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Dear Sirs and Madams,

It is my pleasure to welcome you on the pages of our “Your Laboratory” bulletin, where you can find articles on laboratory diagnostics written by specialists from all over the world, as well as information about our company and our products.

The Cormay company has been active on the medical market for 25 years. This year marks the silver jubilee of our business activity. Some of you had the opportunity to celebrate with us this anniversary on 25th of June in Warsaw. The details are presented further in the bulletin.

The anniversary gives us a chance to reminisce, to make summaries. Throughout all these years, Cormay has transformed from a local company to an international one. Its activity has started with manufacturing of a few reagents for biochemistry, like glucose, cholesterol and some others. However, the company signed soon a licence contract with the biggest Swiss company Hoffmann – LaRoche, which allowed the start of a fully fledged production line for clinical chemistry reagents.

In addition, Cormay was the exclusive distributor of diagnostic analyzers within the distribution contract signed with Hoffmann-LaRoche, which included Integra, and formerly also Cobas Mira, Cobas Micros, Cobas Vega, Cobas Core. PZ Cormay specialises mainly in diagnostic reagents. Cormay has developed a full line of clinical chemistry reagents. Thanks to the aforementioned licence contract signed with the Swiss Hoffmann-LaRoche company, we gained the access to the best manufacturing technologies for biochemical reagents. However, these were solid reagents, which require a freeze drying to be performed.

Since 1996, we have started the design and manufacture a new line of liquid reagents Liquick-Cor® in the stage. These reagents have been developed by our own R&D team, in cooperation with Japanese companies.

In 1998, we started manufacturing haematological reagents. Initially, these were reagents for CellDyn analyzers, and then for Micros, Medonic, Sysmex and other devices. Currently, we focus on the manufacturing of haematology reagents for Mythic 18 and Mythic 22. Our philosophy is the manufacturing of the highest quality reagents. Only the highest quality reagents can give reliable results and the insight into the patient’s health.

The high quality of our reagents is confirmed by the fact, that they are used in over 60 countries throughout the world, including Austria, Germany, the United Kingdom, Italy UAE (also in the medical laboratory of the „Emirates” airlines). In January 2010 we acquired Orphée – a Swiss company, manufacturer of the best performing haematology analysers in the world. This is an important event for us, both because of the experience and the history of success of the Swiss company, as well as the distribution network owned by this company.

After the overtake of Orphée, both companies have 221 distributors in more than 100 countries, combined. Cormay sells its reagents and diagnostic analyzers between the Atlantic Ocean and the Pacific Ocean – in countries such as England, France, Italy, Austria, Romania, and also Turkey, Dubai (UAE), Egypt, Pakistan, Russia, Kazakhstan, China, Vietnam, Philippines, Indonesia.

Orphée has the best developed distribution network in the Switzerland, Western Europe, Middle East countries, in China, India and in many countries of South Asia. Orphée also exports its products to many countries of Latin America – to Brasil, Mexico, Argentina, Uruguay, Guatemala, Dominicana. Cormay and Orphée shall still develop its distribution network and the products portfolio, especially including laboratory diagnostics analyzers.

The Switzerland shall be the headquarters of the Cormay – Orphée company, while the manufacturing shall be developed as it has been – in Poland and in Western Europe.

I encourage you to read the current issue, which is totally covering the diagnostics, diagnosing and monitoring of diabetes, which has become a problem for the society of today.

I hope that this bulletin shall become an advisor and a companion in your every day work, and the information contained therein will account for a more efficient work.

With regards,

Tomasz Tuora
President of PZ. CORMAY S.A.
President and CEO of Orphée S.A.
It has been the wonderful party – we heard from our guests during the night of 25th of June and many times after.

The ceremony started with official part. Management Board formally opened the event, cordially welcoming all guests that came from plenty number of countries. We were visited by the distributors and business partners of Cormay and Orphée from Switzerland, Austria, Italy, France, China, Russia, Turkey, Greece, Ukraine, Belarus, Bulgaria, Romania, Serbia, Macedonia, Czech Republic, Jordan, Algeria, Sudan, Egypt and other countries. Also the employees of companies belonging to the CORMAY’S Capital Group were present: our coworkers from Swiss company Orphée, Russian – Cormay Rusland as well as from Cormay Diana from Belarus.

Amongst 280 invited persons also the highest rank representatives of the Management Board of the Polish Society of Laboratory Diagnostics appeared: President – prof. Dariusz Sitkiewicz, vice-presidents: prof. Grażyna Odroń – Sypniewska and prof. Marek Paradowski. Furthermore the science icons that in the past years contributed to the development of Cormay: prof. Dagna Bobilewicz, prof. Andrzej Brzeziński and dr Hanna Żborowska – they had evaluated performance of the first diagnostics tests manufactured by Cormay. The Chamber of Manufacturers and Distributors of Laboratory Diagnostics was represented by its CEO Józef Oh, what a ceremony so.
Jakubiec and members of the Board: President – Andrzej Banaszkiewicz – also president of Roche Diagnostic Polska – and Elżbieta Wójcik- CEO of Biomerieux Polska. They both were working for CORMAY in the past.

The speeches were endlessly. The eloquence was presented by the chairman of the Supervisory Board – prof. Stefan Jackowski and Domingo Dominguez – Chief Sales Officer of Orphée & Cormay SA.

Then there was time for prizes. The honors and diplomas were granted to the Cormay’s employees and also to the best Clients and Distributors of CORMAY and Orphée. Moreover the members of the Board received special statuettes – to a big amazement of them! These statuettes were funded by the Cormay’s employees honoring their everyday work.

After completing the official ceremony came the time for a surprise for our guests – the outstanding performance of the orchestra “Filharmonia Dowcipu” (“Philharmonic Orchestra of Joke”) which by its bravura show astonished everybody and surprised minute by minute more and more, arousing admiration and laugh. Even though the chief of the orchestra had lost somewhere in the European airports...

After such exciting show guests have a little while for rest during the dinner. Quickly afterwards a band started to play and music made all people come together to dance. When the last of the dancers has left the parquet it was far after the dawn. Despite sleepless night and tiredness everyone remember this party as a delightful moment.
In 1962, Huisman and Dozy reported an increase in one of the minor fractions of haemoglobin in four of their patients with diabetes. They attributed this to all the subjects taking the oral hypoglycaemic drug tolbutamide, but attempts to reproduce this phenomenon in vitro proved unsuccessful. Five years later Rahbar rediscovered this fraction in two patients with diabetes being screened for abnormal haemoglobins. Further investigation found another 47 cases of the abnormal band, all occurring in patients with poorly controlled diabetes, and thus the finding of a "diabetic haemoglobin component" was reported in 1968.

Soon it was demonstrated that the diabetic component had a chromatographic characteristic similar to that of haemoglobin A\textsubscript{1c} (HbA\textsubscript{1c}), which is a minor haemoglobin component described by Schnek and Schroeder in 1961 and found in non-diabetic adults in a proportion of 1–4%. Structural studies later established that the haemoglobin found in patients with diabetes was indeed identical to HbA\textsubscript{1c}.

### HbA\textsubscript{1c} AS AN INDICATOR OF GLYCAEMIC CONTROL

**THE RELATIONSHIP BETWEEN HbA\textsubscript{1c} AND MEAN BLOOD GLUCOSE**

It took almost a further decade before clinical studies started to emerge suggesting that the increased proportions of HbA\textsubscript{1c} in diabetes patients could be used as a reliable index of glycaemic control over the preceding weeks and months. It is indicative of the tools available at that time to assess glycaemic control that glycated haemoglobin was compared with 24-hour urinary glucose excretions, plasma "glucose brackets", daily mean plasma glucose and the area under the curve of the glucose tolerance test. Following these studies there was rapid acceptance of glycated haemoglobin as a useful tool to objectively assess the prior glycaemic control of patients with type 1 and type 2 diabetes.

In some respects the general approval given to the use of the test at that time was ahead of evidence that it reliably reflected the mean glucose of patients with diabetes. However, the relationship was able to be more clearly defined by both the feasibility study of the Diabetes Control and Complications Trial (DCCT) and the subsequent full trial that followed. This was because the patients in the two treatments groups (intensively treated and conventionally treated) not only had HbA\textsubscript{1c} recorded at quarterly intervals, but had a 7-point (pre- and postprandial) glucose profile collected and subsequently measured by a laboratory. As the 1441 patients participated in the trial for an average of 6.5 years, it was possible to make 26056 comparisons between HbA\textsubscript{1c} and a full 7-point profile. The linear relationship found (that mean plasma glucose (MPG, mmol/l) = 1.98 × HbA\textsubscript{1c} (DCCT) – 4.29, r = 0.82) has since been used as the most accurate guide to clinicians and other healthcare workers when discussing glycaemic control with their patients. Nevertheless, the scatter of patients around this regression line meant that an individual with an MPG of, say, 10 mmol/l, could have a HbA\textsubscript{1c} anywhere between about 6% and 11%, and this has obvious implications when using solely HbA\textsubscript{1c} to set glycaemic targets. More recen-
The single linear relationship between MPG and HbA1c has been called into question in that it appeared different between the two treatment groups of the DCCT such that the MPG was 1.2 mmol/l lower at 7% HbA1c in intensively treated patients than in conventionally treated patients, with the difference becoming 4.6 mmol/l at 11% HbA1c. This inferred that the relationship between MPG and HbA1c may not constant but could differ depending on the glycemic control of the population being studied.

Between 2006 and 2007, patients have been recruited to the “mean blood glucose study”, which aims to establish the relationship between HbA1c and blood glucose as definitively as possible. At the European Association for the Study of Diabetes conference in September 2007, preliminary results of the study in 427 patients were presented. The study used continuous glucose monitoring and traditional pre- and postmeal glucose testing to establish the mean glucose of the patients, and used a National Glycohemoglobin Standardization Program (NGSP)-certified method to measure HbA1c. The relationship between MPG and HbA1c was found to be closer than in the DCCT, with a correlation coefficient of 0.91, and more in keeping with the findings of a smaller recent study (r=0.90) that also used continuous glucose monitoring. However, it remains to be seen from any final publication how representative these mean blood glucose study patients are of the diabetes population as a whole, since there were many exclusion criteria to the study that could have removed the very people who might account for much of the scatter around the regression line.

