15.0 ± 2.9 mmol/l), a second when euglycaemia (glucose 4.3 ± 0.3 mmol/L) was achieved after 70100 min and maintained for 20–25 min, and the third taken after another 20–30 min when hypoglycaemia was achieved (glucose 2.4 ± 0.2 mmol/l) and sustained for 20–30 min, depending on tolerability.

The analysis of hemostasis included investigation into induced platelet aggregation, the activity of C and S proteins, and the concentration of antithrombin III and vWF.

Induced aggregation of platelets

Blood samples were taken in vacuum tubes for platelet aggregation analysis,. The anticoagulant hirudin, at a concentration of 15 μg/ml (Verum Diagnostica GmbH, Germany), was used. Blood samples were examined within 20 min after collection. In vitro impedance aggregometry of whole blood using the 5-channel semi-automatic Multiplate system (Verum Diagnostica GmbH, Germany) and reagents from Dynabyte GmbH, Germany was performed. Platelet aggregation was observed in response to collagen (COLtest), thrombin (TRAPtest), adenosine diphosphate (ADPtest), ristocetin (RISTOtest) and arachidonic acid (ASPItest). The area under the aggregation curve was calculated (Figure 1) as the indicator most fully reflecting the platelet activity.

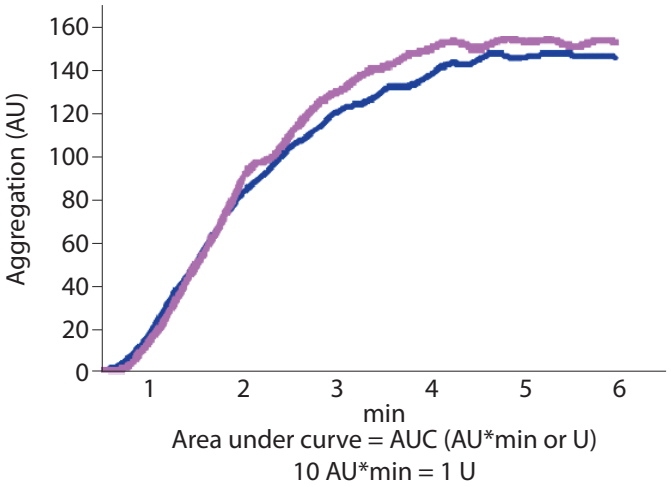

Fig. 1. Aggregation curve using the Multiplate system. Notes: AU – aggregation; AUC – area under curve

Anticoagulants and von willebrand factor

Blood samples were taken in vacuum tubes with 3.8% of sodium citrate. The blood was centrifuged at 2500 g for 15 min and plasma samples were stored at –20°C for up to 2 months. The concentration of anticoagulants and vWF were measured using enzyme immunoassay on the Multilabel Counter (Victor-2) analyser manufactured by PerkinElmer, USA and calculated using MultiCalc software.

Duration of the study

The work was completed in 2017.

Ethical review

Local Committee on Ethics of I.M. Sechenov First Moscow State Medical University. Extract from the minutes No. 04-15 of a meeting of the local committee on ethics dated 15.04.2015.

Statistical analysis

The data was analysed using SPSS v22.0 for Windows. Descriptive statistics are presented as mean and standard deviation (M ± SD), unless otherwise noted. To compare the results of quantitative indicators across three or more related groups, a nonparametric analysis of variance (Friedman test) was used with further pairwise comparison of the groups using the nonparametric Wilcoxon test (W-cr). The critical level of significance (p) was less than 0.05.

RESULTS

Aggregation of platelets

Aggregation with collagen (**COL*test***) leads to the activation of phospholipase C followed by the secretion of thrombocytic granules and the synthesis of thromboxane A2. Amid hyperglycaemia, platelet aggregation to collagen was 36.6% [21.5; 69.2], which was significantly lower than normal (N = 46–117, p = 0.028). At the end of euglycaemia, no significant changes in platelet aggregation to collagen were observed compared to hyperglycaemia (p = 0.48). However, at the end of hypoglycaemia, aggregation to collagen was significantly increased compared with euglycaemia (p = 0.005) and hyperglycaemia (p = 0.005), as shown in Table 2.

