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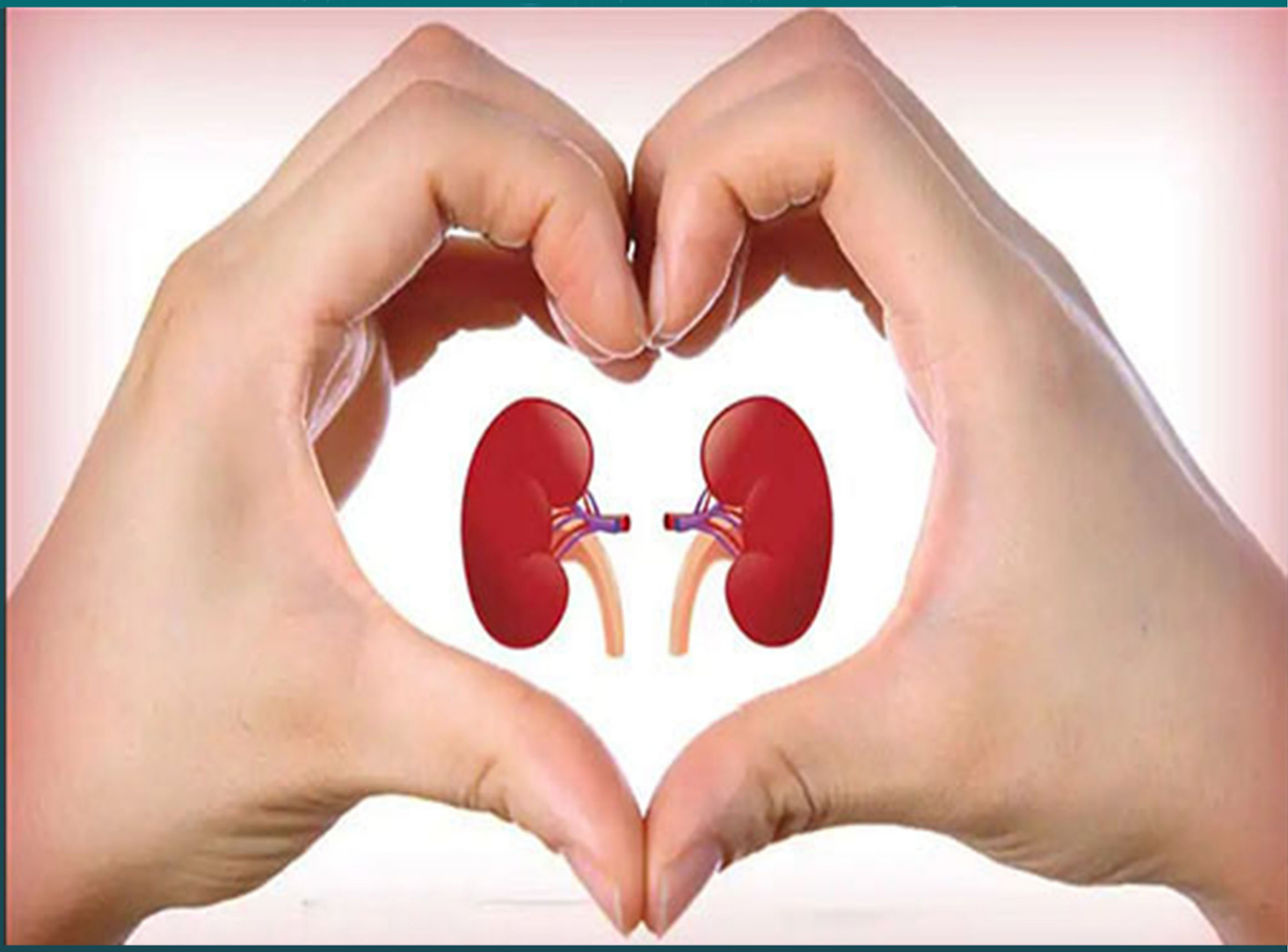

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ARTICLE

Processed Radio Frequency towards Pancreas Enhancing the Deadly Diabetes Worldwide

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ABSTRACT

Diabetes is a chronic and debilitating disease, which is associated with a range of complications putting tremendous burden on medical, economic and socio-technological infrastructure globally. Yet the higher authorities of health services are facing the excruciating cumulative reasons of diabetes as a very imperative worldwide issue in the 21st century. The study aims to relook at the misapplication of the processed radio frequency that frailties in the pancreas within and around the personal body boundary area. The administered sensor data were obtained at laboratory experiments from the selected specimens on dogs and cats in light and dark environments. The study shows the frequent urine flow speed varies with sudden infection due to treated wireless sensor networks in active open eyes. The overweight and obese persons are increasingly affected in diabetes with comprehensive urinary pressure due to continuous staying at dark environment. The findings replicate the increasing tide of diabetes globally. The study also represents the difficulties of physicians to provide adequate diabetic management according to their expectancy due to insecure personal area network control unit.Dynamic sensor network is indispensable for healthcare but such network is at risk to health security due to digitalized poisoning within GPS positions. The study recommends the anti-radiation integrated system policy with user's security alternative approach to inspire dealing with National Health Policy and Sustainable Development Goals 2030.

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1. Introduction

Diabetes is a non-communicable and simple disease. But its media creates phobia belongs to patient's condition. This disease continues long-term condition with a major impact on the lives and well-being of individuals worldwide [1]. This disease also affects persons of all ages and races [2], which is a leading cause of mortality and reduced life expectancy [3]. It is a deadlier disease than coronavirus disease, with 2.5 million people dying from corona in one year but 4.2 million dying from diabetes [4]. Diabetes is one of the deadliest silent diseases in history with a major consequence on the lives and well-being of individuals, families and societies worldwide. It is among the top 10 causes of death in adults and was estimated to be USD 727 billion for global health diabetes expenditure[4]. The cutting-edge sensor technology affects on augmenting causes of diabetes [5]. Wireless sensor networks have unlocked up new scenarios in healthcare systems [6];[7].This network is actually valuable in several medicare applications, which can be inserted into human body for healthcare services [8];[9];[10];[11]. Sensor networks are planned to fulfill the scarcities like measurement, tracking, detection and data classification [12], particularly the field of non-communicable diseases like diabetes. Flexible and wearable health-monitoring provides a revolutionary technology [13];[14], which serves as an alternative to traditional diagnosis methods, putting healthcare data on a path that is more remote, portable, and timely [15];[16];[17];[18]. These healthcare data can be used by a physician to evaluate body condition like diabetes with an artificial intelligence (AI), internet of everything and deep-learning algorithm [19];[20];[21].

Wireless sensor network has a great advantage on diabetes to identify the classical symptoms of polyuria, polydipsia and polyphagia. Diabetes is a chronic progressive debilitating disease that occurs when the pancreas is not adequately enough able to produce insulin or when the body cannot utilize the insulin that produces inside the human body due to insulin resistance [22];[23];[24]. This disease is a pancreatic disorder, whose prevalence is increasing day by day [25]. The effects of the disease have spread rapidly to the human body from the last 20 to 25 years, which has not increased this much in the history of the world in any other decade. The main reason is the misuse of mobile technology with global positioning systems and global navigation satellite systems. The mobile phone is intimately involved with the body, without which we are not. Many people are suffering from this disease, especially as the frequency of radio frequency is increasing. For example, regular painful conditions in the body - unhealthy eating, western lifestyle [26];[27]. Beyond the effects of diabetes, excessive abuse of radio frequency, abnormal thirst and hunger, sudden urinary pressure and urination immediately become cloudy. The causes of diabetes are:

(i) radio frequency consumption [28];
(ii) obesity [29];
(iii) birth [30];
(iv) genitalia [31];
(v) pancreatic abnormalities [32];[33];
(vi) liver abnormalities [34];[35];
(vii) irregular eating and living conditions [36];[37];

Several researches have summarized the progress of flexible electronic devices and their applications in health-monitoring [38];[39];[40];[41];[42];[43]. Human body with diabetes has an augmented risk of increasing a number of severe health problems [44];[45]. Moreover, people with diabetes have an increased risk of developing a number of serious health problems [46]. Diabetes is a condition that impairs the body's ability to process blood glucose[47]. Consistently high blood glucose levels can lead to a high risk affecting the heart and blood vessels, eyes, kidneys, nerves and teeth [22]. According to WHO [23] that the adults with diabetes have a two to three fold augmented risk of the major cause of heart attack and strokes [48]. Severe acute respiratory syndrome coronavirus infection itself might represent a worsening factor for people with diabetes [49]. According to Health Hub [50], the diabetes is a medical condition in which the blood glucose levels remain persistently higher than normal [51] due to exposing to electromagnetic effluence cause higher plasma glucose level [52].

The study aims to assess the misusing of processed wireless sensor networks towards pancreas within the body boundary and GPS position to identify the individual's frequent urinary infection tends to prone diabetes.

2. Research Methods

2.1 Study Site

The study site is at laboratory and GPS field positions

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of Universiti Malaysia Sarawak (UNIMAS), Malaysia. The GPS positions include longitude, latitude and ellipsoid height in dark and light environments. There are several parameters to collect primary and secondary data from diverse sources including ISNAH Experiment, species selection with body mass index, tracking process in light and dark environment, data compilation and analysis. The method was conducted at as PhD research work from October 2014 to October 2017 at the UNIMAS, Sarawak, Malaysia.

2.2 Sample Size and Study Design

The sample size was 14 individuals among two species viz. cat and dog, showed in Table 1. All specimens were housed in a room with controlled temperature 36.4°C in cat and 36.7°C in dog with breathing rates, respiration, blood pressure and feline body mass index [53].

Table 1. Sample category of cat and dog

Specimens	Feline Body Mass Index (FBMI)		
	Underweight	Normal weight	Overweight & Obesity
Cat	3	1	3
Dog	3	1	3

The experimental design were randomly divided into three experimental groups with Feline Body Mass Index: (i) Underweight, (ii) Normal weight, and (iii) Overweight and Obesity. The study design was linked with different parameters, such as: physical parameters (Table 2), specimen's selection, Impact of Sensor Networks towards Animals and Human beings (ISNAH) experiment, data collection and compilation, data analysis and interpretations. The study design showed different parameters in Figure 1.

Table 2. Sample specimens with physical parameters

Specimens	Physical Parameters				
	FBMI	Breathing rate	Respiration	Temperature	Blood pressure
Cat	24.2 (avg)	210 bpm	per minute 23	36.4°C	121/175 mmHg
Dog	24.7 (avg)	192 bpm	per minute 25	36.7°C	122/180 mmHg

It observed the impact of wireless sensor networks towards pancreases among them in the light and dark environments. The study necessitates an integration of methods used in wireless sensor networks towards animals' body and identified its implication. This envisaged the research taking in matter-of-fact research elements to investigate issue hoisted in the study, primarily targeted at SMART devices like telematics' users towards specimens. Telematics is a smart device, consists of a scanner, Global Positioning System (GPS) and Global Navigation Satellite System (GNSS). The fieldwork conducted in the studied area within January 2015 to January, 2017. The tracking design was shown with different components in Figure 1.

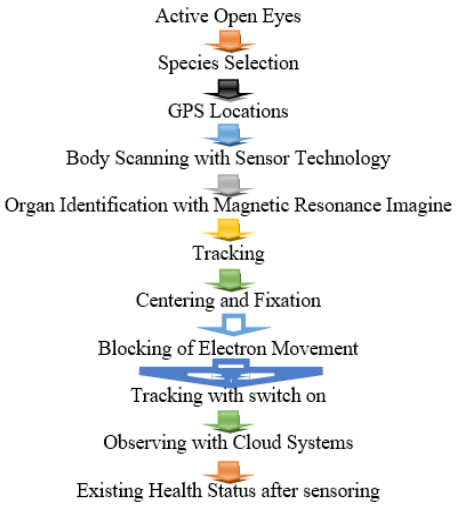

Figure 1. Tracking Design

2.3 Tracking Process

ISNA Experiment implies the experiment on the impact of Sensor Network on Animals (ISNA). The cyber tracker misuses sensor technology to augment non-communicable diseases among animals and human body [54]. The study examined into two specimens, one is dogs and another one is cats among 14 individuals for identification of this misuse application. These animals are available in the study area and suitable for experiment. The study selected sound health two species with Feline Body Mass Index (FBMI), breathing rate, body temperature, respiration and blood pressure measurement in light and dark environment. For the study of FBMI calculation, the study was used web calculator through using rib case circumference and length of the lower back leg from the knee to the ankle [55].

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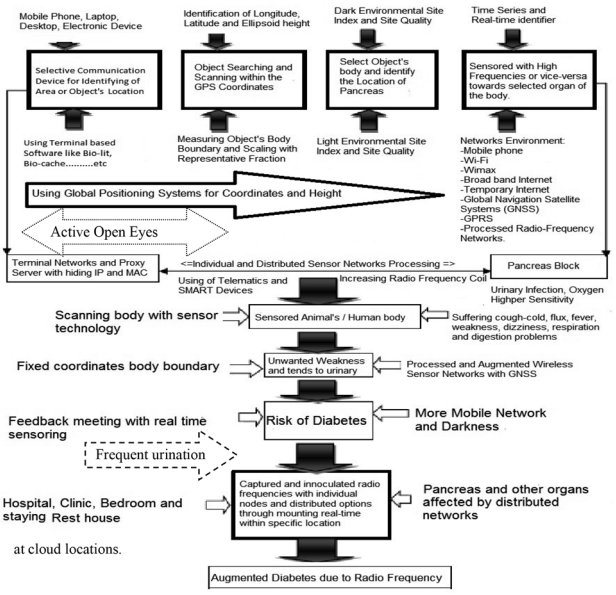

Figure 2. ISNAH Process [5].

The experiment took in dark and light conditions. The specimens stayed in specific geographic location and put the individual inside the iron case (size: 3.5′x 2′x2.5′). Then measurement of individual's coordinates location includes longitude, latitude and ellipsoid height with GPS and GNSS identifiers. From the field observation, the Automated Radio Telemetry System is more effective in dark than light environment. For this purpose, the study has examined the system with on (i) smart cell phone, (ii) telematics device, (iii) iron cage and (iv) individual species separately. The ISNA experiment interlinked with tracking process. This process included several steps which enhanced to fulfill the Sensored observation. The study was observed the physical conditions including non-communicable diseases of animals like diabetes affected by the telematics device through misapplication radio frequency through tracking process as shown in Figure 2. Different stages of Tracking Process of Radio Frequency towards animals are listed as below: (a) Selective communication devices, (b) Searching object and scanning of individuals body organ, (c) Identify body organ and light and dark environment, (d) Sensored the specimens with high, normal and low radio frequency, (e) Observed and compared the specimens status, (f) Feedback meeting and illustrated the consequences at result and discussion.

2.4 Data Accumulation

All primary and secondary bio-sensor data were collected through ISNAH experiments from GPS positions while secondary data were gotten from miscellaneous sources. All accumulated data were assembled with updated software for analysis according to the objectives of the research.

2.5 Data Analysis and Interpretation

The compiled and processed data were involved in the preparation of data master sheet and assimilated into suitable systems used in the results and other segments consecutively. The data were analyzed for presentation and interpretation using standard data analysis software like MS Office Suite 2019, R version 3.4 and SPSS version 26.

All general information regarding the occurrence of specimens, status and affected condition were checked for accuracy from the different sources and sources of information were also verified by the higher authority of University Senate, UNIMAS, Malaysia.

3. Result

The species was identified the fixed location from the combination of retina scanning and GPS sensoring. Then the species was fixed in numbness body from GPS sensor scanning and tracking at pancreas through wireless sensor networks. The study identified species location from sensor device at light and dark environment conditions. Individual's location is recognized with body reflection through sensor technology within GPS coordinates. Using optical distance through processed radio frequency, the sensor device scanned individual's location at species longitude, latitude and ellipsoid heights.

3.1 Pancreas Scanning and Tracking

Due to tracking of processed radio frequency towards pancreas of sensored species in dark and light environments, they felt urination within averages 11 minutes and 18 minutes respectively. When range increasing of processed radio frequency, their urination pressures were also increased and urinated instantly. The experiment observed that the sensor-affected dog and cat felt urinary infections due to dissemination of processed radio frequencies with sensor GPS positions with active eyes within body boundary areas, which as shown in Figure 3. The overweight and obese species suffer in diabetes at more time within dark environment but less in light environment. In the time of medication, the species were altered body temperature and due to pain at sciatica, and blocked pancreas due to fluctuated wireless sensor networks. The findings are also observed from ISNA Experiment that the both species were felt in body complications including: (i) weakness, (ii) sudden weight loss, (iii) reduced nutrition absorbtion, (iv) persistent urinary pain, (v) sudden teeth

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grinding, (vi) frequent comprehensive pressure in micturition.

Once the location of the selected person is determined, his or her entire body is scanned, then the scanned body is sensed to a specific organ (such as the pancreas), then tracked and digitally poisoned at the coordinates point of the GPS location to block blood, air and liquid substances and obstructs the movement of water, air and blood etc. like electrons transmission. The person immediately experiences a urinary tract infection and the urinary comprehensive blood pressure rises, leading to psychological problems such as anxiety and depression. If the person then stays in the designated digital poisoning place, the urinary disorder later turns into diabetes. If the person closes tightly their eyes immediately after feeling a urinary tract infection, wears black sunglasses and dress and quickly moves away, or is placed in another position, the person will not suffer in diabetes. Again, if he/she is suffering from diabetes for any reason, all those involved in its services and treatment, especially doctors, nurses, lab technicians and relatives will wear sunglasses and no one including the patient will be able to keep a mobile phone with everyone. If it is too low, it will have a serious effect on the patient's diabetes. Everyone must be aware of this.

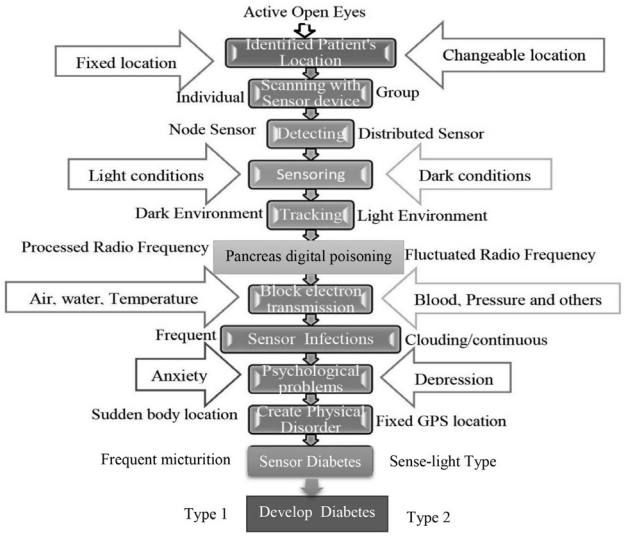

Figure 3. Occurring Sensor Diabetes due to processed wireless sensors

3.2 Light Environment

Individuals suffered in sensor diabetes in light environment, which was observed in cat and dog through the study at longitude, latitude and ellipsoid height within the tracking time. The study illustrated the diabetes occurring period at clouding system, which is indicating the equation through regression analysis. The equation expressed the variable approaches as below:

$$y = -14x^2+54x-25 \quad \text{(i)}$$

$$R^2=1 \quad \text{(ii)}$$

The value 'y' indicates the diabetes occurring period on the pancreas of the species, where the value of 'x' indicates the sensor effect time among underweight, normal and overweight of body mass index at the sufficient light conditions. The (i) equation has stated in the step of R^2 (co-efficient of multiple factor of 1 with standard error of approximation on detected value. The value of R^2 is equivalent to nearly 1, which showed the tracking time was exaggerated towards individual's ampulla of vater in pancreas. So, the definited equation is accepted. The documented equation was formerly active to stirred individual's diabetes responsiveness regarding processed and mixture wireless sensor networks towards underweight, normal weight and overweight individuals. The tracking time also showed the polynomial line in Figure 4. The light environment is suitable for diabetes patients according to polynomian value.

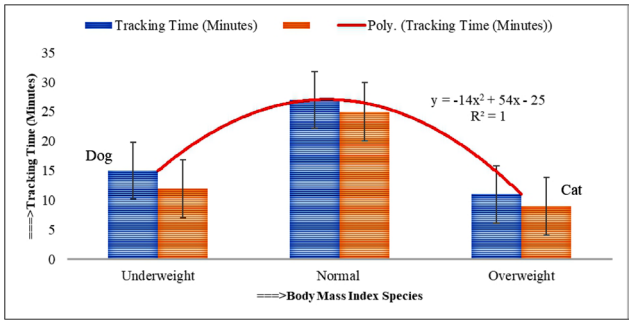

Figure 4. Processed wireless sensor networks tracking time in pancreas of different BMI Individuals at light conditions.