TIME COURSE OF HbA1c FORMATION
Glycation of haemoglobin occurs over the entire 120-day lifespan of the red cell, but within this 120 days recent glycaemia has the largest influence on the HbA1c value. Indeed, theoretical models and clinical studies suggest that a patient in stable control will have 50% of their HbA1c formed in the month prior to sampling, 25% in the month before that, and the remaining 25% in months 2–4. This explains why, traditionally, HbA1c has been thought to represent average glycaemia over roughly the last 6–8 weeks.

**Effect of glucose variability on HbA1c**
Until recently there has been very little evidence to establish whether or not two patients with the same mean blood glucose but very different glucose variability would have similar HbA1c values. However, two recent studies—one using DCCT data—have shown that glucose instability seems to have little influence on the HbA1c result, and rather that it is the mean glucose that appears to be the main determinant, not how that mean is arrived at.

**HbA1c AND DIABETES COMPLICATIONS**
The clinical utility of HbA1c as a tool to assess the risk of diabetes complications was cemented by the publication of the results of the aforementioned DCCT and also the United Kingdom Prospective Diabetes Study (UKPDS); these studies set out to establish the effect of intensive (as compared with conventional) glycaemic control on the development of microvascular complications in type 1 (insulin-dependent) and type 2 (non-insulin-dependent) patients respectively. The original findings from these studies in relation to HbA1c have been reviewed in this journal previously and are only summarised here. Developments since then are included in more detail.

**Microvascular complications**
The microvascular (small vessel) complications of diabetes comprise retinopathy, nephropathy and neuropathy. Patients with diabetes who develop these conditions constitute a large proportion of all subjects who develop blindness, renal failure and/or require limb amputation. The DCCT found that when 1441 patients with type 1 diabetes were randomised to “intensive” rather than “conventional” treatment, their median HbA1c was 7.3% compared with 9.1% throughout the 6.5 years average follow-up period. The subsequent risk of developing retinopathy in the intensively treated group was reduced by 76%, the risk of developing proteinuria was reduced by 54%, and the risk of clinical neuropathy was reduced by 60%. Looked at from the perspective of HbA1c, the risk of microvascular complications in the two patient groups rose exponentially as the HbA1c value increased, with no threshold—short of normal glycaemia—below which patients with type 1 diabetes did not develop microvascular complications at all.

The publication of the UKPDS in 1998 confirmed that a relationship between HbA1c and microvascular complication risk existed in 1867 patients with type 2 diabetes. The difference in HbA1c between the intensive and conventional treatment groups was not as large as in the DCCT (HbA1c 7.0% versus 7.9% over 10 years), but there was still a 25% reduction in microvascular risk. A subsequent analysis of the data has shown that when the two treatment groups are combined, then a similar exponential relationship between rising HbA1c and rising microvascular risk exists as in the DCCT.

After the end of the DCCT, 96% of the patients in the original study agreed to continue to be followed up in a new study known as the Epidemiology of Diabetes Interventions and Complications study. They were no longer in two separate treatment groups. In fact, following the outcomes of the DCCT, it was recommended that all patients follow an intensive treatment regime. It was therefore interesting that, out of a clinical trial scenario, the HbA1c of the previously intensively treated patients rose to an average of approximately 8%, while that of the conventionally treated group tightened up to a similar value. Long-term follow-up of these patients has shown that the benefits of improved glycaemic control during the DCCT on the risk of microvascular complications are maintained in the long term despite the co-
Hypoglycaemia is the main barrier that prevents patients with diabetes achieving “normal” glucose control.

**Diabetes Control and Complications Trial**

The DCCT and the UKPDS have allowed a more “evidence-based” approach to be taken to the target recommendations for HbA1c in patients with type 1 and type 2 diabetes. Prior to these studies, European guidelines tried to account for the lack of standardisation in glycaemic control. In the current UK guidelines, a value of ≤7% is now recommended for patients with type 1 and type 2 diabetes. However, as the relationship between HbA1c and macrovascular complication risk is exponential with no obvious “threshold” value, it means that targets aimed for are still to some extent arbitrary. Indeed, there has been a steady creep towards lower target values in both Europe and the USA. For example, the European Diabetes Policy Group guidelines in 1999 recommended that type 1 and type 2 patients aim for a DCCT-equivalent as-treated mean HbA1c value of ≤7.5%.15 16 and at that time the USA recommended achieving <7.0%, with values >8% suggesting that additional action be taken.17 The current UK guidelines suggest a target between 6.5% and 7.5% (depending on the presence of complications or high arterial risk),18 19 and in the USA a value of <7.0% is now recommended in all situations. This trend to lower targets seems to be continuing with the draft of new UK guidelines in type 2 diabetes encouraging values below 6.5%.20

**HbA1c AS A SCREENING TEST FOR DIABETES**

Interest in using HbA1c as a possible replacement for fasting glucose or the oral glucose tolerance test (OGTT) in diagnosing...
diabetes seldom abates. The appeal is understandable since it would obviate the need for the patient to attend fasting and, if an OGTT was required, would address the problem with poor reproducibility of the 2-hour glucose value. However, repeated studies have shown that the limitation to its use is usually not because a high HbA1c result does not indicate diabetes, but that a “normal” one does not exclude it. Thus, as a diagnostic tool, it is specific but lacks sensitivity. Also, from an analytical perspective, HbA1c is not the most precisely measurable analyte; therefore, an assay showing a 3% coefficient of variation at the critical 6.0% HbA1c value can show a difference in excess of 0.7% HbA1c within the same individual. Added to this is the fact that it has the unenviable task of trying to identify a condition that has two means of being diagnosed (ie, the fasting glucose value and the 2-hour OGTT value), so it is never likely to be able to satisfy the two criteria. There has therefore been a focus on using the test in addition to measuring fasting glucose to either diagnose diabetes or to screen for patients who do or do not need to progress to a full OGTT. In other words, HbA1c is being used as a surrogate for the 2-hour glucose value of the OGTT. Used in this way, the specificity of a raised HbA1c is being exploited in a population already at high risk of having glucose intolerance because of a borderline fasting glucose. One study of individuals at high risk of diabetes found that 43% of those with a fasting plasma glucose <7.0 mmol/l, but a diabetic response to the OGTT, had an HbA1c above the upper limit of the reference interval. Presumably, however, the percentage identified would drop substantially in a lower risk population.

Whatever the technique used to incorporate HbA1c into any diagnostic criteria, it would certainly be feasible to produce an HbA1c threshold that would give a similar proportion of patients the diagnosis of diabetes as the glucose criteria currently do, but it is likely to be a different group of individuals who are identified. Until this group can be shown to be at the same risk of micro- and macrovascular complications as those diagnosed by traditional criteria, and the cost of determining instrumentation is now able to account for fetal haemoglobin and so this issue should now be only of historic interest.

**Interest in using HbA1c as a possible replacement for fasting glucose or the oral glucose tolerance test (OGTT) in diagnosing diabetes seldom abates**

**Abnormal haemoglobins**

Normal adult HbA1c glycates to form HbA1c, but if an abnormal haemoglobin is present then a patient is likely to form other glycated products such as HbS1c, HbC1c and so on, either in addition to or instead of HbA1c. Many glycated haemoglobin analysers can now at least identify the non-glycated portion of these abnormal fractions, but there can still be difficulty in trying to make sense of what a patient’s HbA1c would be were it not for their haemoglobinopathy. This is even more difficult in patients who have haemoglobinopathies where there will be no HbA1c present at all. Some instruments that base their analysis on affinity chromatography can more easily identify glycation on any form of haemoglobin molecule as can immunoassay methods, but even then there is the suggestion that some abnormal haemoglobins glycate at a different rate to native HbA1c and so may give rise to misleading results.

Small proportions of fetal haemoglobin persisting into adult life used to cause considerable problems with some glycated haemoglobin methods because they would co-migrate or co-elute with the glycated haemoglobin fraction leading to an overestimation of the HbA1c or HbA1c result. More recently, it has been shown that urea-derived isocyanate can lead to the formation of carbamylated haemoglobin, which can be indistinguishable from HbA1c when using some glycated haemoglobin methods. However, for most patients the overall effect does not seem too substantial with most HbA1c methods.

**How should HbA1c values be expressed? Standardisation of HbA1c**

In the 1980s and 1990s an important issue for HbA1c measurement was the lack of standardisation of the assay, and this meant that different analysers could have widely differing reference intervals and give varying results with patient samples. As the DCCT and the UKPDS used the same method of analysis in their studies, this was felt to be a useful method to harmonise results against. It also had the added attraction that it meant patients could have their HbA1c results compared directly with those of the subjects who participated in the two trials. In order to develop this international harmonisation an extensive network of reference laboratories was established by the NGSP based in the USA. In the last decade this development has made great strides in making the HbA1c results reported from different laboratories much more comparable. Hence, however, the HbA1c results reported by this means were not the “true” HbA1c values, but simply the best that 1980s technolo-
Diagnostics in the world

Gy could deliver when the DCCT study was conceived. In order to rectify this situation, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) developed a reference method for HbA₁c that first established reference material of purified HbA₁c and HbA₀, and then a highly specific reference method for their measurement. Given this increased specificity it is perhaps not surprising that the results using this technique are between 1.5% and 2% HbA₁c-lower than the NGSP results that relate to the DCCT. As a result, the move over to using these numbers has been resisted in some quarters because of a fear of confusion between the two sets of values, with some evidence from previous changes in HbA₁c numbers that clinicians may either under-treat or over-treat patients because they are still using the older targets.

This led to the suggestion that rather than moving to these IFCC values, there could be a wholesale change to expressing HbA₁c as a “mean plasma glucose equivalent”, or as a “mean plasma glucose” (eAG). The main purported reason for making this change is that patients as being their glucose result when glucose study described above is being used for that purpose. Little has yet been published on this study, but the a priori criteria for acceptable agreement is that at least 90% of patients should have a mean glucose that is within 15% of that derived from the simple regression of MBG on HbA₁c for the study population (ie, within 15% of the eAG). However, even if this criterion is fulfilled it equates to 99% of the population being within ±24% of the estimated average glucose, meaning that in two individuals with an eAG of 10 mmol/l, one patient could have an actual mean glucose of 7.6 mmol/l and another 63% higher at 12.4 mmol/l. Thus, the final results of the study will need to be examined closely to be sure that eAG is not adopted to the detriment of these types of patients.

