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THE EFFECT OF MATERNAL HYPOXIA, PINEAL GLAND, PHYSICAL ACTIVITY AND CIRCADIAN RHYTHM ON SERUM LEVELS OF CHOLESTEROL, INSULIN AND GLUCOSE AND ON THROMBIN TIME

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We suggested influence of epiphysis gland on plasma glucose concentration in rabbit pups. Our study showed maternal hypoxia does not influence the effect of pineal gland on postnatal blood glucose. Our results also included the effect of physical activity on thrombin time and coagulation time and also the role of pineal gland on thrombin time in rats. Furthermore, we concluded that dark cycle decreased the serum level of cholesterol, insulin and glucose. However, more research works in relative biophysical and biochemical fields are needed.

Introduction

The pineal gland (epiphysis) synthesizes and secretes melatonin, a structurally simple hormone that communicates information about environmental lighting to various parts of body. Ultimately, melatonin has the ability to entrain biological rhythms and has important effects on physiological function on many animals. Numbers of investigations indicate the direct and indirect effects of melatonin on coagulation system (1-3). Pinotti, et al in the studies concluded that the chronobiological patterns should consider analyzing activity levels of coagulation factors (4). It is known that physical activity induces modification in blood hemostasis and leads to an activation of blood coagulation and fibrinolysis (5). Physical stress is associated with the activation of blood cell (6). Also there are functional inter-relationships between the beta cells of the endocrine pancreas and the pineal gland (epiphysis) where the synchronizing circadian molecule melatonin originates (7). Animal experiments and human epidemiological data show that a wide range of individual tissues and whole organ systems can be programmed in uterus with adverse consequences for their physiological function later in life (8). Animal studies have also demonstrated that the timing, duration, and exact nature of the insult during pregnancy are important determinants of the pattern of intrauterine growth and the specific physiological outcomes (9). Changes in the intrauterine availability of important material including oxygen, program tissue development and lead to abnormalities in adult cardiovascular and metabolic function in several species (10). Oxygen is implicated in the regulation of trophoblast differentiation and invasion (11) thus induction of intrauterine growth retardation (IUGR) by maternal stress such as hypoxia leads to postnatal abnormalities in cardiovascular, metabolic, and endocrine functions (12). It has been showed that maternal hypoxia leads to postnatal disfunctions in many of laboratory animals (13). Evidences suggest that hypoxia can independently contribute to disorders of glucose metabolism. Hypoxemia is an important stimulus for altering autonomic activity, and with larger desaturations causing greater increases in sympathetic activity can influence glucose homeostasis by increasing glycogen breakdown and gluconeogenesis in rabbits (14, 15).

A circadian rhythm is an approximate daily periodicity, a roughly-24-hour cycle in the biochemical, physiological or behavioural processes of living beings, including plants, animals, fungi and cyanobacteria (16). Circadian rhythms are endogenously generated, and can be entrained by external cues, called Zeitgebers. The primary one is daylight. These rhythms allow organisms to anticipate and prepare for precise and regular environmental changes (16). Circadian rhythms are important in determining the sleeping and feeding patterns of all animals, including human beings. There are clear patterns of core body temperature, brain wave activity, hormone production, cell regeneration and other biological activities linked to this daily cycle (Fig 1). In addition, photoperiodism, the physiological reaction of organisms to the length of day or night, is vital to both plants and animals, and the circadian system plays a role in the measurement and interpretation of day length (17). The classic phase markers for measuring the timing of a mammal's circadian rhythm are;1)melatonin secretion by the pineal gland and 2)core body temperature (18). Melatonin is absent from the system or undetectably low during daytime. Its onset in dim light, *dim-light melatonin onset* (DLMO), at about 21:00 (9 p.m.) can be measured in the blood or the saliva. Both DLMO and the midpoint (in time) of the presence of the hormone in the blood or saliva have been used as circadian markers (Fig 1).