3.3 Dark Environment

From the ISNAH experiment in a dark environment, the study observed that overweight individuals were suffered in diabetes less time. The tracking time was 5 minutes and 7 minutes between cat and dog respectively. But underweight cats affected in diabetes within 6 minutes and dogs in 8 minutes. The normal weight took more time, particularly 12 minutes for cat and 14 minutes for dog, which is shown in Figure 5. The wireless sensor network is prone to active in dark environment towards dog and cat. Here the study stated the tracking method with the following equations as:

$$y = -3.6053x^2+13.342x \quad \text{(i)}$$

$$R^2=0.7778 \quad \text{(ii)}$$

The equations indicate "y" and "x" two variables with

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tracking time towards dog and cat in a dark environment. The individuals were underweight, normal weight and overweight to compare the affected time among them. Equation (i) has an attuned R^2 (co-efficient of multiple determinant of 0.7778 with standard error of estimation on experiential value (Figure 5). The value of R^2 is near to 1, which showed sensored time was affected towards individual's body, specifically in pancreas. So, the definited equation is accepted.

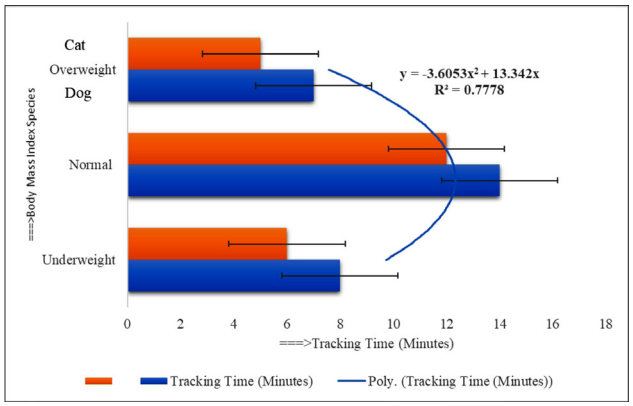

Figure 5. Processed wireless sensor networks tracking time in pancreas of different BMI Individuals at dark environment.

3.4 Occuring Steps of Sensor Diabetes

There are several steps to occur diabetes due to misusing of wireless sensor technology at GPS positions. Firstly, identification of individual's body with GPS, wireless sensor technology and active open eyes. Then the whole body scanning with MRI and GPS Innoculation Sensor and select the pancreas. Again identification the ampulla of vater inside body and blocked its edge with processed radio frequency. As a result, the electron transfer was blocked at pancreas. It obstructs the transmission of bile duct or electron in various parts of the pancreas's body, and impedes the flow of electrons in the body, and it also hampers the optimum growth of insulin in pancreas. When ampulla of vater was blocked by tracking with processed wireless sensor networks within GPS positions, then individual felt uneasy, tiredness, sleepiness, weight loss, weakness and abdominal pain. After certain time, the weak individuals suffer in fluctuating urination within the network boundary of clouding system, which tends to sensor diabetes successively type-1 or type-2 and other problematic functions in pancreas (Figure 6). Again, tracking continues in bladder, she/he suffers frequent comprehensive urination. It is mentioned that ampulla of vater is the 2nd part of duodenum, where pancreatic duct and common bile duct open.

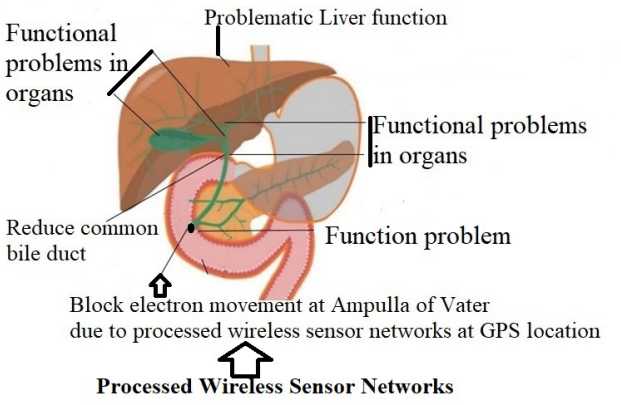

Figure 6. Blocking electron movement at the ampulla of vater with processed wireless sensor networks

Thus, individuals were suffered from symptoms of diabetes, which as shown in Figure 7. In this way, individuals fall ill and tracked several times; eventually expose to die due to processed radio frequencies. Due to wireless sensor tracking, individuals were sick twice times in the light than dark environment. Sensor technology, however, is more sensitive to dark environments than light to make these animals sick. If the time and frequency of the experiment could be increased or doubled, it would have a negative effect on the animals/ human beings.

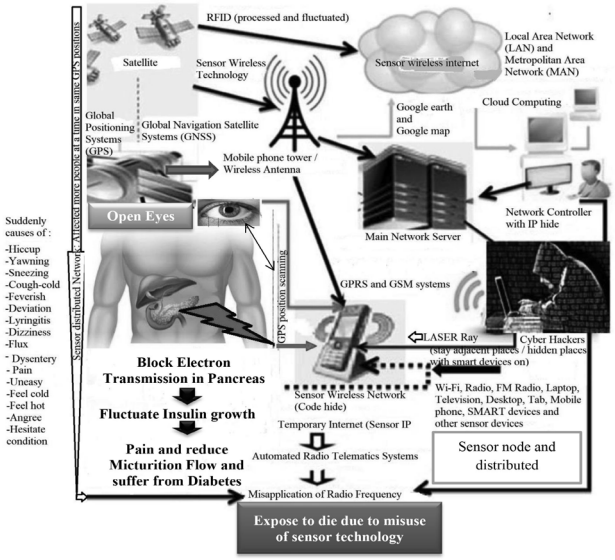

Figure 7. Different Steps to augment Sensor Diabetes due to Wireless Sensor Technology

3.5 Status of Wireless Sensor Network Security

The study continued the collection opinions on the wireless sensor networks security systems stated on participant's perception among three categories including secured security system, restricted location and no

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comment. Approximately 96% of respondents opined for effective secured security systems, 3% restricted location and 1% no comment, which as shown in Figure 8. For sound health and sound minds, the secured wireless sensor networks are essential for the present and upcoming users. Because, we can't move a single moment without WiFi, wireless and mobile phone.The study represents the secure wireless sensor networks for free from different sensor diseases among human beings and animals. The research also illustrates that the secure wireless sensor networks help individual taking effective decision due to free from unwanted tension and sound health network. This sound mind in health connected with neuron networks to help lower blood sugar levels and improves overall glucose tolerance, body's insulin secretion and insulin sensitivity, which can help better control of diabetes.

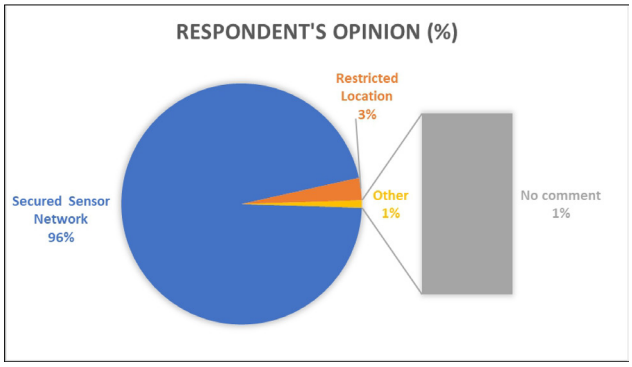

Figure 8. Security perception of wireless sensor network among participants

3.6 Global Diabetes Patients Status

Diabetic patients are increasing every year worldwide due to affecting of processed radio frequency within GPS body boundary. There were about 151 million diabetic patients in the year of 2000, while it was increasing prevelance 463 million in 2019 (Figure 9), which is alarming to all living human beings.

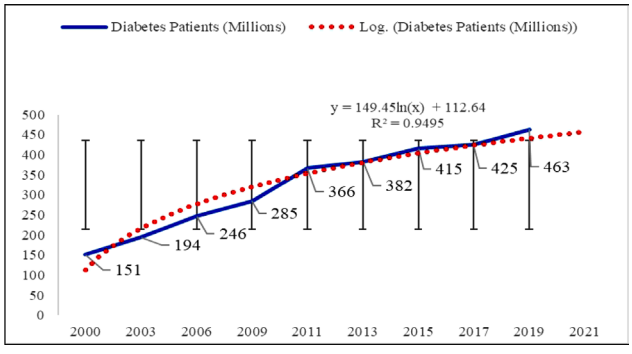

Figure 9. Increase Prevelance of Diabetes Worldwide

Here the study expressed the approach through the following equation,

$$y = 149.45\ln(x) + 112.64 \quad \text{(i)}$$

$$R^2 = 0.9495 \quad \text{(ii)}$$

Where, y is the affected time on sensor users and x is the affected time in diabetes. Sensor users and subscribers increase and diabetes disease also increases in successive year.

Equation (i) has connected with R^2 (co-efficient of multiple determinant of 1 with standard error of estimate on observed mean. The value of R^2 is equivalent to 0.9495, which indicated sensor users/individuals were affected in urinary infection or frequent urination within the stipulated period due to lack of sensor security. So, the stated equation is accepted, which indicated the augmented diabetic patients. The stable equation was then active to stimulate human's diabetes perception regarding processed wireless sensor networks with high frequencies in clouding systems towards, underweight, normal weight and overweight/obese in BMI status. If the value of R^2 is negative, then the approaches were vice versa due to recovery of diabetes.

3.7 Smart Mobile Phone Users

Smart mobile phone users increase in the world due to expansion of wireless sensor technology. In 2015, there were 2.3 billion SP users and 7.1 billion of MP subscribers in the world, but these users and subscribers are increasing 3.2 and 8.3 respectively (Figure 7) unwantedly in 2019 due to cutting-edge technology. Due to lack of effective security, these persons have been surreing in diverse diseases, particularly sensor diabetes during uses of insecure wireless sensor networks.

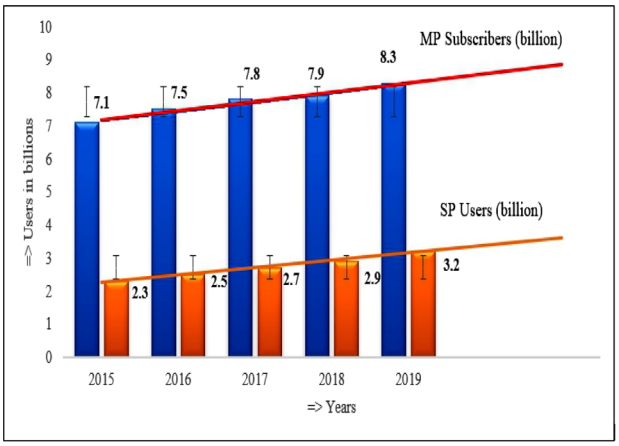

Figure 10. Globally mobile phone users and subscribers from 2015 to 2019.

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4. Discussion

The findings of the study on processed wireless sensor networks towards the ampulla of vater in pancreas cause the frequent urinary infection tends to symptoms with sensor diabetes. The ampulla of vater is purposefully positioned at the flowing together of the pancreatic and common bile ducts, which is the termination and having its own entrance into the duodenum[129];[130];[131];[132]; [135]. This organ position is suitable for blocking with sensor device in active open eyes. The finding symptoms were weight loss, frequent urination, anorexia, tiredness and pain, which are similar with patients in diabetes [131].

The research signifies the sudden comprehensive flow speed of micturition occurred due to tracking of processed wireless sensor networks in active open eyes. From the ISNAH experiment, the study finds that healthy people also suffer from diabetes due to misuse of sensor technology but more risk in patients with overweight and obesity [133];[134]. Therefore, wherever the diabetic patient is located in the world, his GPS location can be known through sensor technology and can be further diseased in various ways, such as: (a) if his eyes are open, (b) if his breathing is in full swing, (c) If he speaks aloud, (d) makes a noise, (e) has a mobile phone with him, (f) has another mobile phone nearby, (g) stays in a designated place for a long time, such as: bedroom, dining room, bathroom, office room or reading room, (h) with GPS device, (i) with sensor device, (j) with CCTV, (k) with Wi-Fi network, (l) electre4ic pole or if the transformer is around, (m) if air is leaked or flatus, (n) if urinal-toilet, (o) if he/she stays in the global navigation satellite system, and (p) if hiccups-sneeze-cough occurs, the current position of the person can be determined, which is shown in Figure 11.

Figure 11. Diabetic Patient identifies through different sensor parameters in GPS location.

4.1 Treatment

Healthy nutrition is an essential component of diabetes management [56]. Diabetes is a debilitating disease that none can treat easily one's at a time due to follow-up sophisticated health maintenance knowledge. Because, diabetes is a minor common problem but someone suffers from this problem, mentally this problem is like a gigantic burden in his thoughts and consciousness. The patient's idea is that he has diabetes, but the disease will never get better. So, he will die in diabetes? But my research says-that person's diabetes will be recovered for a better life. Healthy nutrition is an essential component of diabetes management [56]. It is consequently vital for people with diabetes to eat a diverse and balanced diet to keep their blood glucose levels steady and improve their immune system. Healthy eating, regular exercise, avoiding high calorie foods and smoking, and weight control can help prevent diabetes. If diabetes is not under control, it will be more serious. So, the patient and his family need to be aware to prevent this disease. More than half of individuals with diabetes do not diagnose they have the disease. Experts say that people over the age of 30 are generally at higher risk for developing all types of diabetes due to insulin deficiency. It is possible to prevent such diabetes in 80% of the cases by changing the diet, exercising regularly and careful use of sensor devices [11]. The most urgent awareness for this and doctors, nurses, mobile network companies and telecommunication institutions can play a big role in this work. Specially nurses can make a significant difference in diabetes care if they are proactive [57]. They can teach patients positively how to control their diabetes including blood tests and insulin [58];[59], then diabetes services are likely to change dramatically. There is no substitute for raising awareness if they want to prevent it. If diabetes is a type of sensor technology, its treatment is simple. It is very difficult to get proper treatment due to instant effective initiative. Although various companies and organizations are talking about the discovery of anti-diabetes systems, how effective it will be in curing the disease is a matter of dynamic research. The research has shown that 80% of the disease needs to be treated through psychological and 20% with physical therapy and medicine [11].

Moreover, the advice of a doctor experienced in medical sensors can be taken in this regard to enhancement the field of non-communicable disease care. If a person lives in a remote area where there is no regular registered doctor, he follows the open-close-eyes treatment rules as an alternative arrangement [60]. Moreover, the advice of an experienced doctor in medical censorship can also be taken in this regard. The whole world today is worried about the treatment of diabetes. Therefore, with the joint efforts of doctors, nurses, administration and the general public, we need to move fast to recover from this disease. Therefore,

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the following rules will help to recover of sensor diabetes. The rules are: (a). if a person suddenly suffers frequent urination, hiccup, cyanosis, runny nose, flatus, chills, headache, discomfort, micturition or gasps after being in a certain place, immediately closes his eyes tightly, wears sunglasses (anti-radiation glass) and quickly changes his existing place to a new place. This must do and no mobile phone beside him/her, (b). if individuals feel sick or gruesome, he/she must change the position of the bed or GPS positions from time to time and be very cautious using the smartphone or sensor device for good health. If you do not recover, consult an expert endocrinologist instantly, (c) no other religious activities or entertainment through sensor devices without personal area network control unit. These can be functioned or heard by placing a mobile phone 6 feet away from the boundary of individual's location and be ensured wearing sunglasses. Therefore, mobile phones cannot be used in clouding systems, particularly in religious places, temples, schoolrooms, laboratories, conference rooms, etc., and system security and institutional network control units must be used in these places. Diabetes treatment has suffered dramatic deviations throughout time that can be attributed to breakthroughs in cutting-edge-technology [61]. Various treatment types are patients with diabetes in light and dark environment, but light environment is suitable for adult patients [62]. These treatments include:

(i) open-closed eyes treatment [60],
(ii) technological treatment [63],
(iii) psychological treatment [64],
(iv) physical mentoring-administrative treatment [65],
(v) herbal treatment [66],
(vi) ethical treatment [67];[68],
(vii) environmental treatment [69];[70],
(viii) nutrtional treatment [71];[72];[73];[74],
(ix) yoga treatment [75];[76];[77];[78],
(x) medical treatment [79].

The physicians assist the patients in diabetes treatment options that are appropriate for individuals through the above mentioned rules. The serious patient in diabetes is essential alternative health experts for dynamic treatment team including health scientist, foot doctor, nutritionist, eye specialist and health sensor technologists. Because,due to augmenting causes of sensor diabetes occur in a clouding system according to your movement all day long with is a significant step for the primary disease diagnosis, treatment, and management [80];[81];[82];[83];[84];[85];[86]. Overall, the individual can follow the open-close-eyes treatment systems, which are shown in Figure 12. This system is easy and profitable for effective treatment except physicians.

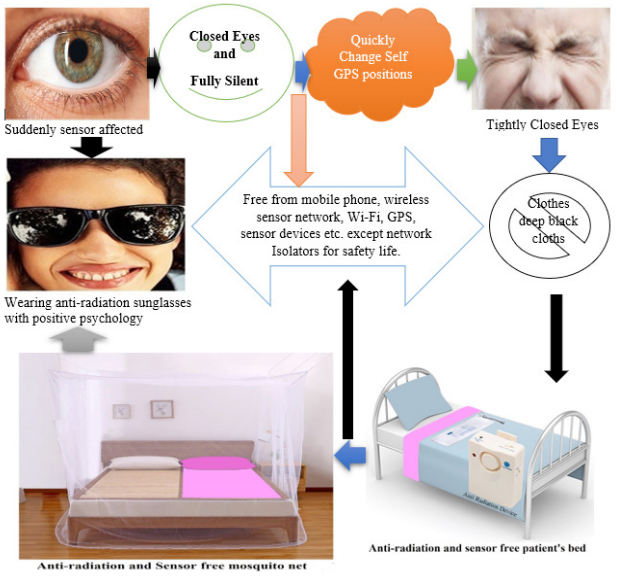

Figure 12. Open-Closed Eyes Treatment System

4.2 Alarming of Augmenting Sensor Diabetes

Wherever you are alive in the world, your diabetes can increase through the misuse of GPS sensor technology through inoculation of sensor particles. Because GPS sensor tracks inside the body [87]. Your position is known through different organs. Many of us are not aware of this. Because, many of us do not know about the effect of radio frequency. These waves do us both good and bad, within certain ranges. Because our body produces certain waves, our body has normal frequency for urination. However, if the amplitude or fluctuated or processed of these waves is different, it has a negative effect on our body. Suppose individuals are staying in the bedroom now (GPS location A), then he/she is going to Amber Khana (GPS location B), from there to Dargah Gate (GPS location C), then to Zindabazar (GPS location D) and finally to Bandarbazar office (destination place) in Sylhet (GPS location-identified with processed frequency), Bangladesh, which as shown in Figure 13. Because individuals have a mobile phone with insecure network, individual's location in these places is known through network graphs. Besides, they have a mobile phone and open active eyes. On the other hand, Cyber hackers have tracking switch-on in clouding systems at GPS and GNSS locations. In these five places affected persons can feel the urination. There are at least five people in these places who abuse radio waves to humans through telematics. So, change the current position quickly from wherever individuals feel like urinating. In this case, individuals do not think that they have diabetes. This has been the effect of applying additional radio frequencies. If we don't have radio frequency in our body,

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we will not urinate. But processed and fluctuated frequencies enhance frequent urination, after certain time it tends to diabetes. We need to urinate because radio frequency is generated, but in the hereafter the radio frequency of the people of Paradise will not be generated, so they will not have it. This is the blessing of Allah (God). And in the life of the world, cyber hackers are involved in these misdeeds. From the study, it is alarming to all for augmenting sensor diabetes due to misusing of cloud sensor technology, which as shown in Figure 13.

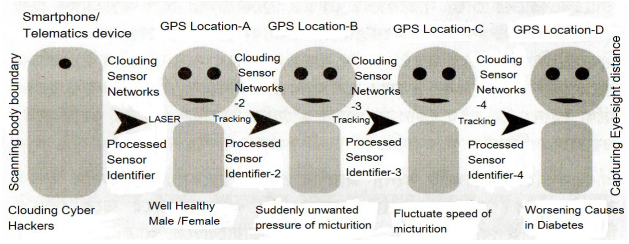

Figure 13. Flow diagram of augmenting diabetes due to misusing of sensor technology within body boundary area

Diabetes is one of the highest wide-reaching public health problems, which can be managed and prevented by dietary factors, insulin therapy and positive psychological catalists for protection of central nervous systems [88];[89];[90];[91];[92];[93];[94]. However, individuals can control the diabetic complications with medication and daily lifestyle changes due to controlling personal wireless network zone [95]. Despite the challenges of cutting edge sensor technology and nutritional research can be helpful to clinicians, patients, and the public [96]. Overall, augmenting consciousness among people for early diagnosis and treatment can recover long-term diabetes [97]. Adult individuals with pre-existing health conditions with diabetes, cardiac arrest, chronic kidney disease and acute respiratory distress syndrome (ARDS) etc. CASID [60] appear to be more susceptible to becoming severely sick with the Coronavirus. When individuals with diabetes affect a common acute sensor infection and disorder (CASID), it can be more difficult to recover due to uncertainties in blood glucose stages [98] and conceivably the complications of existing diabetes.