Since this proposal, the IFCC has taken steps to make sure there is little chance of confusion between its results and those of DCCT values by changing its units from percentages to being expressed as mmol HbA₁c/mol HbA₀. The relationship between percentage and mmol/mol is one of a 10-fold change meaning that, for example, 7% by the IFCC reference system equates to 70 mmol/mol. This would obviously be a large upheaval for users of the test although it is sweeterened, perhaps, by the fact that HbA₁c results could no longer be confused by patients as being their glucose result when expressed in SI units. Unfortunately, for those countries who express glucose as mg/dl it introduces this problem where it did not exist previously.

In the 1980s and 1990s an important issue for HbA₁c measurement was the lack of standardisation of the assay, and this meant that different analysers could have widely differing reference intervals and give varying results with patient samples.

So where from here? Following a meeting on 4 May 2007, a joint consensus statement was issued by the European Association for the Study of Diabetes, the American Diabetes Association, the IFCC and the International Diabetes Federation. It recommended that HbA₁c results be reported worldwide in IFCC units (mmol/mol) and NGSP (ie, DCCT) units (percentage) and, if the ongoing mean blood glucose study fulfills its a priori criterion then the eAG will also be reported as an interpolation of the HbA₁c result.

This author is bemused at how a single test can end up having to be reported in three different ways (four if glucose units are included) on the same report. It therefore seems inevitable that there will be a re-evaluation of this consensus at some stage, with the fear that there may be less rather than more global harmonisation of results in the future if individual groupings of countries choose to take different paths.

CONCLUSIONS

The benefits associated with using HbA₁c to monitor glucose control in diabetes are now being fully realised. However, it is important for clinicians to be aware that there is always likely to be a significant proportion of patients in whom results from the test need to be interpreted with caution.

Reference available upon request.

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• For most patients, haemoglobin A₁c (HbA₁c) measurement has become the most important means of assessing their glycaemic control.
• Despite methodological improvements and the recent harmonisation/standardisation of measurement there remains inherent limitations with the test that healthcare staff and patients need to be aware of.
• There is currently debate about whether HbA₁c should be expressed as a percentage, in millimoles per moles, as an “estimated average glucose”, or as a combination of all three. We are therefore likely to be going through a period of considerable change for the test.

Take-home messages

CORMAY International Bulletin

Whose truth in one drop
Diabetes (diabetes mellitus) is defined as a group of metabolic diseases proceeding with disorders of metabolism of carbohydrates, lipids and proteins, of which chronically remaining hyperglycemia is characteristic. The cause of metabolic disorders in diabetes is decreased insulin production and/or its abnormal functioning (immunity to insulin). Diabetes is still incurable disease causing dangerous peracute and chronic complications, incidence of which in developed countries is 7%-9% adult population – all that determines great social meaning of that disease.

It is estimated that in Poland 1.5 – 2 mln people suffer from diabetes, about 200 mln all over the world. From among forms of diabetes mentioned in etiopathogenetic classification (Table 1) the most frequent is diabetes type 2, being about 90% of this disease cases, however diabetes type 1 includes a few percent of cases.

Laboratory tests are necessary at all stages of looking after diabetics – in recognising and revealing of the disease, monitoring its course and treatment and diagnosing peracute and chronic complications. According to diabetes definition, so far the only laboratory test used in diagnosing of it has been determination of glucose concentration in blood. Recently it has been proposed to use also for that purpose the retrospective glycemia indicator – glycated hemoglobin (HbA1c). Currently used diagnostic criteria include three categories of glucose concentration in blood and, according to American Diabetes Association recommendation from year 2010 HbA1c per-

**Table 1. Etiologic diabetes classification**

<table>
<thead>
<tr>
<th>Type 1 diabetes</th>
<th>Type 2 diabetes</th>
<th>Known etiology diabetes</th>
<th>Gestational Diabetes Mellitus (GDM)</th>
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<tr>
<td>caused by destroy of beta cells of the islets of Langerhans in the pancreas during course of autoimmunizing process (type 1A) or idiopathic (type 1B)</td>
<td>caused by coexistence of tissues insulin resistance and impaired insulin secreton.</td>
<td>cumulative category including among others secondary diabetes in other endocrinopathy (i.e. Cushing’s syndrome), as a result of pancreas resection, in genetically conditioned impaired insulin secretion syndromes and its effect to tissues, including MODY type diabetes.</td>
<td>each form of carbohydrates metabolism disorder diagnosed when pregnant.</td>
</tr>
</tbody>
</table>
percentage (Table 2). For diagnosis of the disease, double identification of any of diagnostic criteria or both independently, is required. It is worth to emphasize that measuring of glucose level for the purposes of diabetes and prediabetes diagnosis must be made in venous blood plasma, in laboratory with accreditation or implemented quality management system. Following, analytic quality criteria of measuring glucose are recommended: inacurateness ≤ 3.3%, analytic bias ≤ 2.5%, overall error ≤ 5.9%. It is inadmissible to diagnose diabetes or prediabetes, basing on results of measuring glucose concentration made in other material and with other methods, i.e. by using glucometers.

Fasting glucose level within 100–125 mg/dl (5.6–6.9 mmol/l) is defined as Impaired Fasting Glucose (IFG). It is second, apart from Impaired Glucose Tolerance (IGT), prediabetes. IGT is diagnosed on the basis of Oral Glucose Tolerance Test (OGTT) – examination often performed wholly in laboratories. Properly performed OGTT includes:

- patient preparation (diet without restrictions of carbohydrates intake, with supply of them at least 150 g/24 hours, consideration of drugs increasing glycemia),
- carrying out the examination in the morning, on an empty stomach, with patient being rested after the night slept through,
- drawing a zero time (baseline) venous blood sample,
- glucose load – patient drinks 75 g (children 1.75 g/kg of body weight) anhydrous glucose dissolved in 250 – 300 ml of water, within 5 minute time frame; adding couple of lemon juice drops does not cause any significant analytical interferences,
- after load leaving the patient in the place of examination performance, at rest, in sitting position,
- drawing the second venous blood sample 120 min. after the load.

Measuring glucose concentration level is performed in venous blood plasma. In the purpose of diabetes or IGT diagnosis only glycemia in 120 min. OGTT is interpreted (Table 2).

Both prediabetes conditions may, but do not have to coexist with the same patient. They are risk factors of developing full-blown diabetes. In this year’s ADA (American Diabetes Association) recommendations it was recommended to use HbA1c determination also to detect increased risk of diabetes occurrence (Table 2). Prediabetes conditions are also independent risk factors of cardiovascular system diseases. Patients with detected IFG and/or IGT require observation and treatment.

Diagnostic criteria based on plasma glucose level serve to diagnosis all kinds of diabetes. Differentiation of individual disease types, mainly type 2 and type 1 is usually possible on the basis of clinical course. In cases of doubts, laboratory tests to detect autoimmune base of pancreas beta cells damaging or to evaluation of tissues insulin resistance may be used in differential diagnosis.

In diabetes type 1 destruction of β cells is made by sensitized lymphocytes and cytotoxic mechanisms, but then in blood there appear autoantibodies oriented against those cells which are recognised autoimmune process markers. Polyclonal islet cells autoantibodies (ICA) are always oriented against cytoplasmic antigens of β cells. They are marked with indirect immunofluorescence method, they appear at 75 – 85 percent of diabetes type 1 and they are regarded as the most sensitive and specific marker of autoimmune diabetes form. Antibodies of glutamic acid decarboxylase (GAD), isozyme of molecular mass 65 kD (anti-GAD-65) appear at 60 – 80 % of diabetes type 1, and tyrosine phosphatases antibodies (Insulimoma-associated antigen, IA-2, IA-28) – at 50 – 70 percent of patients. Insulin autoantibodies (IAA) appear at 90 – 100 % of children under five, being type 1 diabetics, however in the age over twelve their incidence decreases to 40 percent and less. Simultaneous marking of two or three of specified autoantibodies increases the sensitivity and diagnostic specificity of the test to about 100 percent.

Insulin resistance of tissues, being the element of diabetes type 2 pathogenesis, liberates compensatory increase of insulin secretion with its concentration in blood increase. In connection with this, hyperinsulinemia is regarded as easy-to-detect insulin resistance exponent. Increased insulin concentration is, however, temporary phenomenon, only indirectly connected with insulin resistance and does not reflect its degree. Lack of satisfying standardization of immunological insulin determination methods causes additional difficulty. For direct evaluation of insulin resistance tests of glucose distribution in tissues in dependent of insulin are used. Tests include metabolic clamp method. Hyperinsulinemic and euglycemic clamp consist in delivering insulin and glucose to the patient, by intravenous infusion, with doses maintaining their constant concentration in blood; for glucose it is 100 mg/dl (5.6 mmol/l). Quantity of given glucose is directly proportional to tissues insulin sensitivity. Metabolic clamp tests are used mainly for research purposes and are not broadly accessible in clinical practice. Insulin resistance may be easily evaluated with various indicators calculating on the basis of glucose and insulin concentration in blood, such as HOMA-IR in homeostasis model assessment and the quantitative insulin sensitivity check index (QUICK). Breath test, in which excretion of 13C from lungs is examined after “C marked glucose feeding, may be quick, non invasive method of insulin resistance assessment. Within conditions of insulin resistance 13CO2 generation from marked glucose and excretion of it are decreased.