Aggregation with thrombin (**TRAP*test***) reveals the potential ability of platelets to aggregate. The TRAP-6 protein activating the PAR-1 thrombin receptor is an aggregation inducer. Aggregation in response to thrombin in the state of hyperglycaemia was 110.5% [82.2, 139.0], but did not differ from normative indices (N = 84–128). In the analysis of thrombin aggregation during euglycaemia, there were no significant differences in comparison with hyperglycaemia (p = 0.17). However, at the end of the hypoglycaemia period, there was an increase in platelet aggregation compared with euglycaemia (p = 0.027) and hyperglycemia (p = 0.021).

The action of ADP (**ADP*test***) is mediated through binding to platelet receptor P2Y12. During hyperglycaemia, the platelet aggregation against ADP was 60.5% [44.7, 91.7], which was within the bottom quarter of the laboratory's normative indices (N = 57–113). At euglycaemia, the aggregation did not change significantly in comparison with hyperglycaemia. After achieving hypoglycaemia, an increase in aggregation was observed compared with euglycaemia (p = 0.015) and hyperglycaemia (p = 0.028). However, differences from the normative indicators were not achieved.

Aggregation in response to arachidonic acid (**ASPI*test***) is accompanied with the activation of phospholipase C and followed by the subsequent formation of secondary mediators, the mobilisation of intracellular Ca^{2+}, and release of endogenous arachidonic acid. In the state of hyperglycaemia aggregation was 68.0% [48.5; 94.0], significantly lower than the normative indices (N = 71–115, p = 0.031). The end of the euglycaemic period

Table 2. Induced platelet aggregation (%)

Inducer	Hyperglycaemia (15.0 ± 2.9 mmol/L)	Euglycaemia (4.3 ± 0.3 mmol/L)	Hypoglycaemia (2.4 ± 0.2 mmol/L)
Collagen	36.6 [21.5;69.2]	34.5 [21.2;106.2]	62.0* [31.2;121.2]
Thrombin	110.5 [82.2;139.0]	108.0 [88.7;116.7]	137.0** [92.0;147.5]
ADP	60.5 [44.7;91.7]	53.0 [25.0;81.5]	79.0*** [50.7;108.2]
Arachidonic acid	68.0 [48.5;94.0]	64.5 [44.0;137.7]	89.0**** [58.2;134.7]
Ristocetin	24.0 [11.5;50.0]	36.0 [5.0;57.0]	41.0**** [15.5;74.0]

Notes: Aggregation to collagen:$p_{1-3} = 0.005$*;$p_{2-3} = 0.005$*; $p_{1-2} = 0.475$; Aggregation to thrombin: $p_{1-3} = 0.047$**; $p_{2-3} = 0.007$**; $p_{1-2} = 0.169$; Aggregation to ADP: $p_{2-3} = 0.028$***; $p_{1-2} = 0.126$; $p_{1-3} = 0.139$; Aggregation to arachidonic acid: $p_{1-3} = 0.017$****;$p_{2-3} = 0.168$; $p_{1-2} = 0.284$; Aggregation to ristocetin: $p_{1-3} = 0.042$****; $p_{2-3} = 0.628$; $p_{1-2} = 0.241$

Table 3. The level of physiological anticoagulants and von Willebrand factor

	Hyperglycemia (15.0 ± 2.9 mmol/L)	Euglycemia (4.3 ± 0.3 mmol/L)	Hypoglycaemia (2.4 ± 0.2 mmol/L)
Protein C (%)	101.2 [82.8;117.4]	102.9 [83.4;119.7]	103.7 [84.2;130.8]
Protein S (%)	76.3 [61.0;84.5]	77.6 [52.6;90.2]	93.6# [79.2;103.4]
At III (μg/L)	493.3 [291.6;705.3]	414.9* [308.0; 631.2]	426.9* [253.2;574.4]
vWF (U/ml)	0.56 [0.21;0.90]	0.61 [0.26;0.87]	0.49 [0.38;0.74]

Notes: protein S: $p_{1-3} = 0.046^{\#}$; $p_{2-3} = 0.046^{\#}$; antithrombin-III: $p_{1-3} = 0.049^{*}$; $p_{2-}3 = 0.047\#$

and hyperglycaemia measurements were not different (p = 0.24). However, the end of the hypoglycaemia period demonstrated increased aggregation of platelets compared with euglycaemia (p = 0.028) and hyperglycemia (p = 0.037).