4.3 Diabetes with Mental Pollution

Diabetes connects with mental pollution of patients inducing with a thought, critical remark, accusation, visual image, insult, memory [99] disseminated from processed sensor technology. It is exposed to change by several psychological progressions linking with neuroscience [100];[101];[102]. Mental pollution increases in the risk of individual's health complications with diabetes due to presence of wildfire smoke [103];[104]. With the cutting edge sensor technology, people are directly affected to mental pollution with diverse elements of environment to influence diabetes within GPS location [105];[106];[107]. Everyone uses mobile phone, but none can be aware fully its environmental exposures to mental health, which associated to diabetes that have reliably revealed to associate with insulin resistance [108];[109];[110]. Because, insulin resistance is closely related to human health. On the other hand, air pollution is an important global health problem [111];[112], which is increasingly in focus for diabetes epidemiology [113];[114]. Diabetes is a chronic disease that reduces the hormone insulin leading to high blood sugar levels in presence of mental pollution in an unwanted environmental consequences.

4.4 Diabetes in Chief Executives

According to sensor clouding systems, the study stated that any person will be affected in health problems by cyber hackers through misusing of processed wireless sensor technology [60]. For example, President, Prime Minister, Chief Justice, Chief of Army, Inspector General of Police, Vice Chancellor, Chairman, Managing Director, Professor, Principal, Manager, Scientist, Expert, Specislist and or Senior Executives presides the leader in his/her office regularly within a GPS location. When he/she is ready to sign for providing decision or judgement or speech delivery with active open eyes to the audience. As audience, nearby cyber hackers track at his/her bladder. Then he/she suddenly feels uneasy with frequent urination. If he/she changes location instantly to control the unwanted urination. Otherwise, he/she fixes in GPS positions with comprehensive urination feelings, so he/she urinates in his /her chair or sitting place/bed. It is mentioned that the Chief Executives have sufficient security forces, but lack of dynamic sensor security in GPS and GNSS positions, he/she is weak easily through misusing of wireless sensor. Afterward, cyber hackers expose to the media as he/she (Prime Minister / Chief Justice) suffers in diabetes. General public health is in jeopardy due to misuse of advanced sensor technology. Actually, he/she was not suffered in diabetes, but the switch-on of cloud sensor device was active at processed wireless sensor networks. Cyber hackers monitor chief executives and general people with GPS sensor camera due to their open active eyes, making a noise or other voices at dark and light environment, then they select the targeted executive at GPS position for tracking with diabetes or other sensor diseases like CASID.

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4.5 Risks

Frequent urination is a sign of diabetes, which occurs in tracking processed sensor networks [115]. Impact of processed radio frequency is a prime concern to sensor network threats in frequent urination within GPS positions [116]. In-body GPS systems with magnetic resonance imaging (MRI) scans reflect radio signals of the patient [87], which is risky in unrestricted nano-sensor signals. Besides, intrusion detection systems devised for wireless sensor networks [117] with sensor and medical data, which obscures the existing security challenges [118]. Misuse of active eye-sight is also risk due to cutting-edge sensor technology [60]. Devotion to most diets in the longer term for patient is an important challenge [119]. Diabetes research in uncertainty and controversy remains in sudden complications [96], though the proportion of adults with diabetes augmented with age [124]. The vital security encounters in locked health data gathering are confidentiality and reliability of DNA sequencing data for sensor diabetes management. Major risk is misuse of false interface on diabetes test report and media exposure as an infodemic with phobia statement debilitating negative psychological approach towards patients during movement at clouding networks[120];[121];[122];[123];[136]. Cutting edge health sensor technologies can educate patients about diabetes on self-management and awareness [125];[126]. On the other hand, due to initial identification and consequent growing of diabetes are challenges for global healthcare systems [127;128]. Overall, processed wireless sensor networks including clouding systems are at risk for global public health security.

5. Conclusions

From the study, diabetes is indeed measured and cured due to controlling individual's body boundary area. The research has shown the impact of processed wireless sensor networks towards the ampulla of vater for being causes of sensor diabetes. The recovery of diabetes delays to the patients in overweight and obesity in dark environment than other environmental conditions. It is consequently vital for individuals with sensor diabetes to take a specific miscellaneous and balanced diet keeping their blood glucose levels steady for improvement of immune systems. Moreover, human body is in risks due to misapplication of wireless sensor networks with active open eyes in GPS positions. Finally, the study suggests that individuals must use personal area network control units within GPS location and wear sunglasses with tightly closed eyes and black cloths till to recover.

Declarations

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Data Availability

The data are being used to support the findings of this research work are available from the corresponding author upon request.

Competing Interests

The authors declare no potential conflict of interests in this research work.

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Authors Contributions

MRM designed the study. MRM wrote the first draft of the manuscript with MAH, ASR, MSK, MMH, ITR, MSH, CSS, MBU, MTHT, MSA, SIH, AAS, SHC and AKS all co-authors reviewing and amending the initial draft. All authors read and approved the final version of the manuscript.

References

[1] Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A.A., Ogurtsova, K., Shaw, J.E., Brighth, D., Williams. R. and IDF Diabetes Atlas Committee. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for

2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9thedition. Diabetes Research and Clinical Practice,157, 107843, 1-10.
DOI: https://doi.org/10.1016/j.diabres.2019.107843.

[2] Narayan, K.M.V., Zhang, P., Kanaya, A.M., Williams, D.E., Engelgau, M.M., Imperatore, G. and Ramachandran, A. (2006). Disease Control Priorities in Developing Countries. 2nd edition. Jamison DT, Breman JG, Measham AR, et al. [eds]. Chapter 30Diabetes: The Pandemic and Potential Solutions. NCBI. 1-20. URL: https://www.ncbi.nlm.nih.gov/books/NBK11777/.

[3] Lin, X., Xu, Y., Pan, X., Xu, J., Ding, Y., Sun, X., Song, X., Ren, Y. and Shan, P.F. (2020). Global, regional, and national burden and trend of diabetes in 195 countries and territories: an analysis from 1990 to 2025. Scientific Report, 10, 14790.
DOI: https://doi.org/10.1038/s41598-020-71908-9.

[4] IDF. (2020). Diabetes facts & figures. International Diabetes Federation (IDF), Belgium. URL: https://www.idf.org/aboutdiabetes/what-is-diabetes/facts-figures.html (Accessed time on January 19, 2021 at 12:00 pm).

[5] Miah M.R., Khan,M. S., Rahman, A.A.M.S., Samdany, A.A., Hannan, M.A., Chowdhury, S.H., Sayok, A.K. (2020). Impact of Sensor Networks towards Individuals Augmenting Causes of Diabetes. International Journal of Diabetes Research, 9(2),1-10. DOI: 10.5923/j.diabetes.20200902.

[6] Padmapriya, S., Chowdary, V.A. and Dinesh, V.S. (2013). Wireless sensor networks to monitor Glucose level in blood. International Journal of Advancements in Research & Technology, 2(4): 322–326. URL: http://www.ijoart.org/docs/Wireless-sensor-networks-to-monitor-Glucose-level-in-blood.pdf

[7] Miah, M.R., Rahman, A.A.M.S., Khan, M.S., Samdany, A.A., Hannan, M.A., Chowdhury, S.H., Sayok, A.K. (2020a). Impact of Sensor Technology Enhancing Corona Disease. American Journal of Biomedical Engineering, 10 (1), 1-11. DOI:10.5923/j.ajbe.20201002.

[8] Chaudhary, D. and Waghmare, L.M. (2014). Design Challenges of Wireless Sensor Networks and Impact on Healthcare Applications. International Journal of Latest Research in Science and Technology, 3(2):110–114.

[9] Abidi, B., Jilbab, A., and Haziti, M.E.L. (2016). Wireless Sensor Networks in biomedical: wireless body area networks. In: Procedings of the Europe, Middle East and North Africa Conference on Technology and Security to support Learning. EMENA-TSSL, SaidaOujda, Morocco, 3–5.

[10] Wu, F., Xu, L., and Kumari, S. (2017). An Improved and Anonymous two factor authentication protocol for healthcare applications with wireless medical sensor networks. MultimedSyst, 23 (2), 195–205.
DOI: https://doi.org/10.1007/s00530-015-0476-3

[11] Miah, M.R., Rahman, A.A.M.S., Samdany, A.A. and Chowdhury, S.H. (2021). A Dynamic Scientific Model for Recovery of Corona Disease. Frontiers in Science, 11(1), 1-17.
DOI: 10.5923/j.fs.20211101.01, URL: http://article.sapub.org/10.5923.j.fs.20211101.01.html.

[12] Khan, R.I. and Pathan, A.S. (2018). The state-of-the-art wireless body area sensor networks: A survey. International Journal of Distributed Sensor Networks, 14(4):1–16.
DOI: 10.1177/1550147718768994.

[13] Ding, S. and Schumacher, M. (2016). Sensor Monitoring of Physical Activity to Improve Glucose Management in Diabetic Patients: A Review. Sensors, 16, 589: 1–13.
DOI:10.3390/s16040589.

[14] Li, T., Li, Y. and Zhang, T. (2019). Materials, structures, and functions for flexible and stretchable biomimetic sensors. Accounts of Chemical Research, 52(2):288–296.
DOI:10.1021/acs.accounts.

[15] Kim, D. H., Lu, N., Ma, R., Kim, Y. S., Kim, R. H., Wang, S., Wu, J., Won, S. M., Tao, H., Islam, A., Yu, K. J., Kim, T. I., Chowdhury, R., Ying, M., Xu, L., Li, M., Chung, H. J., Keum, H., McCormick, M., Liu, P., Zhang, Y. W., Omenetto, F. G., Huang, Y., Coleman, T. and Rogers, J. A. (2011). Epidermal electronics. Science, 333(6044): 838–843.
DOI:10.1126/science.1206157.

[16] Gao, W., Emaminejad, S., Nyein, H. Y. Y., Challa, S., Chen, K., Peck, A., Fahad, H. M., Ota, H., Shiraki, H., Kiriya, D., Lien, D. H., Brooks, G. A., Davis, R. W. and Javey, A. (2016). Fully integrated wearable sensor arrays for multiplexed in situ perspiration analysis. Nature, 529(7587): 509–514.
DOI:10.1038/nature16521.

[17] Wang, X. W., Gu, Y., Xiong, Z. P., Cui, Z. and Zhang, T. (2014). Electronic skin: silk-molded flexible, ultrasensitive, and highly stable electronic skin for monitoring human physiological signals. Advanced Materials, 26(9): 1309.
DOI:10.1002/adma.201470054.

[18] Sheridan C. (2014). Apple moves on health, drug developers shift into smart gear. Nature Biotechnology, 32(10): 965–966. DOI:10.1038/nbt1014-965a.

DOI: https://doi.org/10.30564/jer.v3i1.2826

[19] Zang, Y. P., Zhang, F. J., Di, C. A. and Zhu, D. B. (2015). Advances of flexible pressure sensors toward artificial intelligence and health care applications. Materials Horizons, 2(2):140–156. DOI:10.1039/c4mh00147h.

[20] Zhao, W. X., Bhushan, A., Santamaria, A., Simon, M. and Davis, C.(2008). Machine learning: A crucial tool for sensor design. Algorithms,1(2): 130–152.
DOI:10.3390/a1020130.

[21] Vu, C. and Kim, J. (2018). Human motion recognition by textile sensors based on machine learning algorithms. Sensors, 18(9): 3109.
DOI:10.3390/s18093109.

[22] IDF (2019). What is Diabetes? About Diabetes.International Diabetes Federation, Brussels, Belgium. URL:https://www.idf.org/aboutdiabetes/what-is-diabetes.html?gcid=EAIaIQobChMInML08.001.8b00497.

[23] WHO.(2018). Key Facts.Diabetes.url: https://www.who.int/news-room/fact-sheets/detail/diabetes (Accessed time on March 16, 2021 at 9:00 am)

[24] Mazid, M.A. (2019, Nov14.). Global Economic Impact of Diabetes. The Daily Asian Age, Dhaka, Bangladesh. url: https://dailyasianage.com/news/204986/global-economic-impact-of-diabetes (Accessed time on March 13, 2021 at 9:00 am)

[25] Murray, C.J.L. and Lopez, A.D.(eds.). (1996). The Global Burden of Disease: A Comprehensive Assessment of Mortality and Disability from Diseases, Injuries, and Risk Factors in 1990 and Projected to 2020. The Harvard School of Public Health on behalf of the World Health Organization and World Bank and distributed by Harvard University Press. 1–43. URL:

[26] Centers for Disease Control and Prevention. (2011). National diabetes fact sheet: national estimates and general information on diabetes and pre-diabetes in the United States, Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention, 2011, Atlanta, GA, U.S.1-12. URL:http://www.diabetesincontrol.com/wp-content/uploads/PDF/ndep_diabetes_facts_2011.pdf (Accessed time on March 16, 2021 at 11:00 am).

[27] Deedwania, P. C. and Fonseca, V. A. (2005). Diabetes, prediabetes, and cardiovascular risk: shifting the paradigm. The American journal of medicine, 118: 939–947. DOI: 10.1016/j.amjmed.2005.05.018

[28] Meo, S., Alsubaie, Y., Almubarak, Z., Almutawa, H., AlQasem, Y. and Hasanato, R. (2015). Association of Exposure to Radio-Frequency Electromagnetic Field Radiation (RF-EMFR) Generated by Mobile Phone Base Stations with Glycated Hemoglobin (HbA1c) and Risk of Type 2 Diabetes Mellitus. International Journal of Environmental Research and Public Health, 12(11),14519–14528.
DOI:10.3390/ijerph121114519.

[29] Algoblan, A., Alalfi, M., & Khan, M. (2014). Mechanism linking diabetes mellitus and obesity. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy, 587.
DOI:10.2147/dmso.s67400.

[30] Bonsembiante B., Dalfrà, M.G., Masin, M., Gallo, A. and Lapolla. A., (2013). Adult-Onset Type 1 Diabetes and Pregnancy: Three Case Reports. Case Reports in Medicine, 1-3.
DOI: http://dx.doi.org/10.1155/2013/920861.

[31] Geerlings, S., Fonseca, V., Castro-Diaz, D., List, J. and Parikh, S. (2014). Genital and urinary tract infections in diabetes: Impact of pharmacologically-induced glucosuria. Diabetes Research and Clinical Practice, 103(3), 373–381.
DOI: 10.1016/j.diabres.2013.12.052.

[32] Sjoberg, R. J. and Kidd, G. S. (1989). Pancreatic Diabetes Mellitus. Diabetes Care, 12(10), 715–724.
DOI:10.2337/diacare.12.10.715.

[33] Meier, J. J. and Giese, A. (2015). Diabetes associated with pancreatic diseases. Current Opinion in Gastroenterology, 31(5), 400–406.
DOI:10.1097/mog.0000000000000199.

[34] Tolman, K. G., Fonseca, V., Dalpiaz, A. and Tan, M. H. (2007). Spectrum of Liver Disease in Type 2 Diabetes and Management of Patients with Diabetes and Liver Disease. Diabetes Care, 30(3), 734–743.
DOI:10.2337/dc06-1539.

[35] Blendea, M. C., Thompson, M. J. and Malkani, S. (2010). Diabetes and Chronic Liver Disease: Etiology and Pitfalls in Monitoring. Clinical Diabetes, 28(4), 139–144.
DOI:10.2337/diaclin.28.4.139.

[36] Sami, W., Ansari, T., Butt, N. S. and Hamid, M. (2017). Effect of diet on type 2 diabetes mellitus: A review. International journal of health sciences, 11(2), 65–71. PMID: 28539866.

[37] ADA (American Diabetes Association). (2003a). Nutrition Principles and Recommendations in Diabetes. Diabetes Care, 27(Supplement 1), S36–S36.
DOI:10.2337/diacare.27.2007.s36.

[38] Takei, K., Honda, W., Harada, S., Arie, T. and Akita S. (2015). Toward flexible and wearable human-interactive health-monitoring devices. Advanced Healthcare Materials, 4(4): 487–500.
DOI:10.1002/adhm.201400546.

[39] Knop, F.K., Vilsboll, T., Hojberg, P.V., Larsen, S., Madsbad, S., Vølund, A., Holst, J.J. and Krarup, T. (2007). Reduced incretin effect in type 2 diabetes: cause or consequence of the diabetic state? Diabetes, 56:1951–1959.
DOI: 10.2337/db07-0100.

[40] Lou, Z., Wang, L. L. and Shen, G. Z. (2018). Recent advances in smart wearable sensing systems. Advanced Materials Technologies, 3(12): 1800444.
DOI:10.1002/admt.201800444.

[41] Khan, Y., OstfeldA, E., Lochner, C. M. and Pierre, A. A. C. (2016). Monitoring of Vital Signs with Flexible and Wearable Medical Devices. Advanced Materials, 28(22): 4373–4395.
DOI:10.1002/adma.201504366.

[42] Zhang, T., Bai, Y.Y. and Sun, F. Q. (2018). Recent advances in flexible self-healing materials and sensors. ScientiaSinicaInformationis, 48(6): 650–669.
DOI:10.1360/n112018-00117.

[43] Li, Y., Zheng, L. and Wang, X. (2019). Flexible and wearable healthcare sensors for visual reality healthmonitoring. Virtual Reality & Intelligent Hardware, 1(4):411—427.
DOI: 10.1016/j.vrih.2019.

[44] Peiris, V. (2013). Highly integrated wireless sensing for body area network applications. The International Society for Optics and Photonics. SPIENewsroom.
DOI:10.1117/2.1201312.005120.

[45] O'Donovan, T., O'Donoghue, J., Sreenan, C., Sammon, D., O'Reilly, P. and O'Connor, K.A. (2009). A Context Aware Wireless Body Area Network. Pervasive Computing Technologies for Healthcare. 1–2,
DOI:10.4108/ICST.PERVASIVEHEALTH2009.5987.

[46] Bilal, M and Kang, S.G.(2017).An Authentication Protocol for Future Sensor Networks. Sensors, 17(5): 979.
DOI:10.3390/s170509.79.

[47] Nall, R. (2018). An overview of diabetes types and treatments.Newsletter on Health. Medical News Today,1(1): 1–5. URL: https://www.medicalnewstoday.com/articles/323627.

[48] Goharimanesh, M., Lashkaripour, A. and Akbari, A. (2015). A Comparison of Fuzzy Types 1 and 2 in Diabetics Control, Based on Augmented Minimal Model. Journal of World's Electrical Engineering and Technology, 4(2): 70–75.
DOI/URL: https://profdoc.um.ac.ir/articles/a/1053908.pdf.

[49] Apicella, M., Campopiano, M.C., Mantuano, M., Mazoni, L., Coppelli, A., Prato, S.D. (2020). COVID-19 in people with diabetes: understanding the reasons for worse outcomes. The LANCET Diabetes and Endocrinology, 8 (9), 782-792.
DOI: https://doi.org/10.1016/S2213-8587(20)30238-2.

[50] Health Hub. (2021). Diabetes Mellitus, Health Hub, Singapore. URL: https://www.healthhub.sg/a-z/diseases-and-conditions/102/topics_diabetes (Accessed time on January18, 2021 at 9:00 am).

[51] Cryer, P.E., Davis, S.N. and Shamoon, H. (2003). Hypoglycemia in diabetes. Diabetes Care, 26:1902–1912.
DOI: https://doi.org/10.2337/diacare.26.6.1902.

[52] Havas, M. (2008). Dirty electricity elevates blood sugar among electrically sensitive diabetics and may explain brittle diabetes. Electromagn. Biol. Med., 27, 135–146.
DOI: 10.1080/15368370802072075.

[53] Sha, H., Zeng, H., Zhao, J. and Jin, H. (2019). Mangiferin ameliorates gestational diabetes mellitus-induced placental oxidative stress, inflammation and endoplasmic reticulum stress and improves fetal outcomes in mice. European Journal of Pharmacology, 859: 172522.
DOI:10.1016/j.ejphar.2019.172522.