Diabetes type 2 constitutes about 90 percent of this disease cases, and it stays not diagnosed in 30 to 50% of patients.
Poland it means that for 1.6 – 1.8 mln diabetics type 2, two hundred thousand persons are not treated. In connection with this, it is recommended to carry out population screening examination for this disease. In this purpose, measuring fasting venous plasma glucose concentration should be made once every three years for every person over forty-five. In persons with type 2 diabetes risk factors screening examination should be started earlier and should be made once a year. Type 2 diabetes risk factors include:

- overweight (BMI ≥ 25 kg/m₂),
- genetic load (type 2 diabetes of parents or siblings),
- little physical activity,
- got over gestational diabetes or birth of a child of weight > 4.5 kg,
- arterial hypertension (> 140/90 mm Hg),
- atherogenic dyslipidaemia: HDL cholesterol level < 45 mg/dl (0.9 mmol/l) and/or triglycerides level > 250 mg/dl (2.82 mmol/l),
- polycystic ovary syndrome,
- cardiovascular system disease.

Gestational diabetes is defined as each carbohydrates tolerance disorder developing or diagnosed for the first time when pregnant. Gestational diabetes occurs in 3-5 percent of pregnant. It constitutes about 90 percent of diabetes in pregnancy, the rest about 10 percent are cases of diabetes that occurred already before becoming pregnant (pre-gestational diabetes). Diabetics complicating pregnancy makes a menace both for mother and developing foetus, hence there comes acknowledged need of detecting it by screening examinations. On the model admitted in Poland, preliminary measuring of fasting plasma concentration often made at the beginning of pregnancy. Gestational diabetes may be then diagnosed on the basis of this examination result or additionally made OGTT (Table 3). If examination made at the beginning of pregnancy was negative, between 24. and 28. pregnancy week optionally screening test is performed – 50g glucose oral load. For performing it pregnant woman has to be fasting and in consist on single measuring of glucose concentration in venous blood plasma drawn an hour after glucose intake (Table 3). OGTT is made in standard way for pregnant woman, with 75 g glucose load. It is worth emphasizing that gestational diabetes mellitus (GDM) is diagnosed at the glucose concentration above 140 mg/dl (7.8 mmol/l) in second test hour.

The purpose of diabetes treatment is to keep patient in near normoglycemia condition, reflected by carbohydrates metabolism indexes close to physiological values in degree enabled by iatrogenic hypoglycemia and proper state of lipid metabolism. Other treatment purposes include maintaining appropriate body weight and proper arterial blood pressure. Achieving this purposes requires strict biochemical monitoring of carbohydrates and lipid metabolic management compensation, by using following examinations:

- glucose concentration in blood,
- glycated hemoglobin (HbA₁c fraction) more rarely glycated albumin (fructosamine),
- glucose and ketone bodies in urine,
- lipid examinations.

Basic examination for assessment of carbohydrates metabolism condition is concentration of glucose in blood controlled in the form of diurnal glycemia profile. It is the result of markings carried out in that day times when terminal values may be expected, dependently on times of food and drugs intake. When interpreting obtained results, it is assumed that glucose concentration in blood ranges in the scope determined by values in glycemia profile. These markings are usually made in the morning, on the empty stomach, 2 hours after meals, before sleep and additionally at midnight and between 2.00 and 4.00

These examinations are made in capillary blood, independently by patients, with using glucometers (self-monitored blood glucose, SMBG). According to clinical recommendations, glycemia self inspection is obligatory for all patients treated with insulin and it is recommended for other diabetics. Measuring within the frames of glycemia self inspection is made with test stripes and glucometers. They are minimized analysers using glucose oxidase or glucose dehydrogenase and amperometric or reflectometric measurement technique. It is worth to emphasize that although full capillary blood is placed on stripe reaction field, measuring is de facto made in plasma and some glucometers show such examination result, while other make recalculation to full blood glucose concentration (10-15 percent less). Glucometers as analysers designed for glycemia self monitoring are to join appropriate analytic quality with the simplicity of operating. Additional convenience for their users is pathologic results signalling, recording in memory up to 500 examination results and possibility of sending them to computer. Analytic quality of measuring with using glucometers has to credible assessment of metabolic compensation of disease and reliable detecting of glycemia requiring immediate intervention, mainly hypoglycemic conditions. Measuring error, defined as difference between the result obtained by using glucometer and referential value (result of the examination with laboratory method or nominal value in control material), expressed as percentage of referential concentration, may range according to various recommendations from 5 percent to 20 percent. Condition of maintaining measurement error within allowable borders is proper education of patients and control of analytic glucom-

---

**Table 3. Gestational Diabetes Mellitus (GDM) diagnosis. 2 hour OGTT - oral glucose tolerance test (2 hour glycemia). Venous blood plasma glucose concentrations.**

<table>
<thead>
<tr>
<th>PREGNANCY BEGINNING</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting glycemia:</td>
<td></td>
</tr>
<tr>
<td>&lt;95 mg/dl (5.3 mmol/l) – normal</td>
<td></td>
</tr>
<tr>
<td>95–125 mg/dl (5.3–6.9 mmol/l) →</td>
<td></td>
</tr>
<tr>
<td>≥126 mg/dl (7.0 mmol/l) (twice) → GDM</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>24. – 28. PREGNANCY WEEK</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening test: optionally (50 g glucose, glycemia after 1 hour):</td>
<td></td>
</tr>
<tr>
<td>&lt;140 mg/dl (7.8 mmol/l) – negative</td>
<td></td>
</tr>
<tr>
<td>140–200 mg/dl (7.8–11.1 mmol/l) →</td>
<td></td>
</tr>
<tr>
<td>≥200 mg/dl (11.1 mmol/l) → GDM</td>
<td></td>
</tr>
</tbody>
</table>

| OGTT (2 hour): |
| <140 mg/dl (7.8 mmol/l) – normal |
| ≥140 mg/dl (7.8 mmol/l) → GDM |

| OGTT (2 hour): |
| <140 mg/dl (7.8 mmol/l) – normal |
| ≥140 mg/dl (7.8 mmol/l) → GDM |
Fig. 1. Glycated hemoglobin production

Table 4. Diabetes metabolic compensation criteria (acc. to Polish Diabetes Association, 2009)

<table>
<thead>
<tr>
<th>Biochemical examination</th>
<th>SI units</th>
<th>Traditional units</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type 1 diabetes, short-lasting type 2 diabetes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting and before meal glycemia</td>
<td>&lt;7,8 mmol/l</td>
<td>&lt;140 mg/dl</td>
</tr>
<tr>
<td>2 hour after meal glycemia</td>
<td>&lt;7,8 mmol/l</td>
<td>&lt;140 mg/dl</td>
</tr>
<tr>
<td><strong>Old age persons with type 2 diabetes, coexisting affections</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting and before meal glycemia</td>
<td>&lt;8,9 mmol/l</td>
<td>&lt;160 mg/dl</td>
</tr>
<tr>
<td>2 hour after meal glycemia</td>
<td>&lt;8,9 mmol/l</td>
<td>&lt;160 mg/dl</td>
</tr>
<tr>
<td><strong>Target lipid profile values</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>&lt;4,5 mmol/l</td>
<td>&lt;175 mg/dl</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>&lt;2,6 mmol/l</td>
<td>&lt;100 mg/dl</td>
</tr>
<tr>
<td>with existing ischemic heart disease</td>
<td>&lt;1,8 mmol/l</td>
<td>&lt;70 mg/dl</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>&gt;1,0 mmol/l</td>
<td>&gt;40 mg/dl</td>
</tr>
<tr>
<td>M: &gt;1,29 mmol/l</td>
<td>K: &gt;50 mg/dl</td>
<td></td>
</tr>
<tr>
<td>’not HDL’ cholesterol</td>
<td>&lt;3,4 mmol/l</td>
<td>&lt;130 mg/dl</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>&lt;1,7 mmol/l</td>
<td>&lt;150 mg/dl</td>
</tr>
</tbody>
</table>

diabetes and patients requiring strict metabolic compensation (i.e., pregnant women), Continuous Glucose Monitoring Systems (CGMS) are applied. They carry out measurements with defined frequency, using glucose correlation in tissue fluid and in blood. They may operate on the base of microdialysis technique — semipermeable catheter placed in subcutaneous tissue serve to gather tissue fluid, in which glucose concentration is measured, and the result is presented in real time and recorded for later analysis. In other system, glucose drawn transdermally by electroendosmosis (reversed iontophoresis).

Blood glucose self monitoring and valid criteria of diabetes metabolic compensation (Table 4), according to which even the greatest target blood glucose concentration does not exceed the renal threshold, practically eliminated glycosuria measurement from treatment monitoring. However, still significant role is played by measuring of ketone bodies in urine, serving to detection of ketosis and diabetes ketoacidosis, being the result of extremely bad metabolic compensation. Ketonuria should be examined with significant hyperglycemia and with ketoacidosis symptoms appearance (nausea, emesis, abdominal pains). Ketone bodies are measured with use of dry stripe tests, in reaction with sodium nitroprusside.

Glycation is two-phase process of non-enzymatic joining carbohydrates molecules to free protein amine groups. During first, faster, reversible reaction aldime shape of glycated protein (Schiff base) is created. Second, slower, irreversible phase is intramolecular Amadori rearrangement with creation of durable ketoamine form, which exists until protein degradation (Fig. 1). The quantity of glycated proteins being created depends on blood glucose level, which justifies their usage in diabetes treatment monitoring as retrospective glycemia indexes. Moreover, it was shown that glycated hemoglobin is independent risk factor of developing chronic diabetes complications. Content (percentage) of glycated hemoglobin, represented by its main measured fraction, HbA1c, reflects average glycemia during three months before examination. According to Polish Diabetes Association (PTD), HbA1c should be measured at each diabetic every three months, and only in case of patients with stable disease course, metabolically compensated, every 6 month.

Glycated hemoglobin is measured with usage of low or high pressure ion-exchange chromatography (HPLC), affinity chromatography and immunochemical methods, mainly immunoturbidimetric. Ion-exchange chromatography is a method of hemoglobin fraction division based on particles charge. Binding N-terminal free amine group to glucose causes that with adequate ionic strength and pH buffer HbA1c loses positive charge owned by non-glycated hemoglobin particles (HbA). Different retention time of individual hemoglobin fraction in column packed with cationic ion-exchange resin enables quantitative measurement of HbA1c. Disturbing influence on measurement with this method may be caused by aldime form of HbA1c, and change of hemoglobin particles charge in uremia, by ethanol metabolites and salicylates.