Aggregation with ristocetin (**RISTO*test***) causes vWF- and GpIb-dependent platelet aggregation. Throughout hyperglycaemia, the aggregation rates for ristocetin amounted to 24.0% [11.5, 50.0] and were significantly lower than normative values (N = 98–180, p = 0.0029). Euglycaemia had no aggregation differences from hyperglycaemia. When hypoglycaemia was achieved, differences with hyperglycaemia became significant. However, the aggregation indices for ristocetin remained significantly below normal.

Physiological anticoagulants

Protein S is a vitamin K-dependent glycoprotein with a mass of 70 kDa. It is mainly synthesised by hepatocytes, but endothelial cells, megakaryocytes and the Leydig cells in the testes can also produce it. Functionally active, the free protein S acts as a co-factor in the activation of protein C [23]. In addition, free protein S independently exhibits anticoagulant activity, slowing the activation of X factor [23]. As shown in Table 3, the activity of free protein S during hyperglycaemia was 76.3% [61.0, 84.5], which was within the limits of the normative indices (N = 60–150). At the end of the euglycaemia period, the activity of free protein S did not change compared to hyperglycaemia. However, at the end of hypoglycaemia, the activity of free protein S increased significantly in comparison with euglycaemia (p = 0.046) and hyperglycaemia (p = 0.046), exceeding the upper limit of the norm.

Protein C plays an important role in the process of protein activation in the blood's coagulation cascade. Its activated form, in connection with protein S, hydrolyses the factors Va and VIIIa on their associated phospholipids [24]. The activity of protein C during hyperglycaemia was 101.2% [82.8, 117.4] and did not differ from the normative indices (N = 70–130). Additionally, during euglycaemia and hypoglycaemia the activity of protein C did not change compared to the initial state and remained within the normative values.

Antithrombin III (AT-III) is a physiological anticoagulant and an inhibitor of all serine proteases (thrombin, factors IXa, Xa, XIIa, kallikrein, plasmin, urokinase) involved with blood coagulation [25]. Initially, the concentration of AT-III was 493.3 μg/l [291.6, 705.3] exceeding the upper limits of the norm by 70.3% (N - up to 290 μg/l, p = 0.0021). Euglycaemia and then hypoglycaemia concentrations decreased by 18%–20% compared with hyperglycaemia, but were still higher than the normative indices.

The von **Willebrand factor** is a carrier protein for factor VIII; forming complexes with it and preventing it from premature proteolytic cleavage [26]. Initially, the concentration of vWF amounted to 0.56 U/ml [0.21, 0.90], and was within the lower limit of normative indices (N = 0.5–1.5 U/ml). When euglycaemia and hypoglycaemia were achieved, vWF activity did not change in comparison with the initial state. It also remained within the lower limit of the normative indices. All data is shown in Table 3.

DISCUSSION

The study demonstrated that in DM1 patients without macro- or micro-vascular complications, a balanced reaction of hemostasis is observed in response to a controlled decrease in plasma glucose level.

During hyperglycaemic states the aggregation rates for the agonists included in this study were lower or within the limits of the norm. Thus indicating sufficient compensatory mechanisms. Physiological anticoagulants, protein S, protein C and vWF were also within normal limits. In contrast, antithrombin-III values significantly exceeded the upper limits of the norm (70.3%). The development of hypoglycaemia activated platelet hemostasis. Moreover, during hypoglycaemia, platelet aggregation rates in response to all agonists increased compared with the rates during hyperglycaemia and euglycaemia. Importantly, no activation of platelets was observed during euglycaemia, and an increase in aggregation rate was only observed in the hypoglycaemic state. Together, these results indicate the importance of increased platelet activity for the rapid development of a hypoglycaemic state, while deemphasising the reduction of glucose levels.