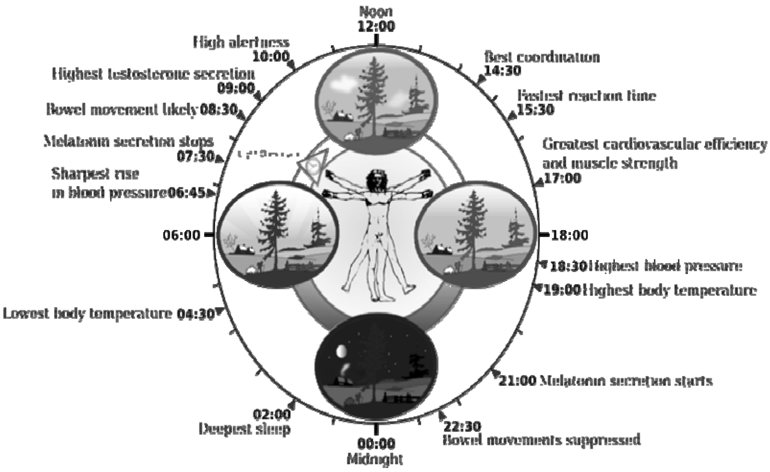

Fig 1. Overview of human circadian biological clock with some physiological parameters

Researchers have discovered that components of the internal molecular clock of mammals have an important role in governing the metabolism of sugars and fats within the body. They found in mice that two of the well-studied proteins in the clock control enable animals to recover from the fall in blood sugar that occurs in response to insulin (19). With attention to these, in this research we studied 1) role of maternal hypoxia interaction with pineal gland effect on blood glucose of rabbit kids 2)effect of physical activity interaction with pineal gland on thrombin time in rats and 3) role of light-dark cycle on serum levels of glucose, insulin and cholesterol.

Materials and Methods

1) Fifteen healthy pregnant rabbits, medium breeds weredivided into three groups. In each group two rabbits were used as controls in which they inspired normal air. The remaining three rabbits as cases were subjected to a 20 minutes daily period of hypoxia for 10 days during first third (1-10 days) of pregnancy for the first group, during second third (11-20 days) for the second group and during last third (21-30) for the third group in which 7% O_2 and 93% N_2 instead of air was passed into the non-poisonous nylon with rubber materials bag. Case animals have been placed in *baro camera* with dimensions; 30, 30, 40cm. Sixteen newborn rabbit pops (8 controls and 8 cases) in each group grew up until 30-days. Epiphysectomy operation was performed on the 31st day of life at all of rabbit pups born from pregnant mothers. Blood samples of rabbit infants were collected from marginal vein of ears and plasma glucose was determined one day before and ten days after epiphysectomy by *Hagedorn-yensen* method.

2) Sixty healthy 30-day-old male rats were selected. All animals were supervised in the animal care facility for at least 15 days before any studies. Animals were used under ethical approval of department. They were divided into two groups; control group without surgical intervention (epiphysectomy) and case group with surgical intervention. In each group subjects were divided into three subgroups; control (without physical activity), short-time (5 minutes physical activity) and long-time (with 20 minutes physical activity). Thrombin time was measured for each tissue. Epiphysectomy surgery was performed by *Aulov* method on case subjects. Animals anesthetized by Ketamine (75 mg/kg) and Xylazine (10 mg/kg)

3) Thirty rats were randomly divided into three groups and treated with three kinds of light (Absolute light, Absolute darkness and Normal light) for 14 days . on 14th day, the levels of serum glucose, cholesterol and insulin were measured by enzymatic method.

Results

1) The mean values for plasma glucose concentrations were 106.71±8.00 and 114.21±13.04 mg/dl in case and control groups in all of thirds of pregnancy respectively before epiphysectomy. After epiphysectomy glucose concentration decreased at all of rabbit pups of both case and control groups (Table 1).

Table 1

Plasma glucose concentration before and after epiphysectomy in all of rabbit pups according to group

Group	Measurement time	Mean	SD	P-value
Case N=24	Before Epiphysectomy	106.71	8.00	P<0.001
	After Epiphysectomy	95.67	10.04	
Control N=24	Before Epiphysectomy	114.21	13.04	P<0.001
	After Epiphysectomy	97.17	19.38	

Total N=48	Before Epiphysectomy	110.46	11.35	P<0.001
	After Epiphysectomy	96.42	15.29	

2) There was a significant difference in thrombin time on all of different tissues according to epiphysectomy. Most difference was observed on liver and least one was found on heart tissue (Table 2). Analysis of data also showed significant differences among subgroups according to physical activity. Both short-time and long-time physical activity decreased thrombin time on different tissues of animals but long-time is more effective than short-time.