Affinity chromatography is method of division based on chemical reaction of all glycated hemoglobin fractions (HbA) with particles of boric acid derivative, connected to background filling the column. HbA1c particles become covalently bounded by creation of cyclic joint with boron atom participation. After HbA1c fraction passage through the column, adding i.e. sorbitol leads to dissociation of HbA1c particles joints, which enables its elution and measurement. Affinity chromatography determinations are not significantly disturbed by aldime HbA1c form, neither by hemoglobin forms: HbF, HbC and HbS. When using this method, however, HbA1c is not determined di-
International HbA₁c measuring standardization is being implemented under the auspice of International Federation of Clinical Chemistry (IFCC)

directly. Because it is HbA₁c determination that is recommended for needs of diabetes treatment monitoring, results of affinity chromatography determination are expressed in the form of “HbA₁c equivalent”.

Immunochemical methods are based on monoclonic specific antibodies against epitopes including N-terminal amino acids of HbA₁c. β chain. These are the most often immunoturbidimetric methods, with usage of latex particles or other antibodies carriers. HbA₁c determination with these methods may be performed with usage of dedicated analysers (i.e. in POCT mode) and also widely accessible in laboratories automatic biochemistry analysers. Monoclonal antibodies use may ensure analytic specificity of individual method, securing against interferences from variants or chemically modified hemoglobins.

In many countries there are national programs of standardization glycated hemoglobin determination, i.e. American, British, Scandinavian, Japanese. In American National Glycohemoglobin Standardization Program (NGSP) ion-exchange HPLC method was assumed as comparative, towards which other methods are calibrated. In this program of international range over 60 methods of glycohemoglobin determination are certified. International standardization of HbA₁c determination is being implemented under the auspices of International Federation of Clinical Chemistry (IFCC). IFCC workgroup defined HbA₁c as Hb with irreversibly glycated one or both N-terminal rests of valines of β globin chains. Two referential methods of determination of glycated N-terminal β chain fragment. They are two-phase methods – first β globin chain is split by specific endopeptidase. Then glycated and non-glycated N-terminal hexapeptides of β chain are determined with the use of HPLC and mass spectrometry or capillary electrophoresis. Results of HbA₁c determination with IFCC method are lower comparing to methods certified in NGSP, which is expressed by empirically derived formula:

\[
\%\text{HbA}1c_{\text{NGSP}} = 0.915 \times \%\text{HbA}1c_{\text{IFCC}} + 2.15\%
\]

Currently there are works being performed on global use of HbA₁c determination standardization according to IFCC. Correctly conducted diabetes treatment should ensure obtaining target values of glycemia and HbA₁c, regarded as metabolic compensation criteria (Table 4.)

Concentration of glycated form of albumin reflects average glycemia during 2 to 3 weeks before determination. Glycated albumin is determined at patients with unstable course of diabetes and in case when it is necessary to obtain strict metabolic compensation, i.e. at pregnant women with diabetes. It is determined as fructosamine created after reaction of glucose with ε-amine group of protein lysine residue. Fructosamine is determined with photometric methods based on its reducing properties in alkaline environment. Referential values range and fructosamine target concentrations are dependent on used determination methodology.

Lipid examinations of diabetics serve both treatment monitoring and cardiovascular risk assessment, that means diabetes macroangiopathy diagnosis. These examinations should be performed initially, after disease diagnosis and then regularly, with time periods of couple weeks to 2 years, dependently on obtained results and cardiovascular risk at individual patient. Examination range includes concentration of total cholesterol (TC), HDL and LDL fractions (HDL-C and LDL-C) and triglycerides. HDL-C and LDL-C determination with direct methods is recommended. If analytic LDL-C determination is not possible, for that purpose Friedewald formula may be used, if triglycerides concentration does not exceed 4.5 mmol/l. As additional parameter of lipid metabolism assessment “not-HDL cholesterol” is used calculated as concentration difference between TC and HDL-C. Fighting dyslipidaemia accompanying diabetes is significant element of treatment of this disease. Target values of lipid parameters of diabetics are close to expected values within the frames of secondary cardiovascular diseases prevention (Table 4).

Literature:
**FOCUS ON DIAGNOSTICS**

**Translating Hemoglobin A₁c into Average Blood Glucose: Implications for Clinical Chemistry**

A recent report (1) establishes a mathematical relationship between HbA₁c and the average glucose (AG) concentration in blood.

David B. Sacks

Measurement of hemoglobin A₁c (HbA₁c) is a fundamental component of the management of patients with diabetes mellitus. HbA₁c measurement provides an indication of chronic exposure to glucose and is extensively used for both monitoring long-term glycemic status and evaluating whether an individual patient has attained adequate metabolic control. The patient’s HbA₁c value is used by clinicians to determine whether glucose-lowering therapy is adequate. A recent report (1) establishes a mathematical relationship between HbA₁c and the average glucose (AG) concentration in blood. The findings presented are likely to have considerable impact on the way HbA₁c is reported by clinical laboratories and used by healthcare providers (and patients). The importance and potential consequences of the study are the focus of this Perspective.

**MONITORING GLUCOSE CONTROL**

The efficacy of therapy to lower blood glucose in patients with diabetes mellitus is assessed by 2 complementary methods, glucose measurement, which is performed by patients, and HbA₁c measurement. Patients perform self-monitoring of blood glucose (SMBG) by using hand-held meters to measure their own blood glucose concentrations. It is recommended that patients on insulin perform SMBG 4 times a day. The dose of the insulin injection is determined by the glucose value. Many modern meters store the results of all the glucose measurements, which can be downloaded and accessed by the physician during the patient’s visit.

The second method for monitoring therapy to lower blood glucose is measurement of glycohemoglobin, most commonly performed by assaying HbA₁c. Glycohemoglobin is formed by the attachment of glucose to hemoglobin by a nonenzymatic process, termed glycation. The erythrocyte membrane is permeable to glucose, which enters the cell, where it binds to hemoglobin. The unstable product, termed an aldime, undergoes an Amadori rearrangement to form a stable ketamine (glycohemoglobin), which persists for the lifespan of the erythrocyte (typically 120 days). The concentration of glycohemoglobin is relatively consistent and does not exhibit the wide diurnal fluctuations seen with blood glucose, which varies substantially with exercise, food ingestion, and other factors. Because the rate of formation of glycohemoglobin is directly proportional to the glucose concentration in the blood, the glycohemoglobin concentration represents the integrated values for glucose over the preceding 8–12 weeks. Interpretation of glycohemoglobin depends on the erythrocyte having a normal lifespan. Any conditions that shorten erythrocyte survival will decrease the concentration of glycohemoglobin.

Several forms of glycohemoglobin have been identified. These include HbA₁a, HbA₁b, and HbA₁c, and total glycohemoglobin (HbA₁ and other hemoglobin-glucose adducts). HbA₁c is formed by the attachment of glucose to the N-terminal valine of the-chain of HbA₁. The clinical value of HbA₁c was unequivocally documented by the Diabetes Control and Complications Trial, which established a direct relationship between blood glucose concentration (assessed by HbA₁c) and risk of microvascular complications in patients with type 1 diabetes. A subsequent study revealed analogous correlations between HbA₁c and microvascular complications in patients with type 2 diabetes. The American Diabetes Association and other clinical organizations recommend routine (at least twice a year) measurement of HbA₁c in all persons with diabetes.

HbA₁c and AG

In the Diabetes Control and Complications Trial, retrospective analysis of data derived from SMBG measurements made by patients identified a linear correlation between HbA₁c and AG concentrations. Although the study population was large (1441 participants), the Diabetes Control and Complications Trial was not designed to determine AG, and the correlation was based on only 7 glucose measurements. A few other studies have examined the relationship, but the limited number of glucose assays raises questions about the assessment of chronic glycemia in these studies. To ascertain the relationship between HbA₁c concentration and long-term glucose values, a multinational study was conducted. The findings were published recently. Study participants were recruited at 11 centers in the US, Europe, Africa, and Asia to obtain diverse racial and ethnic representation. The final study population included 268 patients with type 1 diabetes, 159 patients with type 2 diabetes, and 80 individuals without diabetes. HbA₁c was measured at baseline and monthly for 3 months. To minimize assay variation, all HbA₁c analyses were performed in a single laboratory with 4 different assays certified by the National Glycohemoglobin Standardization Program. AG is more difficult to measure accurately than HbA₁c. For AG evaluation, participants underwent continuous glucose monitoring for 48 h at baseline and monthly for the duration of the study. The monitoring was performed with a MiniMed device, which measures interstitial glucose concentration every 5 min. During the 2 days of continuous glucose monitoring, study participants performed an 8-point assessment of blood glucose with a HemoCue meter. A third measure of glycemia employed SMBG (using the OneTouch Ultra) 7 times per day for at least 3 days per week, for the entire study period. Over the course of the 12-week study, approximately 2700 glucose measurements were performed on each participant.

Statistical analysis of the study data revealed that linear regression between HbA₁c and AG provides the tightest correlation. For example, an HbA₁c value of 6% (equivalent to 7.0 mmol/L) translates into an estimated AG (eAG) of 126 mg/dL. A widely used target...
Several forms of glycohemoglobin have been identified. These include HbA\textsubscript{1c}, Hb\textsubscript{A\textsubscript{1}} (Hb\textsubscript{A\textsubscript{1}} consists of Hb\textsubscript{A\textsubscript{1a}}, Hb\textsubscript{A\textsubscript{1b}}, and Hb\textsubscript{A\textsubscript{1c}}), and total glycohemoglobin (Hb\textsubscript{A\textsubscript{1}} and other hemoglobin-glucose adducts)

for therapy, Hb\textsubscript{A\textsubscript{1c}} of 7%, corresponds to an eAG of 8.6 mmol/L (154 mg/dL). Subgroup analysis indicates essentially uniform results. No significant differences in the regression equation were observed for variations in individuals tested, including sex, presence or absence of diabetes, type of diabetes, age, race, and ethnicity. Thus a single equation can be used for the majority of individuals.

