Relationship between haemoglobin and glucose in type 1 experimental diabetes

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Abstract. 400 million people suffer diabetes around the world. It is the second cause of death in Mexico. The problem is that glucose control is difficult (mainly because of the poor treatment adherence) and the current biomarkers are not efficient enough for the early detection of diabetic complications (before symptoms are present).

The goal of the present study was to use the technique called photoacoustic spectroscopy on blood samples obtained from diabetic male Wistar rats, to detect the optical absorption spectra related to haemoglobin. We assume that such spectra modifications are related to toxic heme, a product of haemoglobin, that could be associated with hyperglycaemia as well as the presence of diabetic complications. We postulate that changes on spectra of blood samples taken after 1 to 10 weeks of diabetes are related to the evolution of diabetes. It is necessary to analyse samples from diabetic animals at longer times, diabetes longer than 10 weeks, to find out if those changes have any relation with complications.

We conclude that photoacoustic spectroscopy of haemoglobin could be used to assess the evolution of diabetes.

1. Introduction

Diabetes mellitus is a group of metabolic diseases caused by hyperglycaemia. The main pathophysiological event is the change in insulin function. Long-term diabetic complications include micro and macro angiopathy, retinopathy, nephropathy and cardiovascular complications which lead to the dead of diabetic patients. [1]

The criterion for the diagnosis of diabetes is based on a fasting glucose greater than 90 mg/dl, a clinical parameter that was chosen because of the appearance of microvascular complications, such as diabetic retinopathy. [1]

Insulin is a hormone produced by the pancreas, and endocrine organ it is located behind the stomach and connected to the digestive tract. Insulin is necessary for metabolism, the process that converts glucose into energy. [2]

Without insulin the glucose produced during food digestion, is not properly used by the organism. In addition, glucose accumulates in the bloodstream and spills into the urine causing glucosuria (the presence of glucose in urine). [2]
Without treatment, the person with diabetes has high levels of glucose in the blood and urine, which over the time cause serious complications in the kidney, the heart, peripheral and central nervous system [2].

There are at least two types of diabetes:

- **Type 1 Diabetes**, known as insulin-dependent diabetes or juvenile diabetes, is characterized by the deficient production of insulin in the body. People with this type of diabetes need daily insulin injections to regulate blood glucose. The cause of the disease is still unknown, but it is known that there is destruction of the beta cells. [1]

- **Type 2 Diabetes**, known as non-insulin-dependent diabetes, in this case the body does not use insulin effectively. The symptoms are very similar to type 1 diabetes, but less intense and there are still cases where these symptoms are not present. In this case, there is no destruction of the beta cells, but people are generally obese, causing some insulin resistance. [1]

The present study was performed on experimental type 1 diabetes because the model (diabetes produced by streptozotocin) is well known and we were interested on the effects of hyperglycaemia and the model is characterized by high levels of glucose.

Haemoglobin is a globular protein, which occurs in high concentrations in red blood cells and is responsible for transporting oxygen to all parts of the body. [3]

The structure of haemoglobin consists of two chains (alpha and beta) and in each of them there is a heme group. The heme group is a porphyrin molecule that contains an iron atom at its centre. This atom is in its ferrous +2 oxidation state and can form five or six coordination bonds depending on the molecular oxygen binding. [3] When haemoglobin is combined with molecular oxygen is known as oxyhaemoglobin and when it does not bind it is known as deoxyhaemoglobin.

There are several laboratory tests to characterize different blood elements. In the present study a non-destructive technique, called photoacoustic spectroscopy (PAS), was performed to obtain the optical absorption spectra of blood and plasma.

The photoacoustic (PA) effect was discovered by Alexander Graham Bell in 1880, but the first theoretical models to explain it appeared until the seventies of the last century. [3]

To observe this effect, gas is confined inside a chamber or cell with the sample inside this cell, it is illuminated with a modulated beam. When the sample absorbs the light, it raises its temperature in a modulated way, causing a flow of modulated heat from the sample to its surroundings, towards the gas confined in the cell. The energy supplied to the gas raises its temperature in a modulated way, causing modulated pressure fluctuations in the gas, which can be sensed by a microphone. [4]

The PA effect in solids gained the interest of some researchers, such as Wilhelm Rontgen and John Tyndall who carried out studies applying the effect in gases, finding that an acoustic signal was produced, when inciting by a modulated light beam in a gas inside the cell. [5]

This technique is very interesting, and a spectroscopic technique was developed with important applications in different areas of knowledge such as physics, chemistry, biology, medicine, among others. [6]

One advantage of this photoacoustic spectroscopy (PAS) technique is the fact that gives a direct measure of optical absorption (it measures the absorbed radiation that relaxes in the form of heat). [3] Another advantage of this spectroscopy is that it is non-destructive, which makes it possible to carry out studies on biological materials "in vivo", as well as electrochemical processes in real time.

Using this technique in medicine, as an application has been compared the information of properties of abnormal body fluids with respect to healthy body fluids.

Different types of biological samples have been characterized, such as the case of blood by Soret in 1883. [7]

The characteristic peaks in blood are shown in Figure 1, the first peaks correspond to the DNA (260 nm) and the aromatic amino acids (280 nm). The second peak corresponds to the heme group (γ)
(420 nm), the third (β) (550 nm) and the last (α) (580 nm) peaks indicate the oxyhemoglobin.