[54] Kays, R., Tilak, S., Crofoot, M., Fountain, T., Obando, D., Ortega, A., Kuemmeth, F., Mandel, J., Swenson, G., Lambert, T., Hirsch, B. and Wikelski, M. (2011). Tracking Animal Location and Activity with an Automated Radio Telemetry System in a Tropical Rainforest. Published by Oxford University Press on behalf of the British Computer Society. The Computer Journal, 1(1): 1–18,
DOI: 10.1093/comjnl/bxr072.

[55] Waltham. (2017). Feline Body Mass Index (FBMI). Waltham FBMI Calculator. 1–2. URL: https://js-calc.z6_io/calc/hORP8x2bWjQU7qxq (Accessed time on March 16, 2017 at 9:00 am)

[56] IDF. (2020a). COVID-19 and diabetes. IDF, Belgium. URL: https://www.idf.org/aboutdiabetes/what-is-diabetes/covid-19-and-diabetes/1-covid-19-and-diabetes.html (Accessed time on January 19, 2021 at 1:00 pm).

[57] Glasgow, R.E. (1995). A Practical Model of Diabetes Management and Education. DiabetesCare, 18(1): 117–126.

[58] Frier, B.M. (2014). Hypoglycaemia in diabetes mellitus: Epidemiology and clinical implications. Nat. Rev. Endocrinol.,10: 711–722.
DOI: 10.1038/nrendo.2014.170.

[59] Burge, M.R., Mitchell, S., Sawyer, A.,Schade, D.S.(2008). Continuous glucose monitoring: the future of diabetes management. Diabetes Spectr.,21:

DOI: https://doi.org/10.30564/jer.v3i1.2826

112–119.
DOI: https://doi.org/10.2337/diaspect.21.2.112.

[60] Miah, M.R., Rahman, A.A.M.S., Khan, M.S., Hannan, M.A., Hossain, M.S., Shahriar, C.S., Hossain, S.A.M.I., Talukdar, M.T.H., Samdany, A.A., Alam, M.S., Uddin, M.B., Sayok, A.K., Chowdhury, S.H. (2021a). Effect of Coronavirus Worldwide through Misusing of Wireless Sensor Networks. American Journal of Bioinformatics Research, 11(1), 1-31. DOI: 10.5923/j.bioinformatics.20211101.01 (URL: http://article.sapub.org/10.5923.j.bioinformatics.20211101.01.html).

[61] Marcus, A.O. (2002). Outpatient Technologies for the treatment and prevention of Diabetes. Diabetes Spectrum, 15(2), 78-80.
DOI: 10.2337.

[62] Khan Y, Han D, Pierre A, Ting J, Wang X C, Lochner C M, Bovo G, Yaacobi-Gross N, Newsome C, Wilson R, Arias A C. (2018). A flexible organic reflectance oximeter array. Proceedings of the National Academy of Sciences, 115(47): E11015–E11024. DOI:10.1073/pnas.1813053115.

[63] Schiel, R., Bambauer, R. and Steveling, A. (2018). Technology in Diabetes Treatment: Update and Future. Artificial Organs, 42 (11), 1017–1027.
DOI/URL: https://doi.org/10.1111/aor.13296.

[64] Delamater,A. M., Jacobson,A. M., Anderson, B., Cox,D., Fisher,L.,Lustman, P., Rubin, R.and Wysocki, T. (2001). Psychosocial Therapies in Diabetes: Report of the Psychosocial Therapies Working Group. Diabetes Care, 24(7): 1286-1292.
DOI: https://doi.org/10.2337/diacare.24.7.1286.

[65] ADA (). (2003). Physical Activity/Exercise and Diabetes Mellitus. American Diabetes Association (ADA) Diabetes Care, suppl 1: s73-s77.
DOI/URL: https://doi.org/10.2337/diacare.26.2007.S73.

[66] Moradi, B., Abbaszadeh, S., Shahsavari, S., Alizadeh,M. and Beyranvand, F. (2018). The most useful medicinal herbs to treat diabetes. Biomedical Research and Therapy, 5(8): 2538–2551.

[67] Azizi, F., Hatami, H. and Janghorbani, M. (2007). Epidemiology and Control of Common Disease in Iran.Tehran: Eshtiagh Press, 1–5.

[68] Gupta, V.K., Gupta, M., and Arora.S. (2017). Diabetes: Ethical Issues. Chapter 159, Medicine Update, India. 742-745. URL: http://www.apiindia.org/pdf/medicine_update_2017/mu_159.pdf. (Accessed time on March 16, 2020 at 9:00 am)

[69] Dazzi, D., Taddei, F., Gavarini, E A., Negro, U.R. and Pezzarossa, A. (2001). The control of blood glucose in the critical diabetic patient: a neuro-fuzzy method. Journal of Diabetes and its Complications, 15: 80–87.
DOI: 10.1016/s1056-8727(00)00137-9.

[70] Grant, P. (2007). A new approach to diabetic control: fuzzy logic and insulin pump technology. Medical engineering & physics, 29: 824–827.
DOI: 10.1016/j.medengphy.2006.08.014.

[71] Forouhi, N.G., Misra, A., Mohan,V., Taylor, R. and Yancy., W. (2018). Dietary and nutritional approaches for prevention and management of type 2 diabetes. BMJ, 361:k2234,1–9.
DOI: 10.1136/bmj.k2234.

[72] IDF (International Diabetes Federation). (2017). Diabetes atlas. 8th ed. IDF, 2017. URL: ww.diabetesatlas.org (Accessed time on March 15, 2020at 9:00 am). URL: https://www.diabetesatlas.org/upload/resources/previous/files/8/IDF_DA_8e-EN-final.pdf.

[73] Johnston, B.C., Kanters, S., Bandayrel, K. Wu, P., Naji, F., Siemieniuk, R.A., Ball, G.D.C., Busse, J.W., Thorlund, K., Guyatt, G., Jansen, J.P. and Mills, E.J. (2014). Comparison of weight loss among named diet programs in overweight and obese adults: a meta-analysis. JAMA, 312(9):923-33.
DOI:10.1001/jama.2014.10397.

[74] Westman, E.C., Yancy, W.S. Jr. and Humphreys, M. (2006). Dietary treatment of diabetes mellitus in the pre-insulin era(1914-1922). PerspectBiol Med,49:77-83.
DOI:10.1353/pbm.2006.0017.

[75] Cronkleton, E. (2018). Yoga for Diabetes. Healthline. URL: https://www.healthline.com/health/diabetes/yoga-for-diabetes (Accessed time on March 15, 2021 at 2:00 pm).

[76] Raveendran, A. V., Deshpandae, A. and Joshi, S. R. (2018). Therapeutic Role of Yoga in Type 2 Diabetes. Endocrinology and Metabolism, 33(3), 307. DOI:10.3803/enm.2018.33.3.307.

[77] Soroka, E. (2019). Yoga for Diabetes: 12 Poses and a Meditation to Mitigate Stress. Yoga Journal. 1-26. URL: https://www.yogajournal.com/practice/yoga-for-diabetes/ (Accessed time on March 15, 2021 at 3:00 pm).

[78] Sharma, M. and Knowlden, A.P. (2012). Role of Yoga in Preventing and Controlling Type 2 Diabetes Mellitus. Journal of Evidence-Based Complementary & Alternative Medicine,17(2) 88-95.
DOI: 10.1177/2156587212438899.

[79] Johnson, J. (2019). A review of therapies and lifestyle changes for diabetes. Medical News Today, 1-2. URL: https://www.medicalnewstoday.com/articles/317074.php (Accessed time on March 10, 2021

at 9:00 am).

[80] Babamiri, B., Bahari, D. and Salimi, A. (2019). Highly sensitive bioaffinityelectrochemiluminescence sensors: Recent advances and future directions. Biosensors and Bioelectronics, 111530. DOI: https://doi.org/10.1016/j.bios.2019.111530.

[81] Hardt, P.D., Brendel, M.D., Kloer, H.U. and Bretzel, R.G. (2008). Is pancreatic diabetes (type 3c diabetes) underdiagnosed and misdiagnosed? Diabetes Care,31 (Suppl 2):S165–S169. DOI: 10.2337/dc08-s244.

[82] Boutry, C. M., Beker, L., Kaizawa, Y., Vassos, C., Tran, H., Hinckley, A. C., Pfattner, R., Niu, S. M., Li, J. H., Claverie, J., Wang, Z., Chang, J., Fox, P. M. and Bao, Z. N. (2019). Biodegradable and flexible arterial-pulse sensor for the wireless monitoring of blood flow. Nature Biomedical Engineering, 3(1): 47–57. DOI:10.1038/s41551-018-0336-5.

[83] Islam, D., Huque, A., Sheuly, Mohanta, L.C., Das, S.K. and Sultana, A. (2018). Hypoglycemic and hypolipidemic effects of Nelumbonucifera flower in Long-Evans rats. Journal of Herbmed Pharmacology, 7:148–54. DOI:10.15171/jhp.2018.25.

[84] Rahimi-Madiseh, M., Karimian, P., Kafeshani, M. and Rafieian-Kopaei, M. (2017). The effects of ethanol extract of Berberis vulgaris fruit on histopathological changes andbiochemical markers of the liver damage in diabetic rats. Iranian Journal of Basic Medical Sciences, 20:552–556. DOI: 10.22038/IJBMS.2017.8681.

[85] Maideen, N.M.P. and Balasubramaniam, R.(2018). Pharmacologically relevant drug interactions of sulfonylurea antidiabetics with commonherbs. Journal of Herbmed Pharmacology.7:200–10. DOI:10.15171/jhp.2018.32.

[86] Rosenstock, J., Seman, L.J., Jelaska, A., Hantel, S., Pinnetti ,S., Hach, T. and Woerle, H.J. (2013). Efficacy and safety of empagliflozin, a sodium glucose cotransporter 2 (SGLT2) inhibitor, as add-on to metformin in type 2 diabetes with mild hyperglycaemia. Diabetes Obes Metab, 15(12):1154–60. DOI: 10.1111/dom.12185.

[87] MIT. (2018). A GPS for inside your body: Wireless system suggests future where doctors could implant sensors to track tumors or even dispense drugs. Computer Science and Artificial Intelligence Laboratory (CSAIL), Massachusetts Institute of Technology (MIT), USA. ScienceDaily, August 20, 2018. URL: www.sciencedaily.com/releases/2018/08/180820085158.html (Acceessed on February 5, 2021 at 12:00 pm).

[88] Wilding, J. P. H., Norwood, P., T'joen, C., Bastien, A., List, J. F. and Fiedorek, F. T. (2009). A Study of Dapagliflozin in Patients With Type 2 Diabetes Receiving High Doses of Insulin Plus Insulin Sensitizers: Applicability of a novel insulin-independent treatment. Diabetes Care, 32(9), 1656–1662. DOI:10.2337/dc09-0517.

[89] Philippe, M.F., Benabadji, S., Barbot-Trystram, L., Vadrot, D., Boitard, C. (2011). Larger E. Pancreatic volume and endocrine and exocrine functions in patients with diabetes. Pancreas, 40(3):359–363. DOI: 10.1097/MPA.0b013e3182072032.

[90] Ershow, A.G. (2009). Environmental influences on development of type 2 diabetes and obesity: challenges in personalizing prevention and management. J Diabetes Sci Technol., 3(4):727–734. DOI: 10.1177/193229680900300418.

[91] Osler, W. and McCrae, T. (1921). The principles and practice of medicine: Designed for the use of Practitioners and students of Medicine. D. Appleton and Company, 1921, New York and London.. URL: https://archive.org/details/principlesandpr00mccrgoog/page/n6/mode/2up (Accessed time on March 16, 2021 at 10:00am).

[92] Wild, S., Roglic, G., Green, A., Sicree, R. and King, H. (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care, 27(5):1047–1053. DOI: 10.2337/diacare.27.5.1047

[93] Fowler, M.J. (2008). Microvascular and Macrovascular Complications of Diabetes. Clinical Diabetes, 26(2):77–82. DOI: https://doi.org/10.2337/diaclin.26.2.77.

[94] Naser, K.A., Gruber, A. and Thomson, G.A. (2006). The emerging pandemic of obesity and diabetes: are we doing enough to prevent a disaster? Int J Clin Pract., 60(9):1093–1097. DOI: 10.1111/j.1742-1241.2006.01003.x.

[95] Frei, P., Mohler, E., Neubauer, G., Theis, G., Bürgi, A., Fröhlich, J., Braun-Fahrländer, C., Bolte, J., Egger, M. and Röösli, M. (2009). Temporal and spatial variability of personal exposure to radio frequency electromagnetic Fields. Environ. Res., 109, 779–785. DOI: https://doi.org/10.1016/j.envres.2009.04.015.

[96] Forouzanfar M.H., Forouzanfar, M.H., Alexander, L., Anderson, H.R., Bachman, V.F., Biryukov, S., Brauer, M., Burnett, R., Casey, D., Coates, M.M., Aaron Cohen, A., Delwiche, K., Estep, K., Frostad, J.J., Astha, K.C., Kyu, H.H., Moradi-Lakeh, M., Ng, M., Slepak, E.L., Thomas, B.A., Wagner, J., Aas-

  DOI: https://doi.org/10.30564/jer.v3i1.2826

vang, G.M., Abbafati, C., Ozgoren, A.A., Abd-Allah, F., Abera,S.F., Aboyans, V., Abraham, B., Abraham, J.P., Abubakar, I., Abu-Rmeileh, N.M.E., Aburto, T.C., Achoki, T., Adelekan, A., Adofo, K., Adou, A.K., Adsuar, J.C., Afshin, A., Agardh, E.E., Al-Khabouri, M.J., Al Lami, F.H., Alam,S.S., Alasfoor, D., Albittar, M.I., Alegretti, M.A., Aleman, A.V., Zewdie A Alemu, Z.A., Alfonso-Cristancho, R., Alhabib, S., Ali, R., Ali, M.K., Alla, F., Allebeck, P., Allen, P.J., Ubai Alsharif, U., Alvarez, E., Alvis-Guzman, N., Amankwaa, A.A., Amare, A.T., Ameh, E.A., Ameli, O., Amini, H., Ammar, W., Anderson, B.O., Antonio, C.A.T., Anwari, P., Cunningham, S.A., Arnlöv, J., Arsenijevic, V.S.A., Artaman, A., Asghar, R.J., Assadi, R., Atkins, L.S., Atkinson, C., Avila, M.A., Awuah, B., Badawi, A., Bahit, M.C., Bakfalouni, T., Balakrishnan, K., Balalla, S., Balu, R.K., Banerjee, A.,Barber, R.M., Barker-Collo, S.L., Barquera, S., Barregard, L., Barrero, L.H., Barrientos-Gutierrez, T., Basto-Abreu, A.C., Basu, A., Basu, S., Basulaiman, M.O., Ruvalcaba, C.B., Beardsley, J., Bedi, N., Bekele, T., Bell, M. L., Benjet, C., Bennett, D.A., Benzian, H., Bernabé, E., Beyene, T.J., Bhala, N., Bhalla, A., Bhutta, Z.A., Bikbov, B., Abdulhak, A.A.B., Blore, J.D., Blyth, F.M., Bohensky, M.A., Başara, B.B., Borges, G., Bornstein, N.M., Bose, D., Boufous, S., Bourne, R.R., Brainin, M., Brazinova, A., Breitborde, N.J., Brenner, H., Briggs, A.D.M., Broday, D.M., Brooks, P.M., Bruce, N.G., Brugha, T.S., Brunekreef, B., Buchbinder, R., Bui, L.N., Bukhman, G., Bulloch, A.G., Burch, M., Burney, P.G.J., Campos-Nonato, I.R., Campuzano, J.C., Cantoral, A.J., Caravanos, J., Cárdenas, R., Cardis, E., Carpenter, D.O., Caso, V., Castañeda-Orjuela, C.A., Castro, R.E., Catalá-López, F., Cavalleri, F., Çavlin, A., Chadha, V.K., Chang, J.C., Charlson, F.J., Chen, H., Chen, W., Chen, Z., Chiang, P.P., Chimed-Ochir, O., Chowdhury, R., Christophi, C.A., Chuang, T.W., Chugh, S.S., Cirillo, M., Claßen, T.K.D., Colistro, V., Colomar, M., Colquhoun, S.M., Contreras, A.G., Cooper, C., Cooperrider,K., Cooper, L.T., Coresh,. J. Courville, K.J., Criqui, M.H., Cuevas-Nasu, L., Damsere-Derry, J., Danawi, H., Dandona, L., Dandona, R., Dargan, P.I., Davis, A., Davitoiu, D.V., Dayama, A., de Castro, E.F., De la Cruz-Góngora, V., De Leo, D., de Lima, G., Degenhardt, L., Pozo-Cruz, B.D., Dellavalle, R.P., Deribe, K., Derrett, S., Jarlais, D.C.D., Dessalegn, M., deVeber, G.A., Devries, K.M., Dharmaratne, S.D., Dherani, M.K., Dicker, D., Eric L Ding, D.E.L., Dokova, K., Dorsey, E.R., Driscoll, T.R., Duan, L., Durrani, A.M., Ebel, B.E., Ellenbogen, R.G., Elshrek, Y.M., Endres, M., Ermakov, S.P. Erskine, H.E., Eshrati, B., Esteghamati, A., Fahimi, S., Faraon, E.J.A., Farzadfar, F., Fay, D.F.J., Feigin, V.L., Feigl, A.B., Fereshtehnejad, S.M., Ferrari, A.J., Ferri, C.P., Flaxman, A.D., Fleming, T.D., Foigt, N., Foreman, K.J., Paleo, U.F., Franklin, R.C., Gabbe, B., Gaffikin, L., Gakidou, E., Gamkrelidze, A., Gankpé, F.G., Gansevoort, R.T., García-Guerra, F.A., Gasana, E., Geleijnse, J.M., Gessner, B.D., Gething, P., Gibney, K.B., Gillum, R.F., Ginawi, I.A.M., Giroud, M., Giussani, G., Goenka, S., Goginashvili, K., Dantes, H.G., Gona, P., de Cosio, T.G., González-Castell, D., Gotay, C.C., Goto, A., Gouda, H.N., Guerrant, R.L., Gugnani, H.C., Guillemin, F., Gunnell, D., Gupta, R., Rajeev Gupta, Rv., Gutiérrez, R.A., Hafezi-Nejad, N., Hagan, H., Hagstromer, M., Halasa, Y.A., Hamadeh, R.R., Hammami, M., Hankey, G.J., Hao, Y., Harb, H.L., Haregu, T.N., Haro, J.M., Havmoeller, R., Hay, S.I., Hedayati, M.T., Heredia-Pi, I.B., Hernandez, L., Heuton, K.R., Heydarpour, P., Hijar, M., Hoek, H.W., Hoffman, H.J., Hornberger, J.C., Hosgood, H.D., Hoy, D.G., Hsairi, M., Hu, G., Hu, H., Huang, C., Huang, J.J., Hubbell, B.J., Huiart, L., Husseini, A., Iannarone, M.L., Iburg, K.M., Idrisov, B.T., Ikeda, N., Innos, K., Inoue, M., Islami, F., Ismayilova, S., Jacobsen, K.H., Jansen, H.A., Jarvis, D.L., Jassal, S.K., Jauregui, A., Jayaraman, S., Jeemon, P., Jensen, P.N., Jha, V., Jiang, F., Jiang, G., Jiang, Y., Jonas, J., B., Juel, K., Kan, H., Roseline, S.S.K., Karam, N.E., Karch, A., Karema, C.K., Karthikeyan, G., Kaul, A., Kawakami, N., Kazi, D.S., Kemp, A.H., Kengne, A.P., Keren, A., Khader, Y.S., Khalifa, S.E.A.H., Khan, E.A., Khang, Y.H., Khatibzadeh, S., Khonelidze, I., Kieling, C., Kim, D., Kim, S., Kim, Y., Kimokoti, R.W., Kinfu, Y., Kinge, J.M., Kissela, B.M., Kivipelto, M., Knibbs, L.D., Knudsen, A.K., Kokubo, Y., Kose, M.R., Kosen, S., Kraemer, A., Kravchenko, M., Krishnaswami, S., Kromhout, H., Ku, T., Defo, B.K., Bicer, B.K., Kuipers, E.J., Kulkarni, C., Kulkarni, V.S., Kumar, G.A., Kwan, G.F., Lai, T., Balaji, A.L., Lalloo, R., Lallukka, T., Lam, H., Lan, Q., Lansingh, V.C., Larson, H.J., Larsson, A., Laryea, D.O., Lavados, P.M., Lawrynowicz, A.E. et al……Murray, C.J. and GBD 2013 Risk Factors Collaborators. (2015). Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks in 188 countries, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet, 386(10010):2287-323.