Some caveats to the study require consideration. The inherent limitation to accurate measurement of glucose with meters and continuous monitors necessitated a wide acceptance range (90% of the values fall within ±15% of the regression line). Although the data meet these a priori criteria for acceptance range (90% of the values fall within ±15% of the regression line), the AG varies among individuals with the same Hb\textsubscript{A\textsubscript{1c}} value. Several factors could account for the scatter. These include measurement error, interindividual variation, imperfect correlation between Hb\textsubscript{A\textsubscript{1c}} and AG, and differences in glycation or heterogeneity in erythrocyte lifespan. Further analysis of selected subgroups in the study may yield insight. Another limitation is the low number of Asian individuals included in the study population (the planned participation of a subgroup of individuals from India did not occur). Therefore, India and China, the 2 countries with the largest numbers of individuals with diabetes, are not represented in the study population. Although no statistically significant differences were detected among ethnic groups, the study was not powered to identify possible differences. The absence of children and pregnant women from the study limits extrapolation of the findings to these groups. Another study constraint was that only diabetic patients with stable glycemic control were included, so the results are confined to this population.

The study is likely to impact both patients and all healthcare workers – ranging from clinicians and nurses to educators and laboratory personnel – who contribute to the management of patients with diabetes. The American Diabetes Association has initiated an extensive campaign to educate clinicians and patients. This education plan includes the establishment of an Estimated Average Glucose Steering Committee, which is working with diverse groups to facilitate implementation. Clinical laboratory personnel will have an important role. It is likely that many laboratories will use the regression equation provided above to calculate an eAG based on the Hb\textsubscript{A\textsubscript{1c}} result. This eAG value would not replace the measured Hb\textsubscript{A\textsubscript{1c}} concentration, which would still be reported, but could be provided in addition to the Hb\textsubscript{A\textsubscript{1c}}. The concept is somewhat analogous to the reporting by many laboratories of eGFR (estimated glomerular filtration rate), which is calculated from the measured serum creatinine concentration. Laboratory information systems will perform the conversion from Hb\textsubscript{A\textsubscript{1c}} and eAG will be reported in International System of Units values (mmol/L) or mg/dL, consistent with the units used to report glucose in the specific laboratory. Some clinicians and numerous diabetes educators have expressed the belief that the concept of AG will be easier to explain to patients than Hb\textsubscript{A\textsubscript{1c}}. Many patients with diabetes do not know whether they had a recent Hb\textsubscript{A\textsubscript{1c}} measurement or its value, and the hope is that an explanation of eAG will replace this lack of knowledge with an indicator of glycemic control that is understandable to patients.

Notwithstanding the limitations mentioned above, the data generated by this study enhance our comprehension of the relationship between Hb\textsubscript{A\textsubscript{1c}} and AG. Measurement of glycohemoglobin is an accepted and integral part of the management of patients with diabetes. It is anticipated that in many laboratories Hb\textsubscript{A\textsubscript{1c}} will continue to be reported, using the same units and reference range currently employed, along with eAG. I sincerely hope that the additional information will facilitate communication between clinicians and patients and improve glycemic control in individuals with diabetes.
Often occurring hypoglycemic states of diabetics, which are the result of antidiabetics action (hypoglycemia factitia, factitious hypoglycemia), are treatment complication, not disease complication itself, however they meet all peracute complication criteria. Two basic peracute diabetes complications are diabetic ketoacidosis (DKA) and hyperglycemic hyperosmolar nonketotic syndrome (HHNS).

Diabetic ketoacidosis occurring in patients with type 1 diabetes as the expression of significant insulin deficiency and predominance of activity of so called anti-regulatory hormones (glucagon, catecholamine, corticosteroids), result of which is practical stoppage of glucose uptake by muscles and adipose tissue, increase of liver gluconeogenesis and lipolysis and ketogenesis with cumulation of acetoacetic and β-hydroxybutyric acid in extracellular fluid and metabolic acidosis development. By intense osmotic diuresis, significant hyperglycemia causes hypertonic dehydration leading to decrease of volume both extracellular and intracellular liquid.

Diabetes treatment monitoring is de facto assessment of its chronic complications development risk.

**Laboratory tests in peracute and chronic diabetes complications diagnostics**

**ROLE OF LABORATORY TESTS IN DKA DIAGNOSIS**

Laboratory tests are indispensable to DKA diagnosis, assessment of disorders secondary to hyperglycemia and intensified ketogenesis and treatment monitoring. DKA with characteristic clinical profile (dehydration symptoms and increased breath frequency) is diagnosed on the base of considerable hyperglycemia, glucosuria, ketonemia/ketonuria, metabolic acidosis with increased anionic gap, serum osmolality

**Table 1. Characteristic abnormalities in laboratory tests in Diabetes Ketoacidosis (DKA) and Hyperglycemic Hyperosmolar Nonketotic Syndrome (HHNS)**

<table>
<thead>
<tr>
<th>Tests</th>
<th>Diabetes Ketoacidosis (DKA)</th>
<th>Hyperglycemic Hyperosmolar Nonketotic Syndrome (HHNS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycemia/glycosuria</td>
<td>↑↑↑</td>
<td>↑↑↑</td>
</tr>
<tr>
<td>Ketonaemia/ketonuria</td>
<td>↑↑↑</td>
<td>N/trace</td>
</tr>
<tr>
<td>Gasometry</td>
<td>↑ pH, HCO₃⁻, pCO₂, N/↑ pO₂</td>
<td>Normal/metabolic acidosis in case of peracute renal insufficiency or lactate acidosis</td>
</tr>
<tr>
<td>Anionic gap</td>
<td>↑↑</td>
<td>N/↑ (in case of peracute renal insufficiency or lactate acidosis)</td>
</tr>
<tr>
<td>Serum osmolality</td>
<td>↑↑</td>
<td>↑↑</td>
</tr>
<tr>
<td>Electrolytes</td>
<td>↑/Na⁺, K⁺, Pi</td>
<td>↑↑↑ Na⁺, ↑/Na⁺ K⁺</td>
</tr>
<tr>
<td>Carbohydrate, creatinine</td>
<td>N/↑</td>
<td>N/↑</td>
</tr>
<tr>
<td>Hematocrit, hemoglobin concent-</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>ration, number of erythrocytes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
growth and dehydration features, like increase of hematocrit, hemoglobin concentration and number of erythrocytes comparing to previous results of blood count (Table 1.)

DKA treatment, consisting in insulinotherapy and completing hydro-electrolyte deficiencies, requires strict laboratory monitoring, including control of glycemia, ketonuria, acidic-basic balance, concentration of potassium (usually considerable decrease during treatment), phosphate and kidneys activity index (peracute renal insufficiency hazard). When interpreting results of examination of ketone compounds in urine one should remember that routinely used stripe tests determine acetoacetic acid and do not detect β-hydroxybutyric acid, which is 80-90 percent of ketone bodies in patients with DKA. It may be the cause of apparent intensity of ketonuria with ketone compounds proportion change proceeding during treatment. Because of that, in monitoring of patient recovering from ketoacidosis, determination of β-hydroxybutyric acid concentration in serum/plasma may be performed with photometric method based on β-hydroxybutyric acid dehydrogenase reaction or dry method with amperometric measurement, adapted to determination in full capillary blood. Required frequency of performing these tests vary between 1 hour (glycemia, ketonuria, acidic-basic balance), through 4-6 hours (electrolytes, blood count) to 12 hours (phosphate, kidneys activity index).

### HYPERGLYCEMIC HYPEROSMOLAR NONKETOTIC SYNDROME (HHNS)

Hyperglycemic hyperosmolar nonketotic syndrome (HHNS) is condition of hypertonic dehydration developing in type 2 diabetics, most often in elderly age, as a result of insulin activity deficiency causing hyperglycemia and osmotic diuresis. Dehydration is usually deepened by insufficient liquid consumption. In clinical picture dehydration symptoms dominate, including disorders of central nervous system activity to hipelmolal coma inclusive. In laboratory tests considerable hyperglycemia is found, often exceeding 900 mg/dl (50 mmol/l), increased plasma osmolality, high concentration of sodium, chloride, carboxide, total protein and blood count changes DKA alike (Table 1.) In HHNS result of acidic-basic balance test is proper, if dehydration and hypotension do not cause lactate acidosis and ketonuria does not exist or is insignificant. Treatment, similarly as in DKA, consists in insulin supply and hydro-electrolyte deficiencies compensation, which also requires precise laboratory and hemodynamic control.

### HYPOGLYCEMIA

Hypoglycemic states proceed with disorders of nervous system activity, for which glucose is basic energy material. Symptoms of neuroglycopenia (glucose deficiency in nervous system cells) develop sequentially, in parallel with glycemia decrease, first covering adrenergic stimulation and then progressing dysfunction of central nervous system (Table 2). Appearing in diabetics hypoglycemia factitia is frequent treatment complication, being consequence of disproporion between hypoglycemic drugs action and glucose consumption on the one hand and carbohydrates consumption on the other hand. It is life hazard condition, requiring early diagnose and intervention. Hypoglycemic states of patients with long-standing diabetes complicated by neuropathy may proceed atypically, with faintly expressed or even without early symptoms of andrenergic stimulation, therefore it is so important to detect them during blood glucose level monitoring or glycemia determination performed temporarily.

Laboratory tests are used in diagnostics of two chronic diabetes complications – diabetes nephropathy and diabetes macroangiopathy, so in cardiovascular risk assessment. It is worth emphasizing that both of these complications are strictly connected. In diagnostics of other chronic complications – diabetic retinopathy and various forms of neuropathy – laboratory tests have no use.

### NEPHROPATHY

Diabetes nephropathy develops in about 35% of patients with type 1 and type 2 diabetes. This complication is characterized by proteinuria occurrence and advancing kidneys functionality impairment, often with accompanying arterial hypertension. Early symptom of diabetes nephropathy is little increase of albumin excretion with urine, defined as microalbuminuria. It is strong risk factor of lasting proteinuria and renal insufficiency occurrence, especially in patients with type 1 diabetes. In type 2 diabetes cause of increased albumin excretion may be arterial hypertension, cardiac insufficiency or renal diseases of non-diabetes origin, that is why intensified proteinuria and other features of advancing diabetes nephropathy take place in 60% of patients with microalbuminuria. According to so-called Steno hypothesis, increased albumin excretion with urine is index of systemic damage and vessel endothelium dysfunction and in connection with that, it is cardiovascular diseases risk factor. That hypothesis finds acknowledgement in clinical experience – in all diabetes nephropathy stages incidence of disease and cardiovascular mortality is increased.