Compared to baseline, the aggregation rate increased during hypoglycaemia in response to thrombin (23.9%), ADP (30.6%), arachidonic acid (30.9%), collagen (69.4%) and ristocetin (70.8%). Aggregation in response to collagen, ADP and arachidonic acid remained within the upper limits of the norm, while aggregation to thrombin exceeded the upper limits, and aggregation to ristocetin remained significantly lower than the lower limit. The increase in aggregation was likely due to a stress reaction during hypoglycaemia. According to Lingenfelser T. [21], a rapid development of hypoglycaemia is accompanied by the release of noradrenaline, adrenaline and other catecholamines, inducing platelet α-adrenoreceptors

[27, 28]. In turn, activated α-adrenoreceptors increase the sensitivity of other platelet receptors to their corresponding agonists [22, 29]. It is also necessary to pay attention to the parameters surrounding platelet aggregation in reaction to ristocetin. In vitro, platelets in the presence of ristocetin bind to vWF by the glycoprotein Ib receptor [30]. As a result, the process of platelet activation and aggregation is initiated [27, 31]. Low aggregation in response to ristocetin was associated with a low concentration of vWF; suggesting the endothelial layer of the vascular bed is preserved.

The analysis of physiological anticoagulants revealed a significant increase in the activity of free protein S. It should be noted that an increase in its activity was observed only during hypoglycaemia and was absent at normal glucose levels. Increased activity in free protein S could be considered a compensatory reaction and related to activated platelets. Free protein S has two mechanisms of anticoagulant effect. First, the activation of platelets induces the release of kinases, which immediately increase the phosphorylation of free protein S and thereby increase its cofactor activity against protein C by 1.5–2.0 times [32]. Phosphorylated protein S has a high affinity for negatively charged phospholipids and thereby improves the contact of the activated protein C with the membrane by forming a complex. In turn, activated protein C is the main inhibitor of the clotting factors V and VIII, which are necessary for the conversion of prothrombin to thrombin, the subsequent formation of fibrin from fibrinogen, and clot formation. Alternatively, free protein S exhibits anticoagulant activity by direct inhibition of X factor activation [23].

The dynamic changes in the concentration of AT-III were of interest. AT-III is the main inhibitor of thrombin, activated IX, X and XII factors and plasmin [25, 33]: Proteins known to have increased activity in DM1 patients [24, 34]. Initially, amid hyperglycaemia, the concentration of AT-III was significantly higher than the normative values. However, it significantly decreased when normal glucose levels were achieved and remained at this level during the transition to hypoglycaemia. Nevertheless, AT-III remained significantly above the upper limits of the norm. The increase in the concentration of AT-III amid hyperglycaemia could be considered as compensatory; intended to bring the coagulation and anticoagulation potential of the blood to equilibrium. Indeed, this cohort's data suggests that the increased activity of AT-III was a reaction to hyperglycaemia, resulting in the normalisation of glucose levels and a significant decrease in its activity, despite values significantly higher than the norm.

No differences were found in the concentration of the vWF antigen, an integral marker of endothelial dysfunction, during the transition from hyper- to normo- and then to hypoglycaemia. This likely indicates the preserved function of the endothelium, as patients included in the study had neither macro- nor micro-vascular complications.

CONCLUSIONS

Thus, the results obtained suggest that in DM1 patients without macro- or micro-vascular complications a rapid decrease in glucose level, from hyperglycaemia to hypoglycaemia, is accompanied by a balanced hemostasis reaction and the equilibrium of coagulating and anticoagulating systems. This remains true even for patients with diminished carbohydrate metabolism (HbA_{1c} = 9.12 ± 2.19). Increased activity of platelet hemostasis is compensated by the activation of free protein S, the preservation of increased AT-III concentration, and without the consumption of coagulation factors.

ADDITIONAL INFORMATION

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Participation of authors. I.R. Yarek-Martynova – data analysis, writing the text; editing the text; M.Yu. Martynov – concept and design of the study, statistical processing and analysis of data, interpretation of results; writing and approval of the final text of the manuscript; K.G. Sarkisova – collection and processing of material, statistical processing of research results, data analysis, writing the text; E.O. Koksharova – typing the material; E.E. Mishina – typing the material; M.V. Shestakova – the concept and design of the study, data analysis, interpretation of the results; approval of the final text of the manuscript; A.N. Yasamanova – data analysis, the text editing.

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