Table 2

Comparison of thrombin time (second) in different tissues according to epiphysectomy status

Group / Tissue	Control (Intact) (N=10)		Case (Epiphysectomized) (N=10)		P
	Mean	SD	Mean	SD	
Blood	29.50	3.88	23.50	5.40	<0.01
Liver	41.00	4.90	18.10	6.80	<0.001
Heart	20.00	2.10	16.50	2.47	<0.01
Spleen	21.10	3.00	14.80	3.85	<0.001
Total	27.90	5.50	18.20	2.620	<0.001

3) Comparison of serum glucose, cholesterol and insulin levels in before of experiment did not showed significant differences among treatment groups i.e. random allocating of the rats to groups was correct (Table 3). Results obtained for the absolute darkness group were significantly different in glucose, cholesterol and insulin levels with that of two other groups.

Table 3

Serum glucose, cholesterol and insulin levels in before, 7th and 14th day of experiment

Experiment	group	Glucose(mg/dl)	cholesterol(mg/dl)	Insuline(μg/lit)
Before	Absolute light	76.80 ± 8.93	63.80 ± 4.85	1.26 ± 0.27
	Absolute dark	83.30 ±13.09	59.00 ± 7.62	1.23 ± 0.30
	Normal light	85.30 ± 9.55	73.50 ± 13.62	1.17 ± 0.39
7th day	Absolute light	114.10 ± 11.84	64.00 ± 3.91	1.19 ± 0.39
	Absolute dark	78.30 ± 9.39	56.10 ± 5.06	1.03 ± 0.26
	Normal light	92.10 ± 9.73	72.20 ± 8.25	1.21 ± 0.42
14th day	Absolute light	130.00 ± 18.86	63.50 ± 6.62	1.35 ± 0.59
	Absolute dark	70.00 ± 10.13	53.40 ± 3.17	0.92 ± 0.10
	Normal light	93.40 ± 11.40	64.10 ± 7.22	1.35 ± 0.46

Discussion

1) The results of our study indicated decreasing effect of epiphysectomy on plasma glucose in 30 days-aged postnatal rabbit pups.

Gorray, et al showed A significant hypersecretion of insulin in the pancreatic islets from the pinealectomized animals (20). Some results substantiated the observation of hyperglycemia because of the application of pineal extracts (21). In another study Single melatonin injection caused hypoglycemia in a newly-hatched parakeet and adult pigeon, and hyperglycemia in a newly-hatched pigeon (22). These data suggest that melatonin can exert an influence on the secretion and/or action of insulin; however, studies on pinealectomised animals have demonstrated contradictory results, such as reduction of blood glucose and hyperinsulinemia, or low basal insulin levels and hyperinsulinemia under certain photoperiod and feeding conditions in pinealectomized animals (23). Also Aliyev, et al showed blood glucose elevation from 24 hour to 15 days after epiphysectomy but it was decreased 20 days after it (24). Some reports on avian species are ambivalent in this respect as well. Whereas John et al. stated in 1983 that melatonin did not lead to an alteration of the blood glucose levels in turkeys, a later result from 1990, on pigeons, confirmed the blood glucose-increasing effect of melatonin after application. Particular attention should be given to those publications that take the age of the animals under examination or the duration of the photoperiod into consideration. For example, melatonin caused hyperglycemia in newborn pigeons, whereas, in the adult bird, hypoglycemia was detected (21). Results of another study indicated plasma glucose elevation by mid-light intraperitoneal injection of melatonin. Thus, melatonin may act directly on the liver to elevate the plasma glucose level, and changes in plasma glucose level itself may in turn affect hepatic melatonin binding (25). Furthermore, it has been published that high melatonin levels, because of blinding, or of exogenous melatonin application, raise blood glucose levels, whereas blood glucose levels decrease and insulin level increases because of pinealectomy (21).

2) The results of our study showed a decreasing effect of pineal gland on thrombin time in rats.The findings provide preliminary support for a protective effect of melatonin in reducing the atherothrombotic risk (26). Wirtz and et al showed a dose-response relationship between the plasma concentration of melatonin and coagulation activity (27). It was showed exogenously administered melatonin normalizes the activated blood coagulation (2). Numerous studies of melatonin, by now widely acknowledged as a circadian rhythm-affecting neurohormone, is capable of promoting platelet production by megakaryocytes, of acting on the latter's ion channels by way of the outward currents, and of performing a physiological anti-aggregation function thus lengthening platelet life span(1).

Results of our study also showed decreasing effect of short-time physical activity on thrombin time in different tissues. Hilberg and et al showed that maximal short-time exercise does not lead to a relevant activation of blood coagulation in healthy young subjects. It is only slightly altered within the normal rang. In this study immediately after exercise, a shortening of a PTT was seen (28). There are conflicting results about the effect of the exercise on prothrombin time and thrombin time. Most of them showed no demonstrable effect on prothrombin time, although some have shown a significant shortening of thrombin time (29). Swimming caused activation of the clotting system by increasing fibrinolytic activity (30).