**Table 2. Symptoms of hypoglycemia/neuroglycopenia**

<table>
<thead>
<tr>
<th>Blood glucose level</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;3.3 mmol/l</td>
<td>Perspiration, tremor, fear, palpitation, hunger</td>
</tr>
<tr>
<td>2.8–3.1 mmol/l</td>
<td>Mood changes, confusion</td>
</tr>
<tr>
<td>2.5–2.8 mmol/l</td>
<td>Slow down, somnolence</td>
</tr>
<tr>
<td>&lt;1.7 mmol/l</td>
<td>Coma</td>
</tr>
<tr>
<td>&lt;1.1 mmol/l</td>
<td>Convulsions, death</td>
</tr>
</tbody>
</table>

Two basic peracute diabetes complications are Diabetes Ketoacidosis (DKA) and Hyperglycemic Hyperosmolar Nonketotic Syndrome (HHNS)

According to diabetes associations recommendations, each diabetic should undergo examination towards diabetes nephropathy, in type 1 diabetes from fifth year of disease, and in type 2 diabetes from the moment of diagnosis. Diagnostic procedure consisting in assessment of albumin excretion with urine (albuminuria) should follow general urine analysis with the purpose of possible explicit proteinuria or infection of urinary system detection. Detected infection should be healed before proper albuminuria examination. As screening examination there may be performed half-quantitative determination of albumin concentration in single urine portion with use of immunochemical stripe tests. As positive result concentration over 20 mg/l is regarded – it indicates the need of quantitative albuminuria assessment.

Laboratory albuminuria assessment may be limited to quantitative determination of albumin concentration in urine, which gives only information about albuminuria intensity; because of which this examination is performed usually in urine sample taken at definite time, which allows to calculate albumin excretion in time unit (Albumin Excretion Rate, AER; Urinary Albumin Excretion, UAE) expressed in mg/24 hrs or in μg/min. For the sake of circadian variability of albumin excretion, measurement in urine is regarded as golden standard in albuminuria as-

**Focus on diagnostics**
FOCUS ON DIAGNOSTICS

assessment. Difficulties in proper diurnal or night urine collection led to searching other albuminuria indexes. Such commonly used and recommended index is proportion of albumin concentration to creatinine concentration in urine (Albumin: Creatinine Ratio, ACR). This index is calculated on the base of quantitative determination of albumin and creatinine concentration in single urine sample, the best if coming from the first urination of the day. Numerous examinations acknowledged good correlation of ACR with diurnal albuminuria. ACR is expressed in mg of albumin/g of creatinine or in mg/mmol. Results conversion may be done according to the formula:

\[ ACR_{mg/g} \times 8.84 = ACR_{mg/mmol} \]

Currently albuminuria examination based on diurnal or night urine collection is usually used in hospitalized patients or for research purposes, while ACR use is convenient in clinic conditions and in screening examinations, in which ARC is test of choice.

After albuminuria diagnosis with the help of any index (Table 3), the same examination should be repeated twice within time of 3–6 months. Diabetes nephropathy diagnosis may be made only after increased albuminuria detection in at least two of three examination performed.

What decides about correctness of AER assessment is collection of all urine excreted by examined person and delivering it wholly to the laboratory. In the case of night collection it is very important to record time of performing (begin and end) with 1 minute accuracy. Albumin is lasting in urine sample kept in refrigerator, in 2-8°C for 7 days and such are recommended material protection conditions. If urine sample has to be stored for longer time, it should be frozen in below -70°C. In case of turbidities or precipitates in sample, it should be centrifugated before frozen.

For albuminuria examination as default immunochemical methods are used – immunoturbidimetric, immunonephelometric and various methods with use of double antibodies, i.e. immunoenzymatic. In these methods various monoclonal or polyclonal antibodies are used, which is the cause of analytical variability resulting from recognition various epitopes of albumin molecule by polyclonal antibodies, and thus, methods based on them may also detect modified molecules or their pieces present in urine. Suitable applications of immunochemical methods are used also for determination in POCT mode. Immunochemical methods detection threshold is 2-10 mg/l. In albuminuria examination also chromatographic methods are used, mainly size-exclusion (SE) HPLC (molecular filtration, gel filtration).

Recently recommended method of GFR estimation is its calculating (eGFR) on the base of creatinine concentration, age and sex

<table>
<thead>
<tr>
<th>Diurnal urine collection mg/24 h</th>
<th>Night urine collection μg/min</th>
<th>Incidental urine portion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normalalbuminuria</td>
<td>&lt;30</td>
<td>&lt;20</td>
</tr>
<tr>
<td></td>
<td>&lt;20 (15)</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
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<tr>
<td></td>
<td></td>
<td>&lt;20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;30</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>30–300</td>
<td>20–200</td>
</tr>
<tr>
<td></td>
<td>20 (15)–200</td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20–200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30–300</td>
</tr>
<tr>
<td>Macroalbuminuria</td>
<td>&gt;300</td>
<td>&gt;200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;300</td>
</tr>
</tbody>
</table>

In most advanced stages of diabetes nephropathy assessment of proteinuria intensity and selectivity is carried out. In that purpose examination of diurnal protein with urine excretion and electrophoretic examination of proteins in urine are made. Kidneys functionality assessment (glomerule filtration, GFR) may be made by measurement of creatinine or Cystatin blood concentration or examination of endogenous creatinine clearance. Recently recommended method of GFR estimation is its calculating (eGFR) on the base of creatinine concentration, age and sex, using MDRD formula:

\[ eGFR = \frac{186}{[\text{Creatinine}]^{1.154} \times \text{age}^{-0.203}} \]

where:

- \( [\text{Creatinine}] \) – creatinine concentration in serum/plasma in mg/dl;
- conversion: mg/dl = μ mol/l/88.4
- for methods of creatinine determination standardized according to IDMS (Isotope Dilution Mass Spectrometry).

DIABETES AND CARDIOVASCULAR HAZARD

Diabetes macroangiopathy, that means large vessels disease, is in diabetes hastened progress of atherosclerosis, increased incidence of cardiovascular events and increased mortality because of cardiovascular system diseases. Macroangiopathy diagnostics boils to cardiovascular risk assessment. Degree of carbohydrate metabolism compensation of patient decides about this – hyperglycemia triggers numerous mechanisms destroying vessels and promoting atherogenesis and thrombus atherosclerosis complications. HbA1c level is acknowledged cardiovascular risk factor and target indexes of carbohydrate metabolism during treatment process serve also to reduction of that risk.

Significant cardiovascular risk factor in diabetes is specific for that disease (mainly type 2 diabetes) atherogenic dyslipidaemia – increase of triglycerides concentration, decrease of HDL cholesterol concentration and increase of little dense LDL content, there may also appear increase of total cholesterol and LDL fraction. Lipid profile assessment is contained within the range of systematic control of diabetes metabolic compensation, and its target values are regarded as connected with little cardiovascular risk.

As mentioned above, diabetes nephropathy increases cardiovascular risk of patient. Increased albuminuria and GFR decrease below 60 ml/min are significant independent factors of cardiovascular events risk. Detection of microalbuminuria creates possibility of therapeutic intervention retarding diabetes nephropathy advance, which translates into decrease of cardiovascular risk.

Finally, in diabetes with no symptoms of ischemic heart disease and persons with stable angina pectoris information about cardiovascular events risk comes from C-reactive protein concentration (CRP determined with methods of high sensitivity – hsCRP), with assumed values of CRP level severance for low (<1 mg/l), moderate (1–3 mg/l) and high risk (>3 mg/l).

In the end, it is worth emphasizing that diabetes treatment monitoring is de facto assessment of its chronic complications development risk. On the other hand, examinations detecting early phase of diabetes nephropathy and evaluating cardiovascular risk should be integrated with treatment monitoring.
Clinical case study

Section Clinical Cases serves for learning, refreshment and reinforcing Your knowledge.

Haematology case study

Clinical data:
36 year-old male with diagnosed HIV infection, being anti-virus treated. Also diagnosed Burkitt lymphoma affecting lungs and bone marrow. Patient was treated with chemotherapy and obtained complete remission after 2 cycles.

Blood count test result:
Thrombocytopenia (42 G/l) without anemia and leucopenia. L2 alarm emphasizes lymphocyte presence among monocytes.

Circulatory blood smear:

Caution: in this case some number of activated lymphocytes was rated as monocytes.

Case description:
Patient in the age of 26, being type 1 diabetic since child. For couple days weakening, increased thirst, during last two days multiple emesis. Unconscious at the moment of admission. In object examination diagnosed dehydration conditions, arterial blood pressure 85/60 mmHg, Heart rate 110/min., increased breath frequency.

Blood tests at the moment of admission:
- Na 137 mmol/l
- Cl 83 mmol/l
- K 4.0 mmol/l
- pH 7.06
- pCO2 13 mmHg
- HCO3 8 mmol/l
- pO2 140 mmHg
- Glucose 842 mg/l (52.3 mmol/l)
- Ketone bodies in urine (++++)
- BUN 12.8 mmol/l
- Lactate 5.1 mmol/l

Patient with diabetes ketoacidosis caused by stoppage of insulin intake. Dehydration, deepened by emesis, caused hypotony with peracute pre-kidney renal insufficiency and decrease of tissue flow, which caused lactate acidosis. Emesis was the cause of hydrons and chlorions loss (metabolic alcalosis). Insulinotherapy and completion of fluids and electrolytes deficiencies and increase of intravascular volume caused glycemia decrease, compensation of hydro-electrolyte disorder and acidic-basic balance along with increase of arterial blood pressure and renal insufficiency withdrawal.

Biochemistry case study

Laboratory tests at the moment of admission:
- Na 137 mmol/l
- Cl 83 mmol/l
- K 4.0 mmol/l
- pH 7.06
- pCO2 13 mmHg
- HCO3 8 mmol/l
- pO2 140 mmHg
- Glucose 842 mg/l (52.3 mmol/l)
- Ketone bodies in urine (++++)
- BUN 12.8 mmol/l
- Lactate 5.1 mmol/l
Care for your health

Does sugar fortify?