 DOI: https://doi.org/10.30564/jer.v3i1.2826

DOI: 10.1016/S0140-6736(15)00128-2.

[97] Clark, N. G., Fox, K. M. and Grandy, S. (2007). Symptoms of Diabetes and Their Association with the Risk and Presence of Diabetes: Findings from the Study to Help Improve Early evaluation and management of risk factors Leading to Diabetes (SHIELD). Diabetes Care, 30(11), 2868–2873. DOI:10.2337/dc07-0816.

[98] Wang J. (2008). Electrochemical glucose biosensors. Chemical Reviews, 108:814–825. DOI: 10.1021/cr068123a.

[99] Bowe, B., Xie, Y., Li, T., Yan, Y., Xian, H. and Al-Aly, Z. (2018). The 2016 global and national burden of diabetes mellitus attributable to PM 2·5 air pollution. The Lancet Planetary Health, 2(7), e301–e312. DOI:10.1016/s2542-5196(18)30140-2.

[100] Fairbrother, N., Newth, S. J. and Rachman, S. (2005). Mental pollution: feelings of dirtiness without physical contact. Behaviour Research and Therapy, 43(1), 121–130. DOI: 10.1016/j.brat.2003.12.005.

[101] Sancar, F. (2019). Childhood Lead Exposure May Affect Personality, Mental Health in Adulthood. JAMA, 321(15):1445–1446. DOI:10.1001/jama.2019.1116.

[102] Reuben A, Schaefer JD, Moffitt TE, et al. (2019). Association of Childhood Lead Exposure With Adult Personality Traits and Lifelong Mental Health. JAMA Psychiatry,76(4):418–425. DOI:10.1001/jamapsychiatry.2018.4192.

[103] Yao, J., Brauer, M., Wei, J., McGrail, K. M., Johnston, F. H., & Henderson, S. B. (2020). Sub-Daily Exposure to Fine Particulate Matter and Ambulance Dispatches during Wildfire Seasons: A Case-Crossover Study in British Columbia, Canada. Environmental Health Perspectives, 128(6), 067006. DOI:10.1289/ehp5792.

[104] Bennett, C. (2020). Pollution and Mental Health. News Medical Life Sciences. URL: https://www.news-medical.net/health/Pollution-and-Mental-Health.aspx (Accessed on March 13, 2021 at 12:00 pm).

[105] Cetin, Y. (2012). Impact of Mental Pollution on Learning and Memory. Procedia - Social and Behavioral Sciences, 46, 5320–5323. DOI: 10.1016/j.sbspro.2012.06.431.

[106] Dendup, T., Feng, X., Clingan, S. and Astell-Burt, T. (2018). Environmental Risk Factors for Developing Type 2 Diabetes Mellitus: A Systematic Review. International Journal of Environmental Research and Public Health, 15(1), 78. DOI:10.3390/ijerph15010078.

[107] Khan, A., Plana-Ripoll, O., Antonsen, S., Brandt, J., Geels, C., Landecker, H., Sullivan, P.F., Pedersen, C.B.,Rzhetsky, A. (2019). Environmental pollution is associated with increased risk of psychiatric disorders in the US and Denmark. PLOS Biology, 17(8), e3000353, 1-28. DOI: 10.1371/journal.pbio.3000353.

[108] Rajagopalan, S. and Brook, R. D. (2012). Air Pollution and Type 2 Diabetes: Mechanistic Insights. Diabetes, 61(12), 3037–3045. DOI:10.2337/db12-0190.

[109] WHO. (2004). Promoting mental health: Concepts, emerging evidence, practice: summary report. World Health Organization. URL : https://apps.who.int/iris/bitstream/handle/10665/42940/9241591595.pdf (Accessed time on March 11, 2021 at 11:00 am)

[110] Brokamp, C., Strawn, J. R., Beck, A. F., & Ryan, P. (2019). Pediatric Psychiatric Emergency Department Utilization and Fine Particulate Matter: A Case-Crossover Study. Environmental Health Perspectives, 127(9), 097006.1-7. DOI:10.1289/ehp4815.

[111] Lelieveld, J., Evans, J.S., Fnais, M., Giannadaki, D. and Pozzer, A. (2015). The contribution of outdoor air pollution sources to premature mortality on a global scale. Nature,525: 367–71. DOI: https://doi.org/10.1038/nature15371.

[112] Brunst, K. J. (2019). Myo-inositol mediates the effect of traffic-related air pollution on generalized anxiety symptoms at age 12 years. Environmental research, 175, 71–78. DOI: https://doi.org/10.1016/j.envres.2019.05.009._

[113] Dzhambov, A. M. (2018). Ambient air pollution and diabetes in China. The Lancet Planetary Health, 2(2), e52–e53. DOI:10.1016/s2542-5196(18)30002-0.

[114] Ruder, K. (2021, January 20). Is There a Link Between Type 2 Diabetes and Air Pollution? Everyday Health. Newsletter. URL: https://www.everydayhealth.com/type-2-diabetes/is-there-a-link-between-type-2-diabetes-and-air-pollution/ (Accessed time on March 13, 2021 at 12:00 pm).

[115] Porter, E. (2018). Is Frequent Urination a Sign of Diabetes? Healthline. URL: https://www.healthline.com/health/frequent-urination-diabetes (Accessed time on February 15, 2021 at 11:00 am).

[116] Agarwal, N. and Hussain, S.Z. (2018). A Closer Look at Intrusion Detection System for Web Applications. Security and Communication Networks, 1–28. DOI: https://doi.org/10.1155/2018/96013.

DOI: https://doi.org/10.30564/jer.v3i1.2826

[117] Butun I., Morgera S. and Sankar R. (2014). A survey of intrusion detection systems in wireless sensor networks.IEEE Commun. Surv. Tutor, 16:266–282.
DOI: 10.1109/SURV.2013.050113.00191.

[118] Butun I., Ra I.H. and Sankar R. (2015). PCAC: Power-and Connectivity-Aware Clustering for Wireless Sensor Networks. EURASIP J. Wirel. Commun. Netw., 1:1–15.
DOI: 10.1186/s13638-015-0321-6.

[119] Bal, A. (2000). Diabetes: ethical, social and economic aspects.The Indian Journal of Medical Ethics, 8:3. PMID: 16323366.

[120] WHO. (2020c). WHO works with the Government of the United Kingdom to tackle misinformation. World Health Organization (WHO). URL: https://www.who.int/campaigns/connecting-the-world-to-combat-coronavirus/how-to-report-misinformation-online (Accessed time on January 11, 2021 at 12:00pm.).

[121] Roozenbeek, J., Schneider, C.R., Dryhurst, S., Kerr, J., Freeman, A.L.J., Recchia, G., vander Bles, A.M. and van der Linden, S. (2020). Susceptibility to misinformation about COVID-19 around the world. Royal Society Open Science, 7: 201199, 1-15.
DOI: http://dx.doi.org/10.1098/rsos.201199.

[122] Giles Keir. (2020, April 9). Beware Russian and Chinese Positioning for after the pandemic. Chatham house.URL:https://www.chathamhouse.org/2020/04/beware-russian-and-chinese-positioning-after-pandemic?gclid=EAIaIQobChMInIPlhsqa7gIVNdWWCh1xeA3uEAMYASAAEgKkCvD_BwE (Accessed time on January 10, 2021 at 3:00 pm.).

[123] Cinelli, M., Quattrociocchi, W., Galeazzi, A., Valensise, C.M., Brugnoli, E., Schmidt, A.L., Zola, P., Zollo, F. and Scala, A. (2020). The COVID-19 social media infodemic. Scientific Report, 10, 16598.
DOI: https://doi.org/10.1038/s41598-020-73510-5.

[124] CDC. (2020). National Diabetes Statistics Report 2020: Estimates of Diabetes and its Burden in the United States, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention (CDC), 1-31. URL: https://www.cdc.gov/diabetes/pdfs/data/statistics/national-diabetes-statistics-report.pdf (Accessed time on March 16, 2021 at 12:00 pm).

[125] Ding, K., Reynolds, C.M., Driscoll, K.A. and Janicke, D.M. (2021). The Relationship Between Executive Functioning, Type 1 Diabetes Self-Management Behaviors, and Glycemic Control in Adolescents and Young Adults. Current Diabetes Report, 21 (10).
DOI: https://doi.org/10.1007/s11892-021-01379-3.

[126] Lyles, C.R., Sarkar, U. & Osborn, C.Y. (2014). Getting a Technology-Based Diabetes Intervention Ready for Prime Time: A Review of Usability Testing Studies. Current Diabetes Report, 14 (10), 534.
DOI: https://doi.org/10.1007/s11892-014-0534-9.

[127] Martin, K., Hatab, A., Athwal, V.S., Jokl, E. and Hanley, K.P. (2021). Genetic Contribution to Non-alcoholic Fatty Liver Disease and Prognostic Implications. Current Diabetes Report, 21(8), 1-8.
DOI: https://doi.org/10.1007/s11892-021-01377-5.

[128] Gu, Y., Rampin, A., Alvino, V.V., G. Spinetti and P. Madedduet. (2021). Therapy for Critical Limb Ischemia: Advantages, Limitations, and New Perspectives for Treatment of Patients with Critical Diabetic Vasculopathy. Currernt Diabetes Report, 21 (11), 1-13.
DOI: https://doi.org/10.1007/s11892-021-01378-4.

[129] Greene, F. L., Compton, C. C., Fritz, A. G., Shah, J. P. and Winchester, D. P. (Eds.). (2006). AJCC Cancer Staging Atlas.
DOI:10.1007/0-387-33126-3.

[130] Nix, G., Wilson, J., Schmitz, P., Dees, J. and Hofwijk, R. (1983). Carcinoma of the ampulla and papilla of Vater. R*ö*Fo - Fortschritte Auf Dem Gebiet Der R*ö*ntgenstrahlen Und Der Bildgebenden Verfahren, 138(05), 531–535.
DOI:10.1055/s-2008-1055779.

[131] Atkinson, M.A., Campbell-Thompson, M., Kusmartseva, I. and Kaestner, K.H. (2020). Organisation of the human pancreas in health and in diabetes. Diabetologia, 63,1966–1973.
DOI: https://doi.org/10.1007/s00125-020-05203-7.

[132] He, X.D., Wu, Q., Liu, W., Hong, T., Li, J.J., Miao, R.Y. and Zhao, H.T. (2014). As-sociation of metabolic syndromes and risk factors with ampullary tumors development: A case-control study in China. World J Gastroenterol, 20(28): 9541-9548
URL: http://www.wjgnet.com/1007-9327/full/v20/i28/9541.htm
DOI: http://dx.doi.org/10.3748/wjg.v20.i28.9541.

[133] Gruberg, L., Weissman, N.J., Waksman, R.F., Fuchs, S., Deible,R., Pinnow, E.E., Ahmed, L.M., Kent, K.M., Pichard, A.D., Suddath, W.O., Satler, L.F., Lindsay Jr. J. (2002). The impact of obesity on the short-term and long-term outcomes after percutaneous coronary intervention: The obesity paradox? J Am Coll Cardiol, 39:578-84.

[134] García-Cano, J. (2016). If you suffer from type-2 diabetes mellitus, your ERCP is likely to have a better outcome. Editorial. RoFo-Revista Espa*ñ*ola

de Enfermedades Digestivas, 108(7),

[135] Li, D. (2011). Diabetes and Pancreatic Cancer. Molecular Carcinogenesis, 51(1), 64–74. DOI: 10.1002/mc.20771.
DOI: http://dx.doi.org/10.17235/reed.2016.4521/2016.

[136] DAB. (2018). Introduction to diabetes mellitus: Epidemiology of diabetes mellitus, Chap. 1. Diabetes Mellitus, 5th Edn, Ahmed, T. (E. Ed.). Certificate Course on Diabetology: Distance Learning Program. Diabetes Association of Bangladesh (DAB), 1–173. ISBN: 984-32-2552-X.

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ARTICLE

Metformin and Lactic Acidosis in Diabetic Patients

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ABSTRACT

Metformin is the basic drug in the clinical treatment of Diabetes, often used in the treatment of Type 2 Diabetes Mellitus (T2DM).Its effect has been fully verified in the clinical treatment of T2DM. However, in the treatment of T2DM with metformin, there is still a certain probability of related lactic acidosis, and the fatality rate is high. Therefore, is the use of metformin drug treatment a direct risk factor for lactic acidosis in diabetic patients? This paper will review the hypoglycemic mechanism of metformin and related studies on lactic acidosis, so as to further explore the relationship between metformin and lactic acidosis in diabetic patients, and provide help and reference for metformin drugs in the clinical treatment of T2DM.

1. Introduction

Metformin is a widely used oral hypoglycemic drugs, have good hypoglycemic effect and the advantages of high safety, strong tolerance, be like the Chinese diabetes society (CDS), Japan diabetes association (JDA), and other academic organizations as type 2 diabetes (T2DM) for the treatment of first-line drugs, at the same time more than academic organization pointed out such as in patients without liver and kidney dysfunction and dose appropriate cases, metformin will always as a treatment of choice for patients with diabetes drug [1-3]. However, some studies have shown that taking metformin may be one of the causes of lactic acidosis, with a mortality rate of up to 50% [4].

Correlation lactic Acidosis is the first-line drugs is often accompanied by one of the serious adverse reactions, clinical trials in recent years, studies have shown that doses of Metformin treatment result in the correlation of lactic Acidosis (MALA) cases are rare, but if the dose is too high, and not considering the circumstances of the body function in patients with abnormal could also lead to plasma lactic acid accumulation, and even cause correlation lactic Acidosis (LA) [5]. This article will focus on the mechanism of metformin associated lactic acidosis (MALA) and its related factors to systematically review, in order to further clarify the relationship between the clinical use of metformin and diabetic lactic acidosis.

2. Hypoglycemic Mechanism of Metformin

Metformin is often combined with sulfonamide drugs in clinical use, which can effectively reduce blood glucose, and the effect of oral treatment is significant in diabetic patients, and the hypoglycemic effect is confirmed [6]. Its hypoglycemic effect mainly includes the following three aspects.

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2.1 Inhibition of Glucose Absorption and Promotion of Glucose Utilization

The amount of blood sugar in human body mainly depends on the extent to which glucose is absorbed and utilized by tissues or organs. Glucose is absorbed mainly through the intestines. Glucose absorption by intestinal wall and other tissues is mainly through active transportation and glucose uptake by the carrier and ATP [7]. Several recent studies have shown that endotoxin derived from gut microbes may increase the disruption of intestinal barrier function and increase glucose uptake, leading to the development of type 2 diabetes. Some studies have shown that [8]metformin can affect the microflora of mice and reduce the abundance of endotoxin-producing mucin-degrading G (-) anaerobes Akkermansia muciniphila in the intestinal tract of mice, thereby inhibiting excessive glucose absorption and thereby reducing the incidence of type 2 diabetes [9, 10]. Glucose use is regulated mainly by hormones, the most important of which is insulin. Experimental results show that the oral metformin can improve mice to mice pancreas litres of blood sugar hormone kind of peptide 1 (glp-1) content, but its mechanism is still unclear, glp-1 have promote insulin secretion, enhance tissue sensitivity to insulin, alpha cells glucagon secretion inhibition, and by increasing the body's insulin levels, in turn, promote the use of glucose [11-13]. Therefore, metformin, on the one hand, can reduce the absorption of glucose in the intestinal wall, and on the other hand, it can promote the utilization of glucose by regulating GLP-1 to increase the level of insulin.

2.2 Inhibition of Liver Glucose Output

In the regulation of human blood glucose, the liver plays an extremely important role in maintaining blood glucose balance. It can not only reduce blood glucose concentration through metabolic methods such as liver glycogen synthesis, but also increase blood glucose concentration through liver glycogen decomposition and gluconeogenesis. Metformin reduces liver glucose production primarily by adenosine activated protein kinase (AMPK). Main effect of metformin on the respiratory chain complexes I to block the mitochondrial respiratory chain reducing intracellular ATP production, in turn, activates AMPK, its possible mechanism is: ① After metformin into cells, by influencing the LKB1 / AMPK signalling to reduce the concentration cAMP, promote response element binding protein 2 (CRCT2) phosphorylation, inhibition of sugar dysplasia the expression of related genes, thereby reducing glycogenesis [14]. ② AMPK can activate liver deacetylase (SIRT1) and promote the acetylation and ubiquitination degradation of CRTC2, thereby inhibiting the transcription of genes related to gluconeogenesis [15]. ③ Metformin activated AMPK upregulates the expression of orphan nuclear receptor (SHP), which competes for the binding site of CRTC2 and inhibits the formation of transcriptional complexes, thereby inhibiting the expression of glycogenic related genes [14]. In addition, metformin can also reduce gluconeogenesis through other pathways, the possible mechanisms of which are as follows: ① Mitochondrial respiratory chain is blocked by acting on respiratory chain complex I and ATP production is reduced, and ATP has allosteric inhibition on key enzymes in glycolysis [16]. ② The decrease of ATP is accompanied by the increase of AMP, which inhibits the increase of blood glucose by inhibiting the glucagon signaling pathway [17]. ③ Metformin directly inhibits mitochondrial glycerol-3-phosphate dehydrogenase, enhances cytoplasmic reductive state, and inhibits the conversion of glycerol and lactic acid to glucose.

2.3 Improve Insulin Sensitivity and Insulin Resistance (IR)

After absorption into blood, metformin can act on organs around the liver, increase the number of insulin receptors and tyrosine kinase activity, and also improve the ability of insulin receptors and insulin binding, so as to improve the responsiveness of tissues and organs around the liver to insulin. Carolina et al. showed that when metformin was applied in clinical treatment, it could increase the insulin responsiveness of tissues and organs around the liver, so as to improve the utilization of glucose by tissue cells guided by hypoglycemic hormone [18]. Metformin also increases glucose transporter 4(GLUT-4) gene expression in skeletal muscle cells, thereby increasing the number and activity of GLUT-4 in skeletal muscle cells, thereby reducing peripheral insulin resistance (IR) [19]. In adipose tissue, metformin inhibits the phosphorylation of carbohydrate response element (ChREBP) and sterol binding element -1c (SREBP-1c) through AMPK signaling pathway, promotes the reconversion of free fatty acids (FFA) to triglycerides and inhibits the decomposition of triglycerides. AMPK can also directly reduce acetyl-CoA carboxylase (ACC) activity and β-oxidation, thereby indirectly improving IR [20].

3. Metguanidines and Lactic Acidosis

Lactic acidosis is a type of anion gap metabolic acidosis that occurs when lactic acid production is increased or metabolic pathways are blocked. When the disease is severe, it often involves multi-system organ function and

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has a high-risk of death [21-22]. The hypoglycemic effect of guanidine drugs is mainly realized by inhibiting liver glucose production and glucagon secretion and other mechanisms. It can play a role in lowering blood glucose in diabetic patients with normal or abnormal insulin secretion, but has no significant lowering effect on blood glucose in non-diabetic patients [23]. The most common adverse reaction in the treatment of formin drugs is lactic acidosis, and MALA is a rare adverse reaction of metformin with a high fatality rate up to 50% [24], which is caused by the enhanced effect of formin drugs on the metabolism of reducing glucose. Metformin has been recognized as one of the first choice drugs for the treatment of type 2 diabetes due to its superior hypoglycemic effect, good tolerance and high safety. Metformin reduces lactic acid gluconeogenesis, thereby reducing the production of glucose, thereby accumulating lactic acid and thereby increasing the possibility of lactic acidosis in diabetic patients [4,23,25].

In clinical studies, cases of MALA are rare. There have been reports of cases of LA in type 2 diabetic patients taking metformin, but these reports do not have a large number of data to show a clear causal relationship between metformin and lactic acidosis, and medical evidence is lacking. However, a large number of previous meta-analyses have shown that the use of metformin has no significant correlation with the incidence of lactic acidosis and the mortality caused by lactic acidosis [23]. Previous studies have shown that MALA patients are often associated with secondary diseases that accelerate metabolic decompensation, usually infection, acute kidney, liver failure, or heart failure [26-28]. Although a possible role cannot be ruled out, most researchers agree that metformin does not consistently correlate with the degree of acidosis. It is generally believed that the associated exacerbation of high-risk diseases such as heart, kidney, and respiratory failure is the cause of the high mortality of lactic acidosis rather than the effect of metformin [29]. Scale et al. believed that compared with metformin, the organic changes of tissues and organs caused by diabetes itself were more of a risk factor for LA, and the possible mechanism was that microvascular lesions caused by diabetes caused tissue hypoxia, thus increasing the risk of LA [30]. However, there are no substantial data showing a clear causal relationship between appropriate doses of metformin and lactic acidosis in patients without liver or kidney impairment.