Our results also showed long-time physical activity has a more decreasing effect on thrombin time than short-time physical activity. The effect of muscular exercise on blood coagulation has been the subject of several investigations in both man and laboratory animals and relative results have indicated that coagulation is accelerated immediately after muscular exercise (31). Several studies have shown that strenuous exercise leads to a shortening of the activated partial thromboplastin time (32).

3) The investigators demonstrate a role for the circadian clock proteins, Bmal1

and Clock, in regulating the day-to-day levels of glucose in the blood. Suppressing the action of these molecules eliminates the diurnal variation in glucose and triglyceride levels. In addition, they found that a mutated Clock gene protected mice from diabetes induced by a high-fat diet. Some years ago, a team led by senior author Garret FitzGerald, MD, Chairman of Penn's Department of Pharmacology, discovered a molecular clock in the heart and blood vessels. "We noticed a variation in the recovery of blood glucose with clock time," says Dan Rudic, PhD, a Research Associate in the Department of Pharmacology and a lead author on the current study. Food is also an important cue in directing the daily oscillations of metabolism and blood-sugar levels. The molecular clock genes work somehow to orchestrate this complex system. However, when this finely tuned scenario is upset, all-too-familiar diseases arise: diabetes when there is too much sugar; hypoglycemia when there is too little (19).

What's more, the researchers found that a high-fat diet amplified the oscillation in blood sugar over a 24-hour period and that disabling the Clock gene markedly reduced this effect. Poor dietary habits and a sedentary lifestyle have been linked to diabetes, high blood fats, and high blood pressure, all characterized in an epidemic called metabolic syndrome, which is reaching alarming proportions in both developed and developing countries, says FitzGerald. This work adds to the understanding of physiological control of metabolism and therefore possibilities of working with the body's natural rhythms to fight disease (19).Melatonin significantly reduced cholesterol absorption in rats fed on HCD and caused significant decreases in total cholesterol, TG, VLDL- and LDL-cholesterol in the plasma and contents of cholesterol and TG in the liver. The level of HDL cholesterol was significantly increased after melatonin. These results suggested that inhibition of cholesterol absorption caused by melatonin could be a mechanism contributing to the positive changes in plasma cholesterol, lipoprotein profile and the lipid contents in the liver (33).

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EPİFİZ VƏZİ, FİZİKİ YÜKÜN, SİRKAD RİTMİN HİPOKSİYADAN SONRA QAN PLAZMASINDA XOLESTERİNİN, İNSULİNİN, QLÜKOZANIN SƏVİYYƏSİNİN DƏYİŞMƏSİNƏ VƏ TROMBİN VAXTINA TƏSİRİ

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XÜLASƏ

Epifiz vəzinin ifraz etdiyi melatonin hormonu mühitin işıqlanma amilinin bədənin müxtəlif hissələrinə təsirinə cavab olaraq sintez olunur.

Bu sahədə apardığımız təcrübələrimizdən məlum olur ki, postnatal ontogenezdə hipoksiya şəraitində qanda şəkərin miqdarında, fiziki işin və epifizektomiyanın təsirindən sonra toxumalarda trombin vaxtında işıq və qaranlıq fazasından sonra qanda xolesterin, insulin, qlikozanın sirkad ritmində statistik qanunauyğun dəyişikliklə müşahidə olunur.

ВЛИЯНИЕ ЭПИФИЗА, ФИЗИЧЕСКОЙ НАГРУЗКИ, ЦИРКАДНОГО РИТМА НА ИЗМЕНЕНИЕ УРОВНЯ ХОЛЕСТЕРИНА, ИНСУЛИНА, ГЛЮКОЗЫ И ТРОМБИНОВОГО ВРЕМЕНИ В ПЛАЗМЕ КРОВИ ПОСЛЕ ГИПОКСИИ

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РЕЗЮМЕ

Мелатонин, выделяемый эпифизом, синтезируется в ответ влияния све-тових факторов среды на различные органы иткани организма.

Эксперименты, проведенные в этой области, показали, что в постнатальном онтогенезе в условиях гипоксии количество сахара в крови, после физической нагрузки и эпифизэктомии тромбинового времени в тканях, при различных условиях освещенности на циркадный ритм холестерина, инсулина, глюкозы крови наблюдаются статистические закономерные измерения.