Sugar is for many people included to the group of substances defined as "white death". It is commonly known that sugar harms, some claim that also strongly makes addicted. We often eat it involuntarily, and stoppage of taking it – even if we do not feel addicted – may be very difficult.

Tadeusz Baranowski

Sugar fortifies* tells magnificent prewar slogan of Melchior Wankowicz authorship, encouraging to eating this – as it was regarded at that time – healthy substance, product of domestic sugar refineries. In addition to that slogan, later humorous rhyme was invented: "vodka better". In recent years some of scientists started to prove that such juxtaposition of sugar and vodka, apart from the fact that one and other "fortifies", may have another sense – both substances make addicted.

Sugar harms

It is worth starting from the fact that may be safely called as unquestioned – sugar is harmful. Apart of visible at first sight results of its abuse, such as obesity or tooth decay, also other may be mentioned, some more oblique. Sugar disturbs acidic-basic balance, irritates intestines and stomach, slowing digestion. Besides, sugar in refined version, as a source of pure carbohydrates, delivers empty calories to the organism. It is devoid of any additional values, pure. And eating such causes metabolism problems.

Of course great menace resulting from eating great amounts of sugar is diabetes. When organism gets great amounts of sugar and its concentration in blood increases, pancreas produce large quantity of insulin to lower that level. As a result glucose concentration decreases below normal and appetite for sweets grows. Physical and intellectual efficiency also lowers, for which sweets greatly help. That is vicious circle leading to diabetes and addiction.

Is it addiction?

Majority of nutrition experts is against talking about something like sugar addiction. Argument for such approach is that eating sugar does not meet addiction criteria. They are: so strong attraction to addictive substance that it disturbs normal functioning, impossibility of leaving it, strong mental reaction for the lack of it and also substance intake in continuous and uncontrolled way despite damages causing by it.

It is argument to agree with, but it is possible to make effort to refute it. Actually, it may be very difficult for persons eating much sugar to give it up – this is the trace pointing that still it may be the addiction. In great majority of cases normal functioning disorders are not visible – average Polish eats a lot of sugar, but he does not behave strange because of that, he does not have problems with functioning within the society. But if we consider health results, we may hazard a guess that organism of person abusing sugar does not function normally.

Some scientists – those for whom term "addiction" is proper – indicate apparent neurological changes appearing in sugar eaters and similarity to changes in brains of drug addicts. For sure it is known that something connects sugar consumption with using of other improving mood stimulants. These are reactions in brain. Consumption of something sweet causes in organism the increase of serotonin, one of so-called happiness hormone. Sweets improve mood, cause bliss or positive stimulation. When their effect ends, mood is worsening rapidly and this often causes reaching for the next chocolate bar...

Bitter separation

Decision about giving up sugar is not easy. And not only because that simple reason that we are addicted to coffee or tea sweetening or eating sweets. With less or more pain we may give up this all. The biggest problem stays somewhere else. Sugar is present in places where ordinary consumer does not expect it at all. Giving up of consciously used sugar still will not eliminate it from our diet because we will keep eating it unaware of that.

Real life without sugar is great challenge. It requires aware and vigilant shopping, refusing when on visit and picking out in menu in restaurant. It requires a lot of strong will because even if it is not addiction, this is so tasty and nice... Is it worth this? From the health point of view for sure it is. Giving up white sugar will bring only benefits for our health!
Diabetes incidence in general population has significantly increased during last 20 years. The latest world data show that number of diabetics, which is presently about 140 mln, may double by the year 2025. According to Dutch research, 6.8% Europeans in the age 45-47 has got diagnosed diabetes. Causes of such prognoses are population aging and improper nutrition leading to obesity (too much carbohydrates and fats in the diet) and sitting way of life. Persons, whose fasting blood glucose concentration is in the range of hyperglycemia, constitute high risk group of type 2 diabetes and its complications development. Modification of way of life, including changes in everyday diet by restriction in sugar consuming and products with high hyperglycemic index (potatoes, white bread, pasta, cornflakes, rice, cola type drinks), lowers risk of diabetes development. According to recommendation concerning saccharose consumption by diabetics with proper body weight, may be even up to 10% of daily calories number with assumption that it is distributed to individual meals.

Meal consumption causes increase blood glucose concentration, and next insulin secretion, which intensifies glucose assimilation by tissues and simultaneously decreases its intralivery synthesis. Other neuroendocrine hormone – amylin, synthesized also by pancreas, stops after meal glucagon secretion, and thus, glucose liberation from hepatocytes. Amylin slows down digestion processes in stomach and its emptying, but it also influences on the brain centre, causing feeling of satiation. In type 2 diabetics basic level of amylin is normal, as insulin, but increase of its secretion after meal is not observed.

Excessive hunger, being the cause of too many food consumption during meals and snacks with high content of carbohydrates in them, leads to overweight and obesity with many complications, with more incidence concerning females than males. In females, especially those with overweight, seasonal differences in amount of consumed calories, while in males larger consumption of carbohydrates is observed in autumn-winter season. The cause of increased carbohydrates consumption is lowered catch of serotonin by afferent neurons and centres in brain responsible for appetite regulation. There exist many evidences for connection between alcohol and sweets consumption. It concerns may have partially natural genetic background. This especially concerns alcoholics, of whose it is often family load. Participation of serotonin, opioids and dopamine is suggested as physiologic mechanisms responsible for that phenomenon. In American research it was proved that only one per four persons characterized by prediabetes (fasting blood glucose level 100-125 mg/dl; HbA1c concentration 5.7-6.4%) is aware of that fact. Lack of adequate diagnostics, especially for early stage of prediabetes condition, is serious problem. However, glycated hemoglobin concentration in persons with no diabetes in the range 5.5 – <6.0 % causes increase of ischemic heart disease risk average to 1.23 and ischemic stroke to 1.7, however HbA1c in “high normal” range 6.0-<6.5% increases this risk correspondingly to 1.78 and 2.22. Too much saccharose consumption in diet, independently of its originating source (pure form or as fruite, vegetables or sweets ingredients) is connected with increase of ischemic heart disease risk, especially for women, which was proved in large EPICOR research that included about 48 thousand people (Siri and co. Arch Int Med 2010, 170, 640). Comparing to women with low carbohydrates consumption and to men, those women whose consumption of sugar and products with high glycemic index was much, had ischemic heart disease risk twice higher. It is suggested that this may be caused by differences in metabolism of insulin, insulin-like growth factors, dependent on sex. It is also thought that estradiol influences energy balance and women calories consumption ranges dependent on the phase of menstrual cycle.

Differences dependent on sex were also proved when analysing influence of physical activity on body weight decrease, which is definitely greater if concerning men than women. The way in which physical activity influences concentration of hormones regulating energy balance and appetite is different for men and women. Physical effort concerning women causes increase of acylated ghrelin, stimulating appetite and decrease of insulin concentration, while when talking about men, such effect is not observed (Hagobian and partners Exerc Sport Sci Rev. 2010, 38, 25). Authors explain this phenomenon by defense mechanism, which aim is prevention of energy deficit and maintaining mass of fat tissue for preserving reproduction functionality.

Carbonated drinks sweetened with sugar significantly increase hyperuricemia risk and act disadvantageously on kidney functions, decreasing degree of glomerule filtration (Bomback and partners. Kidney Int 2009). But not only too much content of saccharose and also fructose in diet causes undesirable effects. Fructose, very frequently added to various kinds of drinks, may cause metabolic disorders, increase of uric acid and be chronic kidney disease risk factor (Perez-Pozo Int J Obesity 2009). Giving 200 g fructose to adult men everyday during two weeks caused increase of blood pressure and triglycerides concentration and HDL cholesterol decrease. Despite glycemia being without changes, increase of fasting insulin level and index of resistance to insulin – HOMA.

On the other hand very interesting research results were published this year in March in “Eur Heart Journ” by Bujisje and partners. They proved that regular consumption of dark chocolate with 70% cocoa content is connected with lower brain stroke and heart failure, which may be partially explained by decreased blood pressure. It is estimated that 57% of chocolate consumers chose milk chocolate, while dark chocolate with over 70% cocoa content is chosen by 24% and only 2% choses white chocolate. Flavonoids present in cocoa have advantageous action, so the more their content, the better. Flavonoids contained in chocolate cause nitrogen oxide liberation, contributing to decrease of blood pressure, decrease oxidative stress and advantageously influence blood platelets functions. It was proved that advantageous effects, 48% decrease of brain stroke hazard and 27% decrease of heart failure hazard may be achieved by consumption of only one block (about 6g) of dark chocolate daily. Awareness is necessary that 100 g of dark chocolate contains about 500 kcal and milk chocolate even more, including great amount of carbohydrates and fats.

Type 2 diabetes incidence increases also among children, which results from significant percentage of overweight and obesity within this age group. Diabetes among children, similarly as adult increases complications risk, such as: hyperglycemia, arterial hypertension, albuminuria (Wong and partners Diabetes Care 2010, 33, 512). Results of many-years American research carried out among children of school age, The Bogalusa Heart Study, proved that fasting glucose concentration >-86 mg/dl is predictive factor of appearance of prediabetes or type 2 diabetes in adult age. Such children glucose concentration, defined as “high normal” was accompanied by higher body weight, lower LDL cholesterol concentration and higher of triglycerides. Incidence of prediabetes or diabetics in adult age among children with glycemia >-86 mg/dl is 3.5 and 2 times higher than among those with glycemia of below this value. Interesting is fact that previous research carried among adults gave very close result of optimal fasting glycemia value, that means < 87 mg/dl (NEJM 2005, 353, 1454; Am J Med 2008, 121, 519).

On the base of results of many so far research it may be said that regular glycemia monitoring, both among children, especially those obese and adults has significant meaning, it allows prevent complications suitably earlier.
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Technical data:

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- External and internal barcode readers
- Determination of urgent samples
- Large, color LCD touch screen
- External computer keyboard (option)
- Only 3 reagents for 22 parameters analysis
- Monitoring level of reagents