3.1 Factors Influencing Metformin - Associated Lactic Acidosis (MALA)

As mentioned earlier, metaguanides are associated with an increased risk of lactic acidosis in diabetes treatment. However, due to the differences in molecular structure and chemical properties, metformin does not inhibit the release and metabolism of lactic acid. Therefore, the possibility of LA occurrence caused by metformin is much lower than that of other metformin drugs, and it is a relatively safe drug. According to many clinical studies, the occurrence of LA in the treatment of diabetes with metformin is very rare, and most of the cases are often accompanied by cardiopulmonary insufficiency, renal dysfunction and other diseases. Meanwhile, the incidence of lactic acidosis caused by metformin is also related to age and dosage [31].

3.2 Renal Insufficiency and MALA

Metformin is mainly excreted after the formation of water-soluble compounds through the treatment of oxidative reducing water and other processes of the kidney. In general, metformin is mainly excreted through renal tubules, so as to ensure an appropriate level of metformin in the body. Thus, when renal function is normal, clinical treatment with medical doses of metformin does not result in lactic acidosis. When kidney dysfunction occurs, metformin cannot be effectively excreted, leading to metformin deposition in the body, which leads to elevated lactic acid levels in the body. Tian Hui et al. carried out an experiment, selected 243 elderly patients with T2DM, and observed the blood lactic acid level of patients before and after metformin alone, combined with other oral hypoglycemic agents or combined with insulin, and found that there was no statistical significance in the fluctuation of blood lactic acid content in patients, and no lactic acidosis event was observed [32]. Our guess is that severe kidney failure leads to a significant increase in lactate levels in the body.

3.3 Hypoxia and MALA

3.3.1 Cardiac Insufficiency and MALA

Most clinical studies have shown that the probability of metformin directly causing LA in patients is very small, while the probability of lactic acidosis in an anoxic state is sharply increased [33]. Diabetic patients are far more likely to suffer from heart failure than healthy people. If acute heart failure, myocardial infarction and other diseases occur in the body, it will lead to cardiac pumping dysfunction, which will lead to the decline of human circulation function, resulting in hypoxia of tissues and organs and lead to functional disorders. Therefore, the ability of important organs such as liver and kidney to remove lactic acid may decrease, and the lactic acid content may be affected and tends to rise, and even lactic acidosis may occur in the human body. Moreover, due to the increase of lactic acid accumulation, the K^+ channel of cardiomyo-

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cytes is stimulated to expand and the K^+ outflow degree is increased. The adverse effect is inhibited on the Ca^+ channel, resulting in the decrease of cellular ion concentration, and then the weakening of cardiac systolic function, forming a chain of adverse reactions [34-35].

Therefore, metformin is often contraindicated for heart failure patients in clinical practice. Studies the selection of diabetes patients with heart failure in 1997-2006 (n = 10920), these patients through the use of metformin or sulfonylureas fall blood sugar, such as drug therapy, patients at the same time of voluntary participation in research study until the end of 2006, finally found a total of 6, 187 (57%) patients died, the process and no lactic acidosis patients in experimental [36]. Evans et al., Roussel et al., also reached the same conclusion in the same year [37,38]. Relevant literature has shown that metformin plays a certain role in antioxidant protection of cardiovascular disease [39]. Therefore, when the body does not have hypoxia caused by acute cardiac dysfunction, metformin can be used in patients with diabetes and cardiac dysfunction, but follow-up experimental studies are still needed to verify this.

3.3.2 Alcohol Intake and MALA

Long-term alcohol intake may lead to liver damage and even terminal liver disease. Liver is the main place for lactic acid metabolism, and metformin also causes the increase of lactic acid by inhibiting the metabolism of lactic acid in the liver. Therefore, in the case of liver injury, the use of metformin will lead to the increase of lactic acid content in the body, leading to lactic acidosis. Krzymie ń, etc, according to a study in 29 patients with correlation lactic acidosis (8 cases of metformin treatment, 21 cases of other glucose-lowering drugs or treatment), 12 cases of alcoholic patients, 5 cases of death cases, 3 cases of metformin treatment, therefore the literature suggests that patients with diabetes with correlation of lactic acidosis with metformin use has no obvious relation, and with greater [40] the correlation of alcohol abuse. The specific mechanism is that alcohol stimulates the intestinal wall and produces a large amount of lactic acid accumulation, which needs to be transformed with the help of the liver. If the patient is alcoholized and the lesion involves the liver, ethanol metabolism in the liver cells has a synergistic effect with the conversion of pyruvate to lactic acid, and it can also inhibit the degree of glycoeogenesis of pyruvate. Therefore, chronic alcoholism may impair liver parenchyma, thereby attenuate hepatic gluconeogenesis. Metformin has a similar effect to alcoholism, both promoting lactic acid production and preventing lactic acid metabolism [41]. They work in synergy. Therefore, heavy alcohol intake and chronic alcoholism may increase the incidence of MALA.

3.4 Age and MALA

With the increase of age, the liver and kidney functions of the elderly gradually decrease; meanwhile, the long-term lesions of the microvessels in the elderly diabetic patients tend to cause hypoxia in the surrounding tissues, resulting in the increase of metformin and lactic acid content, which eventually leads to lactic acid accumulation and poisoning [33]. Based on the data analysis of 12 diabetic patients with lactic acidosis caused by clinical treatment with metformin by Hua Zhong et al., it was shown that MALA was mainly characterized by older age, more liver and kidney insufficiency, irregular medication, etc [42,43]. Again, age was strongly associated with the incidence of MALA. Therefore, in the treatment of elderly diabetic patients, patients with serious liver and kidney dysfunction should be careful to take biguanidine drugs.

4. Summary and Prospect

A rare lactic acidosis occurs during the use of metformin. Is it metformin that is the culprit? Comb through the literature at home and abroad were reviewed, found that most of the research results show that has no direct correlation between metformin and lactic acidosis, at the same time a large number of clinical randomized controlled support metformin, there is no direct causal relationship between lactic acidosis and [44-45], but the real relationship also need to develop a large number of clinical trials conducted in-depth research for them. What's more, metformin can significantly increase the incidence of lactic acidosis when used in patients with liver and kidney insufficiency and heart failure. Although the incidence of lactic acidosis is very low, the characteristics of high mortality still need to be paid attention to by clinical workers. Therefore, patients' conditions, risk factors and physical conditions should be fully evaluated before the use of metformin in the treatment of patients, in order to effectively reduce the adverse reactions of metformin in the clinical treatment of diabetes, including metformin associated lactic acidosis (MALA).

References

[1] American Diabetes Association.Standards of medical care in diabetes-2011［J］.Diabetes Care，2011，34(5) : 11-60.

[2] Nathan DM,Buse JB,Davidson MB,et al.Medical management of hyperglycaemia in type 2 diabetes mellitus: A consensus algorithm for the initiation and

DOI: https://doi.org/10.30564/jer.v3i1.2849

adjnstment of therapy: a consensus statement from the American diabetes association and the European Association for the study of diabetes [J]. Diabetolgia, 2009, 52(1) : 17 -30.

[3] Yang WY. Guidelines for the prevention and treatment of type 2 diabetes in China [M]. Beijing: Peking University Medical Press, 2010:21.

[4] Almirall J, Briculle M, Gonzalez clemente JM. Metformin-as-sociated lactic acidosis in type 2 diabetes mellitus : incidence and presentation in common elinical practice. Nephrol Dial Trans-plant[J], 2008,23:2436- 2438.

[5] Xianbin Cai,Xi Hu,Bozhi Cai,et al. Metformin suppresses hepatocellular carcinoma cell growth through inductionof cell cycle G1/G0 phase arrest and p21CIP and p27KIP expression and downregulationof cyclin D1 in vitro and in vivo[J]. Oncology Reports,2013,30(5).

[6] KlarenbachS,CameronC,SinghS,etal.Cost-effectiveness of second-line anti -hyperglycemictherapy in patients with type 2 diabetes mellitusinade quately controlled on metformin [J].CanMedAssoc J,2011,183(16):E1213-1220.

[7] Yang K. Study on the mechanism of sea-buckthorn seed flavonoids promoting glucose uptake in fat and skeletal muscle cells [D]. East China Normal University, 2017.

[8] Hur KY,Lee MS.Gutmicrobiota and metabolic disorders Diabetes MetabJ[J],2015,39;198-203.

[9] Everard A, Lazarevic V, Gaia N, Johansson M, Stahlman M, Backhed F, Delzenne NM, Schrenzel J, Francois P, Cani PD. Microbiome of prebiotic-treated mice reveals novel targets involved in host response during obesity[J]. ISME J, 2014;8:2116–2130.

[10] de Kort S, Keszthelyi D, Masclee AA. Leaky gut and diabetes mellitus: what is the link?[J], Obes Rev 2011;12:449–458.

[11] Mu Yiming, Ji Linong, Li Chunlin, et al. Expert consensus on the clinical application of metformin (2018 edition). Chinese Journal of Diabetes,2019, 27 (3): 161-173.

[12] Maida A,Lamont BJ,Cao X,et al.Metformin regulates the incretin receptor axis via a pathway dependent on peroxisome proliferator-activated receptor-α in mice. Diabetologia,2011,54(2):339-349.

[13] Cho YM,Kieffer TJ. New aspects of an old drug: metformin as a glucagon-like peptide 1 (GLP-1) enhancer and sensitiser[J]. Diabetologia,2011,54(2): 219-222.

[14] Viollet B,Guigas B,Sanz Garcia N,et al.Celluar and molecular mechanisms of metforminan overview. Clinical Science,2012,122:253-270.

[15] Caton PW,Nayuni NK,Kieswich J,et al.Metformin suppresses hepatic gluconeogenesis through induction of SIRT1 and GCN5.J Endocrinol,205:97-106.

[16] Foretz M,Hebrard S,Leclerc J,et al.Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state[J]. The Journal of Clinical Investigation,2010,120(7):2355-2369.

[17] Miller Russell A,Chu Q,Xie J,et al.Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP[J]. Nature,2013,494(7436).

[18] Carolina Gutiérrez-Repiso,Isabel Moreno-Indias,et al. Mucosa-associated microbiota in the jejunum of patients with morbid obesity: alterations in states of insulin resistance and metformin treatment[J]. Surgery for Obesity and Related Diseases,2020:1878-7533.

[19] Qingling Meng, Xiuping Jin, Naijun Wu, Weibo Yang. Effects of metformin on the expression of adenylate activated protein kinase and glucose transporter 4 in insulin-resistant mouse embryonic fibroblasts [J]. Chinese Journal of Diabetes,2014,22(02):177-180.

[20] Wang Zhanjian, Tan Lixin. Metformin improves insulin resistance [J]. Drug Evaluation, 2010,7(13):17.

[21] Hu Baoji, Bo Lulong, Deng Xiaoming, Duan Hongwei. Chinese Journal of Medical Review,2018,15(03):22-25.

[22] Kajbaf F, Lalau JD. The criteria for metformin-associated lactic acidosis: the quality of reporting in a large pharmacovigilance da-tabase[J]. Diabetic Med, 2013, 30(3) : 345-348.

[23] Lu ZQ. The value of metguanidine hypoglycemic drugs in the treatment of type 2 diabetes mellitus [J]. Journal of Clinical Therapeutics,2010,8(04):33-38.

[24] Inzucchi,SE,BergenstalRM,Buse JB,et al.Management of hyperglycemia in type2 diabetes,2015:A patient-centered approach:Update to a position statement of the American Diabetes Association and the European Association for the study of diabetes[J]. Diabetes Care, 2015,38:140-149.

[25] McAlister FA, Majumdar SR, Eurich DT et al. The effect of specialist care within the first year on subsequent outcomes in 24 232 adults with new-onset diabetes mellitus: popula- tion-based cohort study.Qual Saf Health Care, 2007, 16(1) : 6 -11.

[26] Salpeter S, Ureyber E, Pasternak U, et al. Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus[J]. Cochrane Database Syst Rev, 2010,4: CD002967.

[27] Inzucchi SE, Lipska KJ, Mayo H, et al. Metformin in patients with type 2 diabetes and kidney disease a systematic review.JA- MA,2014, 312(24)

 DOI: https://doi.org/10.30564/jer.v3i1.2849

: 2668-2675.

[28] Richy FF, Sabido-Espin M, Guedes S, et al. Incidence of lactic acidosis in patients with type 2 diabetes with and without renal impairment treated with metformin: a retrospective cohort study. Diabetes Care, 2014, 37(8) : 2291-2295.

[29] Aharaz A, Pottegard A, Henriksen DP, et al. Risk of lactic aci- dosis in type 2 diabetes patients using metformin: A case control study. PloS One, 2018, 13(5) : e0196122.

[30] Scale T, Harvey JN.Diabetes,Metformin and lactic acidosis [J]. Clin Endocrinol (Oxf), 2011, 74(2) : 191-196.

[31] Nathan DM ,EuseIB ,Davidzcn MS, et al.Medicai management of hyperglycaemia in type 2 diabetes mellitus; Acon-sensus algorithm for the initiation and adinsmen of therapy:a consensus statement from the American diabeies asseciation and the European Association for the study of diabeteg[J]. Diabetolgia, 2009,52(1);17-30.

[32] Tian Hui, Li Chunlin, Yang Guang, et al. Safety evaluation of metformin in elderly patients with type 2 diabetes mellitus. Chinese Journal of Internal Medicine, 2008,47:914-918.

[33] Wang Xiaobing, Ye Shandong. Research progress of metformin related lactic acidosis [J]. Anhui Medical Journal,2014,35(01):130-133.

[34] Deng Shiman.Study on the correlation between hemoglobin A1c, serum lactic acid level and cardiac dysfunction [J]. China Contemporary Medicine,2019,26(1): 45-47.

[35] Chen Haiyan, Wang Yinghuo. Clinical significance and prognosis evaluation of serum lactic acid monitoring in patients with cardiogenic shock [J]. Chinese Journal of Traditional Medicine,2020(03):92-95.

[36] Andersson C, Olesen JB, Hansen P R, et al. Metformin treatment is associated with a low risk of mortality in diabetic patients with heart failure: a retrospective nationwide cohort stud [J]. Diabetologia, 2010, 53 (12) : 2546 -2553.

[37] Evans JM, Doney AS, AlZadjali MA, et al. Effect of Metform in on mortality in patients with heart failure and type 2 diabetes mellitus [J]. Am J Cardiol, 2010, 106 (7): 1006-1010.

[38] Roussel R, Travert F, Pasquet B, et al. Reduction of Atherothrombosis for Continued Health (REACH) Registry Investigators. Metformin use and mortality among patients with diabetes and atherothrombosis[J]. Arch Intern Med, 2010, 170 (21) : 1892 -1899.

[39] lalau JD,Race J.Lactic acidosis in metfoxmin-tx-eaied pa-tients. RPrognosiic value of axiexial lsciate levels and plasmameifoxmis concentrations[J]. Drug Sef ,1999 ,20(4):.371-384.

[40] Krzymień J, Karnafel W. Lactic acidosis in patients with diabetes[J].Pol Arch Med Wewn, 2013, 123(3): 91 -97.

[41] McAlister FA, Majumdar SR, Eurich DT, et al. The effect of specialist care within the first year on subsequent outcomesin 24 232 adults with new-onset diabetes mellitus: popula-tion-based cohort study[J]. Qual Saf Health Care, 2007, 16(1) : 6-11.

[42] Yu Huazhong, Li Zhangping, Wu Chaoming, Cheng Junyan, Chen Shouquan. Analysis of the characteristics of lactic acidosis in diabetic patients taking metformin [J]. Journal of Practical Medicine,2006(07):804-805.

[43] Lin YC, Lin LY, Wang HF, Lin HD. Fasting plasma lactate concentrations in ambulatory elderly patients with type 2 dia- betes receiving metformin therapy: a retrospective cross-sec- tional study[J]. Chin Med Assoc, 2010, 73 (12) : 617-622.

[44] Salpeter SR ,Greyber E, Pasternak GA.et al. Risk of fatal and nonfatal lactic acidosis with metformin use in type 2 diabetes mellitus. Cochrane Database Syst Rev, 2010, 4:CD002967.

[45] Cryer DR. Nicholas SP, Henry DH.et al. Comparative outcomes study of metformin intervention versus conventional approach the COSMIC Approach Study. Diabetes Care, 2005 ,28:539-543.

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ARTICLE

Effect of Metformin on Lactate Metabolism in Normal Hepatocytes under High Glucose Stress in Vitro

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ABSTRACT

Objective To study the effect of metformin on lactate metabolism in hepatocytes in vitro under high glucose stress. In vitro LO2 cells, liver cells were randomly divided into blank control group, 25 tendency/L glucose solution, 27 tendency/L glucose solution, 29 tendency/L glucose solution, 31 tendency/L glucose solution, 33 tendency/L glucose solution, 35 tendency/L glucose solution treatment group, the optimal concentration of 31 tendency after L, use 30 tendency for L metformin solution, and then divided into blank control group, the optimal concentration of glucose solution, normal liver cells + metformin solution normal liver cells. The optimal concentration of glucose solution normal liver cells + metformin solution respectively in the 12 h, 24 h, 48 h on cell count plate to calculate the number of liver cells, and using lactic acid determination kit the optimal concentration of glucose solution + normal liver cells and normal liver cells + the optimal concentration of glucose solution + metformin solution respectively in the 12 h, 24 h, 48 h of cell cultures of lactic acid value. There was no significant change in the lactic acid concentration but significant increase in the number of surviving hepatocytes in the high-glycemic control group compared with that in the high-glycemic control group without metformin. Metformin has no significant effect on lactic acid metabolism of hepatocytes under high glucose stress in vitro, and has a protective effect on hepatocytes under high glucose stress. Based on this, it is preliminarily believed that metformin is not the direct factor leading to diabetic lactic acidosis.

1. Introduction

With the change of people's lifestyle and habits, the incidence of diabetes has been on a straight rise. At present, 1.01 million new diabetes patients are newly diagnosed in China every year. It is predicted that the number will reach 59 million in 2025, and more than 90% of the patients will be type II diabetes mellitus (T2MD) [1-3]. China

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has the largest number of diabetic patients [4]. Because the occurrence and development of diabetes involve a variety of factors, the pathogenesis is complex, and there is a variety of damage mechanism interaction, so far there is no cure.

Clinically, traditional hypoglycemic drugs have played a good role in controlling blood glucose and delaying the occurrence of complications, but there are still some limitations and adverse reactions. Metformin is an oral metformin antidiabetic drug, which is widely used in clinic because of its significant hypoglycemic effect and multi-faceted clinical application value. Metformin is also regarded as the preferred hypoglycemic drug in the world. Home and DDP studies have demonstrated the safety and efficacy of metformin in the treatment of diabetes [5]. However, with the increasing application of metformin in clinical practice, some side effects have been gradually shown in the use of metformin as a hypoglycemic agent, among which lactic acidosis (LA) has been a concern of clinicians. Patients taking metformin caused by lactic acidosis, called metformin correlation lactic acidosis (MALA), MALA is the use of metformin treatment in the process of unusual and severe adverse reactions, is due to the metformin hindered the pathways of the lactic acid to glucose in the mitochondria, the cause of lactic acid in the body to generate too much or too little, caused the body to produce the metabolic disorders, the mortality of the disease is very high [6-7]. Studies have shown that the lactic acidosis caused by metformin may be related to the severe diseases of diabetic patients [8], which is mainly related to renal insufficiency, cardiac insufficiency and hypoxia of patients. Based on this, this paper will elaborate the effects of metformin intervention on lactic acid metabolism of normal liver cells under high glucose stress in vitro, and then explore whether metformin has a direct correlation with lactic acid metabolism, so as to provide reference for the clinical treatment of diabetes and the rational use of metformin.

2. Materials and Methods

2.1 Main Reagents and Instruments

Reagents: LO2 liver cell line, DMEM medium, trypsin, penicillin-streptomycin double antibody mixed solution, metformin, 10% fetal bovine serum, lactate assay kit (all purchased from Haikou Ruike Biological Technology Co., Ltd.) were selected.

Instruments: microscope, ultra-clean biological platform, CO_2 cell incubator.

2.2 Experimental Methods

2.2.1 Cell culture

LO2 cells with 10% fetal bovine serum DMEM culture completely, at 37 ℃ and 5% CO_2 incubator in the breeding, cultivation to the cell wall, routine cell culture, 1 ~ 2 days in a fluid, when the culture cell coverage was 80% ~ 90%, subculture, repeated operation, will have a bottle of the represented the cells cryopreserved, in order to avoid accident cause lack of LO2 liver cells to experiment.