From these peaks, there is a certain relation between the ratio of the optical absorption of the characteristic peaks of blood γ/β and γ/α whose values, has been reported that they are inversely proportional to the concentration of haemoglobin. [7]

![Figure 1. Band of Soret in absorption spectroscopy [3]](image)

2. Materials and methods
2.1 Experimental animals
Type 1 diabetes was produced in male Wistar rats by the administration of streptozotocin (STZ, 65 mg/kg, intraperitoneal). Diabetes was confirmed 72 hours later by blood glucose levels > 200 mg%.

Metabolic control was performed by a time-course of body weight and glycaemia measured weekly from 1 to 10 weeks after STZ. For every time-course experiment were n=3 controls and n=3 diabetics rats.

Blood samples taken at weeks 1, 3, 4, 8 and 10, they were treated with heparin and frozen on dry ice. Photoacoustic measurements were performed in 100 µl.

2.2 Experimental PAS setup
The photoacoustic spectrometer consists of a Xenon lamp, as a light beam source, a monochromator, for the selection of the different wavelengths coming from the lamp, a mechanical optical chopper, to obtain a modulated monochromatic light, at 17Hz fixed frequency. And optical fiber guides the monochromatic and modulated light to the photoacoustic cell. Inside the cell, there is an electret microphone, which captures the pressure variations in the cell, produced by the photoacoustic effect. The sound wave sensed by the microphone is converted to an electrical signal, amplified and sent to a Lock-in amplifier. Finally, the photoacoustic signal is sent and stored on a PC. [8]

![Figure 2. Diagram of the equipment of photoacoustic spectroscopy. [8]](image)

For the blood samples the optical absorption spectra was obtained as a function of the incident wavelength, from 300 nm to 800 nm, the obtained PA signal is directly proportional to the absorption of samples.
3. Results and discussion

Figure 3. Shows statistical study in rats diabetes and control, A) weight and B) glucose

- **Figure 3a**) Weight was assessed by a statistical study two-way (ANOVA) followed by Bonferroni post hoc comparison between diabetic rats and control rats.
  
  By using the PRISMA software the figure 3) was obtained. In figure 3a) it is possible to observe that for 4, 8 and 10 weeks there is a probability value less than 0.01, which is considered as statistically significant [11] and shows that control animals had a significant higher body weight than diabetic animals. Changes are explained by the lack of insulin, which affects the entrance of glucose to the cells and the whole metabolism. In this way the organism eliminates the unusable glucose through the urine, consequently, the patient tends to lose weight. [8]

- **Figure 3b**) Glucose was assessed by a statistical study two-way (ANOVA) followed by Bonferroni post hoc comparison between diabetic rats and control rats.
  
  Figure 3b) shows that diabetic animals had significant higher concentrations of glucose in blood because of the lack of insulin [1] and therefore there is a big difference between the diabetes and the control with there is a probability value 0.0001 it is considered as statistically significant [11]

Figure 4. Shows the spectra of blood for control and diabetic in A) Week 1, B) Week 3, C) Week 4, D) Week 8 and E) Week 10
In Figure 4, the photoacoustic spectra, in a range of 300 nm to 800 nm, in which the Soret peak $\gamma$ (419 nm ± 4 nm), $\beta$ (521 nm ± 5 nm) and $\alpha$ (580 nm ± 3 nm) are observed. There is no apparent difference between the spectra of diabetic compared to control rats before the tenth week, when an increase in the optical absorption spectra at alpha and beta peaks was noticeable in diabetic rat. It was also possible to observe another peak around 300-340 nm which, according to the literature, implicates a change in the configuration of the hemoglobin molecule that goes from Hb-oxygenated to Hb-deoxygenated [12].

Figure 5. The peak ratios A) $\gamma/\alpha$ B) $\gamma/\beta$ were evaluated by means of a statistical study of Two-way ANOVA followed by Bonferroni post hoc comparisons between diabetic and control rats.

Figure 5. Note that for week 10 there is a probability value less than 0.01, which is considered as statistically significant [11].

The PAS spectra in the blood sample show the result in the blood. Note that the ratios $\gamma/\beta$ and $\gamma/\alpha$ in control rats are larger than in diabetic rats, this means, that these ratios are inversely proportional to the amount of haemoglobin. In other words, diabetic rats have a higher concentration of haemoglobin. [7]

In diabetes, this is often associated with elevated levels of haemoglobin, which in turn is associated with increased stress in the kidneys. [8, 9]

The high concentration of haemoglobin could increase blood viscosity [10] resulting in unappropriated distribution of oxygen and consequently stressing the cardiovascular system. Therefore, free haemoglobin could play an important role on the development of diabetic complications.

4. Conclusion

Changes on haemoglobin spectra detected by photoacoustic spectroscopy were evident 10 weeks after diabetes was produced in Wistar rats. Since rats remain hyperglycaemic all over the study (from 1 to 10 weeks after STZ), changes are not related to hyperglycaemia. It is known that complications are developed in the model that we used, 12 weeks after STZ. It is necessary to continue the studies (longer time) after STZ to find out if haemoglobin changes detected by photoacoustic spectra are related to diabetic complications.

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