2.2.2 Group Model Establishment Control Experiment

2.2.2.1 Establishment of High Glucose Model

Before the beginning of this experiment, LO2 liver cells were pretreated with high Glucose, and the optimal concentration of Glucose (G) was tested. The concentration gradient of G was set as 0 mmol/L, 25 mmol/L, 27mmol/L, 29mmol/L, 31 mmol/L, and 33 mmol/L. The preexperiment obtained 31 mmol/L as the optimal concentration, and the lactic acid content was measured with the lactic acid kit.

2.2.2.2 Establishment of Metformin Model

In this experiment, metformin hydrochloride tablets were diluted to 30mmol/L into cell culture medium to pretreat LO2 hepatocytes with metformin for 12 h, 24 h and 48 h.

2.2.2.3 Establishment of Intervention Model

In this experiment, 30mmol/L of metformin hydrochloride culture solution and 31mmol/L of glucose solution were added into the culture flask. After culture for 12 h,24 h and 48 h, the lactic acid content was measured with the lactic acid kit.

2.2.3 Hepatocyte Proliferation was Determined by Cell Count Method

Trypsin was used to decompose the cells, and after digestion for a period of time, the culture solution was added to stop digestion, and then the culture solution was transferred to the counting plate with pipetting gun, and the cells were counted in strict accordance with the cell counting rules.

2.2.4 Lactic Acid in Samples was Detected by Kit Method

In addition, metformin solution and high-glucose treated cell solution were added and put into carbon dioxide cell incubator for culture for 12 h, 24 h and 48 h, and the lactic acid value in the cell culture medium was measured by the lactic acid kit respectively, so as to judge the effect

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of dimethyldiplastema on cells under high glucose.

2.3 Statistical Methods

Statistical analysis SPSS25.0 statistical software was used for data processing. Sample t test was used for sample mean and one-way analysis of variance was used for multiple mean. $P < 0.05$ was considered statistically significant.

3. Results and Analysis

3.1 Selection of the Most Suitable Liver Cells with High Glucose Concentration

After this experiment, the most suitable concentration of G in LO2 liver cells under high glucose stress was explored, as shown in Table 1. When the concentration of G was 31 mmol/L, the most appropriate concentration was found.

3.2 Effects of Metformin on Lactic Acid Metabolism of Normal Liver Cells under High Glucose Stress

30 mmol/L metformin was prepared in the medium, and the control group and the experimental group were set. Normal liver cells, high glucose + normal liver cells, metformin + normal liver cells, and high glucose + metformin + normal liver cells were designed respectively. The changes of lactic acid concentration measured at 12 h, 24 h and 48 h of culture were shown in Table 2.

According to the experimental group and control group, the metformin itself does not cause liver cells to produce lactic acid, under the environment of high sugar, according to the results of the experiment concerning group contrast, before adding metformin, the concentration of lactic

Table 1. Selection of the most suitable liver cells with high glucose concentration (normal liver cells)

The concentration of added glucose (mmol/L)	25	27	29	31	33	35	0
Number of surviving liver cells (12h)	1.12×10*7	1.06×10*7	1.02×10*7	0.98×10*7	0.88×10*7	0.40×10*7	1.30×10*7
Number of surviving liver cells (13h)	2.04×10*7	1.97×10*7	1.95×10*7	1.92×10*8	1.72×10*8	1.25×10*8	2.60×10*7
Number of surviving liver cells (14h)	3.99×10*7	3.92×10*7	3.79×10*9	3.76×10*9	3.63×10*9	3.19×10*9	5.20×10*7
Number of surviving liver cells (15h)	1.68±0.29	2.46±0.79	3.27±1.38	3.48±1.42	4.63±1.63	5.34±2.26	0
Number of surviving liver cells (16h)	3.89±1.42	4.79±1.68	5.24±2.17	5.89±2.64	6.23±2.79	8.98±2.86	0
Number of surviving liver cells (17h)	6.72±2.33	8.43±2.56	9.14±2.99	9.28±3.11	10.87±3.34	11.27±3.39	0

Table 2. Effects of metformin on lactic acid metabolism of normal liver cells under high glucose stress

	Group 1 (liver cells in normal group)			Group 2 (normal + high glucose)		
	12h	24h	48h	12h	24h	48h
Add glucose concentration mmol/L	/			31		
Add metformin concentration (mmol/L)	/			/		
Lactic acid concentration (mmol/L)	0	0	0	3.48±1.42	5.89±2.64	9.28±3.11
Number of surviving liver cells (1)	1.30×10*7	2.60×10*7	5.20×10*7	0.98×10*7	1.92×10*8	3.76×10*9
	Group 3 (normal group + metformin)			Group 4 (hepatocytes + high glucose + metformin)		
	12h	24h	48h	12h	24h	48h
Add glucose concentration mmol/L	/			31		
Add metformin concentration (mmol/L)	30			30		
Lactic acid concentration (mmol/L)	0	0	0	3.30±1.39	5.83±2.63	9.17±3.08
Number of surviving liver cells (unit)	1.30×10*7	2.60×10*7	5.20×10*7	1.15×10*7	2.24×10*7	4.79×10*7

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acid in culture medium of high glucose + normal liver cells increased from 1.42 mmol/L to 3.48 mmol/L in 12 h, and the highest value increased to 5.89 mmol/L after 24 h, After 48 h, the increase rate of cell number began to decrease significantly, and the lactic acid concentration also increased to 9.289 mmol/L, and after adding metformin, There were no significant changes in lactate concentration and cell number compared with those under high glucose stress alone.

4. Discussion

A large number of studies have shown that lactic acidosis is a rare and serious complication of diabetic patients, most of which occur in patients who take guanidine drugs and are accompanied by liver and kidney insufficiency, heart failure, etc [9-10]. In recent years, studies have suggested that Metformin Lactate Acidosis (MALA) caused by normal therapeutic doses of Metformin is rare, but it may also lead to elevated plasma lactic acid levels and even Lactate Acidosis (LA) if it is improperly used in clinical practice [11]. Liver is an important organ of glucose metabolism. Liver can absorb and use glucose to reduce blood sugar, and can convert glucose into liver glycogen for storage. Patients with cirrhosis have increased insulin resistance, which will affect glucose metabolism and cause hepatogenic diabetes. Diabetes can also affect the liver, especially patients with type 2 diabetes who are prone to liver function damage and non-alcoholic fatty liver disease [12]. As a traditional hypoglycemic agent, metformin can promote the metabolism of glucose, increase its anaerobic colysis, improve the level of lactic acid and lead to lactic acidosis. In addition, metformin can inhibit the utilization of lactic acid by the liver and muscle and inhibit gluconeogenesis, thus reducing the production of glucose, and thereby increasing the risk of lactic acid poisoning by accumulation of lactic acid [13-15].

In this study, it was found that metformin had little effect on the metabolism of lactic acid in liver cells under high glucose environment, and there was no significant difference in the content of lactic acid measured between the experimental group and the control group after adding different levels of metformin, but different levels of metformin could promote cell proliferation. High glucose environment can inhibit the proliferation of liver cells, the reason may be that high glucose induces the expression of STC2 in liver cells, and overexpression of STC2 can further enhance the proliferation inhibition ability of liver cells induced by high glucose [16]. In addition, high glucose has also been shown to promote the secretion of inflammatory cytokines such as TNF‐α, IL‐6, and regulate the expression of apoptosis-related molecules B lymphoma 2 and Bax, thereby inducing apoptosis of hepatocytes[17]. Metformin promotes the proliferation of hepatocytes, possibly because it inhibits the secretion of inflammatory cytokines and the activity of nuclear factor-κB (NF-κB) through AMPK-dependent pathways, so as to promote cell proliferation [18].

In conclusion, metformin has no significant effect on lactic acid metabolism of hepatocytes in high glucose environment, but different concentrations of metformin have a protective mechanism for hepatocytes and can promote cell proliferation.

References

[1] Mu Yiming, Ji Linong et al. Expert consensus on clinical application of metformin (2016 edition) [J]. Chinese Journal of Diabetes, 2016,24 (10) :871-884.

[2] Dang Yongyan, Ye Xiyun, Shen Jie. Journal of East China Normal University, 2010, (5) : 143-148. (in Chinese with English abstract)

[3] Su Hongwei, Wei Xun, Pan Hailin et al. Blood Lactic Acid Level and Its Influential Factors in Patients with Type 2 Diabetes [J]. Diabetes mellitus, 2015,32 (2) :215-218.

[4] NingGuang. The status and prospect of diabetes prevention and treatment in China [J]. Science China (Life Sciences), 2018,48 (8) : 810-811.

[5] Lilian Beatriz Aguayo Rojas,Marilia Brito Gomes. Metformin: an old but still the best treatment for type 2 diabetes[J]. Lilian Beatriz Aguayo Rojas;Marilia Brito Gomes,2013,5(1).

[6] Zhang Weina, Yu Min, Sun Meiyun, et al. Lactic acidosis caused by metformin: a case report [J]. China Rural Medicine 2020,27(01),43-44.

[7] Qiu Y P. Be careful to take metformin in special population [J]. Shanghai Journal of Traditional Chinese Medicine 2019-08-09 004 Medication guidelines.

[8] Lin Jian, Zhou Zhiguang. Diagnosis, treatment and progress of diabetic lactic acidosis. Journal of Clinical Internal Medicine, 2017, 34:159-161.

[9] Richy FF, Sabido-Espin M, Guedes S, et al. Incidence of lactic acidosis in patients with type 2 diabetes with and without renal impairment treated with metformin: a retrospective cohort study. Diabetes Care, 2014, 37(8) : 2291-2295.

[10] Aharaz A, Pottegard A, Henriksen DP, et al. R isk of lactic aci- dosis in type 2 diabetes patients using metformin: A case control study. PloS One, 2018, 13(5) : e0196122.

[11] Xianbin Cai,Xi Hu,Bozhi Cai,et al. Metformin suppresses hepatocellular carcinoma cell growth through inductionof cell cycle G1/G0 phase arrest

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and p21CIP and p27KIP expression and downregulationof cyclin D1 in vitro and in vivo[J]. Oncology Reports,2013,30(5).

[12] Guo Yali, Lu Shuming, Li Chunyan, Liu Lina. The relationship between insulin resistance and liver function in patients with cirrhosis and diabetes mellitus [J]. Journal of Dalian Medical University,2017,39(04):378-382.

[13] Almirall J, Briculle M, Gonzalez clemente JM. Metformin-as-sociated lactic acidosis in type 2 diabetes mellitus : incidence and presentation in common elinical practice. Nephrol Dial Trans-plant , 2008 ,23:2436- 2438.

[14] Lu ZQ. The value of metguanidine hypoglycemic drugs in the treatment of type 2 diabetes mellitus [J]. Journal of Clinical Therapeutics,2010,8(04):33-38.

[15] McAlister FA, Majumdar SR, Eurich DT et al.The effect of specialist care within the first year on subsequent outcomes in 24 232 adults with new-onset diabetes mellitus:population based cohort study [J]. Qual Saf Health Care,2007,16 (1) : 6 — 11 .

[16] Tao Wenyu, Chen Jiaoli, Li Xiaojin, Xiong Yuxin, Hong Chao, Wang Xiaoling. Journal of Applied Medicine,2019,35(14):2220-2224+2229.

[17] LI Yan-chen, HUANG Cai-bin, LIU Yao, XIA Wenyan, XU Rong. The role and mechanism of high mobility group protein B1 in human hepatocyte malignant transformation induced by high glucose microenvironment [J]. Chinese Journal of Diabetes,2019,27(02):137-142.

[18] Li Z, Ding Q, Ling LP, et al.Metformin attenuates motility, contraction, and fibrogenic response of hepatic stellate cells in vivo and in vitro by activating amp-activated protein kinase[J].World J Gastroenterol, 2018, 24 (7) :819-832

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REVIEW

Covid-19 Mutations and the Effect of Different Vaccines on Immune Memory

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ABSTRACT

We traced the coronavirus classification and evolution, analyzed the Covid-19 composition and its distinguishing characteristics when compared to SARS-CoV and MERS-CoV. Despite their close kinship, SARS-CoV and Covid-19 display significant structural differences, including 380 amino acid substitutions, and variable homology between certain open reading frames that are bound to diversify the pathogenesis and virulence of the two viral compounds. A single amino acid substitution such as replacing Aspartate (D) with Glycine (G) composes the D614G mutation that is around 20% more infectious than its predecessor 614D. The B117 variant, that exhibits a 70% transmissibility rate, harbours 23 mutants, each reflecting one amino acid exchange. We examined several globally spreading mutations, 501.V2, B1351, P1, and others, with respect to the specific amino acid conversions involved. Unlike previous versions of coronavirus, where random mutations eventually precipitate extinction, the multiplicity of over 300,000 mutations appears to have rendered Covid-19 more contagious, facilitating its ability to evade detection, thus challenging the effectiveness of a large variety of emerging vaccines. Vaccination enhances immune memory and intelligence to combat or obstruct viral entry by generating antibodies that will prohibit the cellular binding and fusion with the Spike protein, restricting the virus from releasing its contents into the cell. Developing antibodies during the innate response, appears to be the most compelling solution in light of the hypothesis that Covid-19 inhibits the production of Interferon type I, compromising adaptive efficiency to recognize the virus, possibly provoking a cytokine storm that injures vital organs. With respect to that perspective, the potential safety and effectiveness of different vaccines are evaluated and compared, including the Spike protein mRNA version, the Adenovirus DNA, Spike protein subunits, the deactivated virus genres, or, finally, the live attenuated coronavirus that appears to demonstrate the greatest effectiveness, yet, encompass a relatively higher risk.

1. Coronavirus Evolution

The corona virus is a positive RNA virus enveloped by a membrane that was first identified in Wuhan in 2019 (SARS-CoV2 or Covid-19). It is classified under the beta coronaviruses category along with SARS-CoV (Severe Acute Respiratory Syndrome) and MERS CoV (Middle East Respiratory Syndrome). There are other beta type of

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human coronaviruses that cause enteric and upper respiratory tract infections, experienced during the common cold, such as the HCoV-OC43 and HCoV-HKU1. On the other hand, HCov-229D and HCoV-NL63 come under the alpha classification. Feline (FCoVs) and canine corona viruses (CCoVs) are also sorted under the alpha group. The remaining coronaviruses fall under the genera of gamma and delta categories that primarily affect poultry, wildlife and other birds, although rather sparce information is available regarding the delta division [1-2]. Very different types of viruses such as the Bafinivirus infects fish, while the Aterivirus is isolated in specific species including mice, monkeys, horses and pigs [3].

The highly contagious Covid-19 pandemic, resulting in 172,666,073 cases and 3,711,545 deaths globally by May 2021, has been one of the primary focuses of scientific research and review. Unlike the MERS-CoV that uses adenosine deaminase complexing protein 2, also known as Dipeptidyl peptidase-4 (CD26), as its primary receptor, both SARS-CoV and Covid-19 (SARS-CoV2) target and fuse with the angiotensin-converting enzyme 2 (ACE2) that serves as the viral portal into the cell. During their multiplication process, all viruses evolve to outlive the immune counterattack. Interestingly, these mutations appear to enhance the effectiveness and transmissibility of Covid-19, in contrast to other types of coronaviruses which are adversely affected by repeated mutations, eventually becoming extinct.

2. Coronavirus Composition

The genome of corona viruses is composed by sequences of around 26,000-32,000 variations of the ribonucleic acid (RNA) nitrogenous bases: adenosine, cytosine, guanine, and uracil. It harbours 6-11 open reading frames (ORFs), 67% of which encode 16 non-structural proteins (nsps) that direct virus assembly, transcription and replication in connection with the host, and the rest encode the accessory and structural proteins. Structural proteins include the main surface trimeric Spike glycoprotein (S) that binds and fuses with the ACE2 receptor, a key to lock process that releases the viral RNA into the cells; the smaller surface envelope (E) protein, the membrane (M), and the nucleocapsid (N) viral protein that defensively surrounds the genome. The N protein offers protection and signature sequences, equipping the virus with adaptation skills that enable it to survive the adversities of the host's environment. The immune defence assembled against the antigen is composed by: A/ antibodies (innate immune system) that prophylactically cover the ACE2 receptor to obstruct the Spike's contact and fusion with the human cells; and B/ the adaptive immune response comprised by a number of cells including cytotoxic T-killer cells or $CD8^+$ that clasp onto the infected cells' antigens, and obliterate them by releasing perforin and granzyme that is absorbed through the cellular pores [4-5].

2.1 The Kinship between SARS-CoV and Covid-19

The SARS-CoV and Covid-19 genomes are similar, however, there are important differences that may hinder upon the Covid-19 rate of infectiousness and virulence. Covid-19 is missing the 8a accessory protein that is present in SARS-CoV. The Covid-19 8b accessory protein has 37 more amino acids than SARS-CoV; and the SARS-CoV 3b has 132 more amino acids than Covid-19. Overall, SARS-CoV and Covid-19 have significant structural differences that amount to 380 amino acid substitutions; for example, the non-structural nsp3 protein has 102 variations in its amino acid sequence, the nsp2 has 61 amino acid conversions, and the Covid-19 Spike protein has 27 different amino acids' exchanges than SARS-CoV [6-7]. Therefore, despite their kinship, the two viral configurations, SARS-CoV and Covid-19 are packed with different messages that are bound to have important implications pertaining to their pathogenetic effects.

The S gene that encodes the Spike protein in Covid-19 is approximately 24% different from the S gene in SARS-CoV, and 65% different from the S gene in MERS-CoV. The N gene that encodes the nucleocapsid protein has around 90% homology between SARS-CoV and Covid-19, but only 48% between MERS-CoV and Covid-19. The E and M proteins' genes of Covid-19 have a 94% and 90% similarity with the SARS-CoV E and M genes respectively. Moreover, a comparison between Covid-19 and SARS-CoV has unveiled significant differences between the open reading frames ORF3a, ORF6, and ORF8, with homology rates of only 72%, 68%, and 40% respectively. The ORF7a appears to have the highest similarity rate, approaching 85% [8-10].

The trimeric spike (S) protein undergoes a structural transformation while binding with the ACE2 receptor. As a result, the S1 subunit sheds, allowing the S2 subunit to fuse with the receptor. This process is common in both SARS-CoV and Covid-19. The structural transformation of the S1 subunit consists of two conformation states, the up that exposes and makes the viral receptor available and the down that closes off the receptor. However, in SARS-CoV the down conformation of the receptor binding domain clicks into the N-terminal domain of the trimeric Spike (S) protein, while in the Covid-19, the down receptor fits into the central cavity of the trimer. [11-12]. This may be of significance under the assumption that the N-terminal domain remains available, in light of recent findings that

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certain human antibodies bind with the N-terminal domain (NTD). NTD targeting human antibodies can be added to those binding with the receptor binding domain (RBD) for greater therapeutic efficiency [13-14].

2.2 Covid-19 Neutralizing Antibodies

Covid-19 neutralizing antibodies are Y shaped proteins that can recognize the S1 RBD and fit into the viral antigens like a key to a lock. This prohibits the virus from binding with the ACE2 cellular receptor, thus preventing viral entry. Other antibodies can neutralize the heptad repeat 2 (HR2) domain to impede S2 fusion with the ACE2 receptor, so even if the Spike protein can bind with the ACE2 receptor, the second step of antigen/receptor fusion is compromised, disallowing Covid-19 entry into the cells, without which the virus can neither replicate, nor spread inside the body [15].

A recent study experimented on a powerful monoclonal antibody, LY-CoV555, that binds with the Covid-19 spike protein obstructing it from fusing with the cells' ACE2 receptors. The results of 309 patients who received the LY-CoV555 antibody treatment were compared to 148 patients who received placebo. Eighty percent of all 452 participating patients had mild Covid-19 symptomatology. By the 11th day of clinical observation both experimental and placebo group had a significantly reduced viral load, with the treated patients exhibiting a modest advantage. The experimental group patients who received a 2800mg antibody dose had a -0.53 difference from the placebo group ($p=0.02$ / $p<0.05$), which is a statistically significant result; notably, however, neither a lower dose of 700mgs ($p=0.38$) nor a higher dose of 7000mgs ($p=0.7$) were statistically significant. Importantly, when the rate of hospitalizations was examined on the 29th day, the percentage of the viral load in the experimental group that was treated with LY-CoV555, was 1.6%, contrasted with the significantly higher viral load of the placebo / control group that was 6.3%. Further analysis focusing on high risk aged (>65) and obese (BMI>35) individuals denoted a diminished hospitalization rate of 4.2% for those receiving LY-CoV555, when compared to 14.6% of non-treated patients [16].

2.3 Immune Memory

Immune memory that develops from milder forms of coronaviruses such as the HCoV variants (229E, NL63, OC43, HKU1) which cause the common cold, may be a significant factor contributing in the activation of immune defences to obstruct the virus, and/or reduce the viral load that diminishes contagion [17-18]. Immune memory pertains to antibodies that are secreted by B cells, the adaptive immune system killer-T cells ($CD8^+$) that obliterate infected cells, and helper T cells ($CD4^+$) that are in charge of activating cytokines [21]. Unfortunately, recent studies have shown that individuals infected with milder forms of coronavirus related to the common cold do not develop neutralizing antibodies that can be useful in Covid-19 [19]. However, the cytotoxic $CD8^+$ T cells have indicated a longer lasting SARS-CoV viral recognition, as illustrated by research evidencing that $CD8^+$ T cells were reminiscent in 60.9% of SARS-CoV recovered patients for at least six years, whereas these patients' B cells specific memory, that is crucial in generating antibodies, appeared to be negligible or absent [20]. Obviously, the longer lasting the immune memory, the superior the immune intelligence. This is the purpose of vaccination: to enhance inherent immune memory by presenting the Spike protein that informs the immune system of the malevolence of the enemy, thus prohibiting future binding and fusion between the Spike trimer and the ACE2 receptors. This type of innate immune "education," designed to obstruct viral entry altogether, is particularly useful in the elderly, whose adaptive immune response is compromised due to aging, and who will be inherently more vulnerable in combating the antigen via the adaptive immune defences, once Covid-19 has invaded human cells and has started multiplying [21].

2.4 Robust $CD4^+$ T and $CD8^+$ T cells Memory

A recent study has identified $CD4^+$ T cells which appeared to be reactive to Covid-19 Spike (S) glycoprotein of 83% of Covid-19 patients, targeting epitomes in both the N and C terminals of S, as well as in 34% of healthy controls, despite the fact that the target was limited to the C terminal. These investigators entertained the possibility that the $CD4^+$ T cells found in healthy controls that were reactive to Covid-19 S protein, may be the result of previous exposure to the common cold variant virus under the HCoV umbrella [22]. Another recent study revealed that Covid-19 patients have 70% of reactive $CD8^+$ cytotoxic cells and 100% of $CD4^+$ helper cells, which appeared to recognize the Spike, Membrane and Nucleocaspid proteins, as well as certain non-structural proteins (nsp3, nsp4) and open reading frames (ORF3a and ORF8). Additionally, they identified 50% of $CD4^+$ T helper cells and 20% of $CD8^+$ T cytotoxic cells reacting to the Spike, Membrane, and non-structural proteins in individuals that had tested negative to Covid-19, again suggesting that these cytotoxic and helper T cells' reactivity was obtained from previous exposures to milder coronavirus forms such as HCoV-OC43 and HCoV NL63 which, as previously noted, are responsible for the "common cold." The CD4+ and CD8+ cells' reactivity to open reading frames (ORFs) is significant, considering that

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ORFs encode both non-structural and structural proteins as well as assembly ones [23].

The immune memory of $CD4^+$ and $CD8^+$ T cells is an encouraging finding. However, allowing Covid-19 to enter the system which will occur in the absence of antibodies, may be already too late, especially in the elderly, or immunosuppressed individuals. The optimum method of fighting Covid-19 is focusing on antibodies that can block viral invasion in the first place. This is important for two reasons: a/ Preventing viral entry into the cells is the safest option. Once Covid19 enters the cells, the $CD8^+$ cells must exterminate the infected cells, a necessary intervention, but a casualty nevertheless, that can often injure the host. b/ There is evidence that coronavirus inhibits the interferon type I production and therefore, it suppresses the ability of the adaptive immune system to recognize the virus, possibly leading to the destruction of healthy cells. This is illustrated by the cytokine storm that indiscriminately attacks and rampages the host's vital organs [24-26]. What seems to happen during the cytokine storm is that the adaptive immune system is informed about the lethal danger, but has difficulty identifying the enemy that is evasive and imperceptible due to insufficient availability of Interferon I. As a result, immune counterattack is persistently fierce yet, undifferentiating, with deleterious consequences for the human body. The inhibition of the interferon type I production that compromises adaptive efficiency can be particularly detrimental to aged individuals with compromised immunity, who are faced by viral influx, and rely on the adaptive immune system for protection. This is why neutralizing antibody treatments have become so promising in the treatment of older Covid-19 patients.

3. Vaccines

Covid-19 messenger ribonucleic acid (mRNA) based vaccines, like the two-dose Pfizer/BioNTech and Moderna vaccines, that have now received emergency use authorization from the FDA, are developed by first sequencing the gene of the S protein, followed by a transcription of its mRNA, and finally encapsulating the nucleotide-modified messenger in a lipid nanoparticle that is subsequently delivered within a sterile saline solution, acting as a dilutant, into the muscles of the host's upper arm. mRNA is a single stranded molecular sequence that can be read by the host's ribosomes. The intention is to introduce the immune system to the configuration of the Spike protein, provoking it to produce the specific antibodies that can defensively wrap around the Spike protein to prohibit viral binding, fusion and entry into the human cells [27]. The Moderna vaccine encodes the Covid-19 S1 subunit of the spike trimer that binds with the ACE2 receptors, as well as the S2 one that fuses with the ACE2 receptors, releasing the viral RNA into the cells. For additional safety, the S2 subunit is stabilized by substituting two amino acids at two consecutive positions, 986 and 987, by prolines which are secondary amines that do not contain the amino-group -NH, often used in the biosynthesis of proteins.

The vaccine's effect on children under twelve years of age, pregnant women and individuals with specific pre-existing conditions is currently unclear, since the above mentioned populations were excluded from the clinical studies. Additional unknowns involve the vaccine's interaction with a wide range of medications; the durability of immune protection against viral infection; and the vaccine efficiency against new viral mutations [28].

Two other vaccines are produced by inserting the Adenovirus' DNA which also contains an S protein, after deleting part of its genetic sequence, hence rendering it unable to replicate. The AstraZeneca vaccine uses the Adenovirus found in chimpanzees, while the Johnson and Johnson vaccine uses an Adenovirus derived from humans. Other vaccine research companies like the Novavax and Sanofi-GlaxoSmithKline produce the spike protein vaccines in insect cells out of recombinant baculovirus [29]. Protein subunit vaccines utilise an isolated protein, in this case the Spike protein, which is purified from any viral infectious components to establish safety, and provide immunocompromised individuals with the best alternative. The problem arises when the isolated protein becomes denatured, losing its quaternary, tertiary or secondary structure as well as its functionality, thus failing to stimulate the immune production of the necessary antibodies that can ultimately protect the system against Covid-19. Therefore, its high safety may be undermined by its potentially compromised efficiency [30].

An alternative method is vaccination with a Covid-19 virus that has been deactivated and therefore, it is unable to reproduce, like for example, CoronaVac, a deactivated vaccine, produced by the Beijing based biopharmaceutical company, Sinovac. Deactivated vaccines' research started by obtaining different Covid-19 strains from hospitalized patients around the world including China, Italy, Switzerland, United Kingdom and Spain, and has chemically inactivated the hazardous viral features, leaving a purified, disarmed Covid-19 version that can no longer assail the body. Introducing the sight of the inactivated virus prepares the body to anticipate future viral invasion and encompass immune defences by eliciting potent antibodies, which have so far demonstrated an ability to neutralize at least 10 viral mutations in mice, rats and nonhuman primates. The PiCoVacc was formed by deactivating the CN2 strain and testing it against CN3, CN5 and OS6, as well as the CN1 and

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OS1, which are closely related to the Covid-19 mutations observed in Wuhan that evinced severe clinical symptoms. These investigators report that the purified inactivated virus exhibited genetic stability, despite multiple passages. The comparison of the different purification stages unveiled minor amino acid substitutions in the Envelope protein - residue 32, which replaced Alanine (A) with Aspartate (D). It also presented an interchange between Threonine (T) with Isoleucine (I) in the non-structural protein nsp10 - residue 49. Genetic stability persisting despite inactivation, signifies that the immune system should be able to recognize and create antibodies to potentially protect the cells from future mutations. Immune recognition should occur despite future alternations of the Spike protein, designed to disguise it, so that it eludes antibodies, inconspicuously succeeding in infecting the cells [31-32].

3.1 Vaccines' Comparison

Theoretically, the protein subunit, as well as the RNA / DNA vaccines appear to demonstrate both safety and effectiveness. The Spike mRNA can only express the S protein, which is only one out of around 29 primary proteins that compose Covid-19, thus making it impossible for the virus to replicate. The DNA vaccine is not even based on the Covid-19 virus and its genetic sequence is altered to disable reproduction within the cell. As a result of the vaccination, the B cells can produce antibodies for the particular S protein configuration presented, thus obstructing the Covid-19 spike protein from targeting and fusing with human cells. However, any formation of immune memory resulting from this process can be rendered ineffective by a viral mutation that substantially disguises the S protein to be unrecognizable by the immune system. On the other hand, the genetic stability of inactivated vaccines could perhaps offer protection against several mutated strains; however, it is unclear whether accurately examining and mapping certain current strains can extend to future emerging ones. Additionally, it is unclear how many vaccine dosages will be warranted with the inactivated virus vaccines; and what will be their final level of effectiveness and durability [33].

Live attenuated virus vaccines are based on whole viruses that have been modified and hence weakened. A single dosage can stimulate immune responses against a wide variety of viral proteins, without infecting the body with the disease. However, a mutation in live attenuated viral compounds could potentially reinstate their harmful potency; or they may have deleterious consequences in individuals with compromised immunity. Moreover, in light that Covid-19 is excreted in the feces, there is a risk of transmitting the attenuated viral compound to healthy individuals, and the potential of viral fusion with alternative wild-type Cov versions [34].

4. Covid-19 Mutations

Covid-19 represents a mutation with a higher contagion rate when juxtaposed against SARS-CoV. Covid-19 Spike (S) protein binds with the ACE2 receptor in a manner that is 10 times more secure and steadfast than the juncture formed between the SARS-CoV spike protein with the ACE2 receptor. This finding pertains to both the Covid-19 increased rate of viral infection and transmissibility, explaining the speed with which Covid-19 has spread globally [35]. Since it first appeared in Wuhan, Covid-19 has mutated from 614D to 614G, basically exchanging the amino acid Aspartate (D) with the amino acid Glycine (G) in the genome's 614 position [36]. Korber et al. (2020) looked at single amino acid changes in 28,576 sequences of the trimeric spike (S) protein that included both the subdomain Spike 1 (S1), which mediates the binding with the ACE2 receptor, and the Spike 2 (S2) subdomain that accomplishes the membrane fusion, ultimately resulting in the release of viral contents into the cell. These investigators found that the Spike variant D614G that exchanges Aspartate with Glycine had a significantly higher rate of transmissibility globally, when compared to its predecessor 614D. Several countries were affected including Eurore, the USA, Canada and Australia. This new mutation D614G varied only 0.3% from the original 614D Covid-19 sequence that was identified in Wuhan. Interestingly, this mutation that was solely based on a single substitution of the amino acid Aspartate (D) by the amino acid Glycine (G) in Spike's 614th amino acid position, resulted in an increase of at least 20% in the viral infectiousness rate [37]. While 614D only involves one conformation, D614G exhibits two to three Spike protein conformations, thus increasing the probability of contagion [38].

Covid-19 genome has undergone several mutations, usually based on one or two amino acid substitutions that reflect the virus' adaptation to each host's diverse biological apparatus to maximize viral survival and transmissibility. Some of these changes may not be as important as others. Van Dorp et al (2020) reported changes in the non-structural proteins Nsp6, Nsp11, Nsp13 as well as the trimeric spike [39]. However, if instead of the Nsp6, Nsp11 and Nsp13, the non-structural proteins Nsp7, Nsp8, and Nsp12 in association with Nsp14 were involved, the antigen's capacity to replicate long viral RNA would have been compromised. Research on previous versions of the antigen indicated that the mutation of the nsp8 residues P183 and R190 that are involved in the interphase between nsp8 and nsp12 as well as K58 had deleterious effects for the virus [40].

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Another variant, the A222V that substitutes Alanine with Valine on the 222nd position, was linked to Italy and Spain and appeared to represent about 11.2% of the genetic sequences collected from Covid-19 patients between June and October 2020. This mutant seemed to primarily affect immune recognition since it occured in the S protein segment that binds with the ACE2 receptor [41-42]. Overall D614G was manifested in around 86.5% of Covid-19 infections, while other mutants, such as the A222V, were relatively less frequent. D614G was often present along with A222V, as well as other common mutations such as the S477N that exchanged Serine (S) with Asparagine (N) at the 477th position and L5F. Several other mutations have been identified such as the L18F reflecting a Leucine (L) exchange with Phenylalanine (F), the A262S involving a switch between Alanine (A) and Serine (S) and other far less frequent ones such as the T632N (Threonine to Asparagine), V3G (Valine to Glycine), D574Y (Aspartate to Tyrosine), P272L (Proline to Leucine), D1163Y (Aspartate to Tyrosine), and others. The S protein contains around 98,699 amino acid sequences, each consisting of around 1273 amino acids. Around 3205 of these amino acid sequences that compose the Spike protein are unique, suggesting that Covid-19 has evolved significantly, obtaining genetic diversity from previous versions of coronavirus. [43].

Recently, a highly contagious new mutant, the B117 appeared in the UK in September 2020, currently accounting for around two thirds of the UK cases, only after three months. Since then it has already spread to around 24 USA states, and was recently identified in a few cases in Hong Kong. The B117 may have emerged as the virus multiplied within an infected immunocompromised patient, and it appears to be up to 70% more transmissible [44]. It combines around 23 mutants, primarily affecting the Spike protein, that include substitutions of amino acid Asparagine (N) with Tyrosine (Y) at the 501 position forming the mutant N501Y; amino acid Alanine (A) with Aspartate (D) at the 570 position, composing A570D; the D614G variant discussed above that involves replacement of Aspartate (D) with Glycine (G) at the 614 position; P681H entailing an interchange of Proline (P) with Histidine (H) at the 681 position; T716I reflecting a switch of Threonine (T) with Isoleucine (I) at the 716 position; the S982A featuring an exchange between Serine (S) and Alanine (A) at position 982; D1118H representing a commutation between Aspartate (D) with Histidine (H) at position 1118, etc [44]. Seventeen out of these 23 alterations appear to have occurred simultaneously, expressing a successful viral transformation with the purpose to disguise viral proteins compromising the antibodies' efficacy against the infection. The mutation N501Y modifies the structure of the S protein camouflaging it from immune detection and threatening to eventually render ineffective all vaccines targeting the S protein. What is remarkable is that after undergoing around 300,000 mutations, Covid-19 mutants have evolved into being more effective and contagious rather than disintegrating into extinction, which is what would be expected if such accumulated mutations were random. As if there is some calculated intelligent programming within Covid-19 that renders mutations purposeful – something that has never been encountered previously in naturally occurring coronaviruses [45-46].

The 501.V2 variant which is at least as dangerously transmissible as the UK B117 has also been spreading globally. It is known to have three main substitutions of amino acid Lysine (K) to Asparagine (N) at the 417 position (K417N); the variant E484K that involves Glutamate (E) being switched into Lysine (K) at position 484; and the mutant N501Y where Asparagine (N) is replaced by Tyrosine (Y) at the 501 position of the genetic sequence. The N501Y that is shared by both the B117 and the 501. V2 and which enhances the affinity between the Spike protein and the ACE2 receptor appears to be compromised by the K417N and E484K variants, possibly rendering the African mutation less infectious and effective than the British one. These two transformations' deleterious effects are based on the action of E484K and K417N resulting in disrupting the salt bridges developed by E484 and K417 after these two variants have been transformed into 484K and 417 N that do not support salt bridges with BD23 R108 / H11013 R52 and C105 E96/E99 respectively [47].

Additional highly contagious mutations like the 501Y. V2 or, as otherwise termed, B1351, that shares a lot of the variants composing B117, and the P1 from Brazil have recently emerged. The 501Y.V2 includes 19 mutations, with nine of them located in the Spike protein including the N501Y (Asparagine being replaced by Tyrosine), the E484K (Glutamate being replaced by Lysine) and K417N (Lysine being exchanged by Asparagine). It also lodges mutations on the N-terminal domain, including L18F - replacing Leucine (L) with Phenylalanine (F) in the 18th position, D80A – transposing Aspartate (D) with Alanine (A) at the 80th position, and D215F that substitutes Aspartate (D) by Phenylalanine (F) at the 215 position [48]. The P1 mutation displays 17 unique amino acid reversals, four nucleotide insertions and four deletions. P1, B117 and 501Y.V2 all harbour the N501Y mutation that enhances the contact affinity between the Spike protein and the ACE2 receptor, rendering the variant significantly more contagious.A new variant that was first detected in India is now spreading around the world, diminishing the effectiveness of the Pfizer/BioNTech and AstraZeneca vaccines from

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93% to 88% and from 66% to 60% respectively.

5. Conclusions

Viral mutations have been long considered as random events, or mistakes during the RNA replication of a virus. Usually, what can go wrong will go wrong; therefore, repeated mutations lead to the extinction of a virus. However, with Covid-19 the opposite has occurred. The aggregate result of over 300,000 Covid-19 changes has expanded the virus' transmissibility and infectiousness. Covid-19 mutations have not degraded the virus; they have empowered and facilitated its disguise to evade detection; which poses the question: Is this really a random mindless process or are we witnessing the unfolding of a learning expedition driving this virus to adjust and adapt, thus ensuring its maintenance and survival?

Moreover, there is the hypothesis that Covid-19 inhibits the interferon type I production, compromising adaptive immunity from recognizing the virus; eventually leading to the deleterious consequences of the cytokine storm where the $CD8^+$ killer cells injure the vital organs of the host. It may appear improbable that there is a premeditated strategy to debilitate key systemic defences and prevail, yet, it may not be entirely impossible that evolution has programmed Covid-19 with an adeptness, that distinguishes it from all previous coronavirus versions. If that is the case, the danger is exponentially increasing and our current observations are no more than a prelude to more elusive, inconspicuous, and far more sophisticated versions of this pandemic.

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Conflict of Interests

The author has no conflicts of interests to disclose.

References

[1] Li, F., 2016. Structure, function, and evolution of coronavirus spike proteins. Annual review of virology, 3, pp.237-261. https://doi.org/10.1146/annurev-virology-110615-042301

[2] Decaro, N. and Lorusso, A., 2020. Novel human coronavirus (SARS-CoV-2): A lesson from animal coronaviruses. Veterinary Microbiology, p.108693. https://doi.org/10.1016/j.vetmic.2020.108693. http://www.sciencedirect.com/science/article/pii/S0378113520302935

[3] Schütze, H., 2016. Coronaviruses in aquatic organisms. In Aquaculture Virology (pp. 327-335). Academic Press. https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/deltacoronavirus

[4] Zhou, P., Yang, X.L., Wang, X.G., Hu, B., Zhang, L., Zhang, W., Si, H.R., Zhu, Y., Li, B., Huang, C.L. and Chen, H.D., 2020. Discovery of a novel coronavirus associated with the recent pneumonia outbreak in humans and its potential bat origin. BioRxiv. doi: 10.1038/s41586-020-2012-7. / DOI: https://doi.org/10.1101/2020.01.22.914952

[5] Surjit, M. and Lal, S.K., 2010. The nucleocapsid protein of the SARS coronavirus: structure, function and therapeutic potential. In Molecular Biology of the SARS-Coronavirus (pp. 129-151). Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-03683-5_9

[6] Wu, A., Peng, Y., Huang, B., Ding, X., Wang, X., Niu, P., Meng, J., Zhu, Z., Zhang, Z., Wang, J. and Sheng, J., 2020. Genome composition and divergence of the novel coronavirus (2019-nCoV) originating in China. Cell host & microbe. https://doi.org/10.1016/j.chom.2020.02.001

[7] Ruan YJ, Wei CL, Ee AL, Vega VB, Thoreau H, Su ST, Chia JM, Ng P, Chiu, KP, Lim L, Zhang T, Peng CK, Lin EO, Lee NM, Yee SL, Ng LF, Chee RE, Stanton LW, Long PM, Liu ET. 2003. Comparative full-length genome sequence analysis of 14 SARS coronavirus isolates and common mutations associated with putative origins of infection. Lancet 361: 1779–1785. https://doi.org/10.1016/S0140-6736(03)13414-9.

[8] Yang ZY, Werner HC, Kong WP, Leung K, Traggiai E, Lanzavecchia A, Nabel, GJ. 2005. Evasion of antibody neutralization in emerging severe acute respiratory syndrome coronaviruses. Proc Natl Acad Sci U S A 102:797–801. https://doi.org/10.1073/pnas.0409065102.

[9] Grifoni A, Sidney J, Zhang Y, Scheuermann RH, Peters B, Sette A. 8 April 2020. A sequence homology and bioinformatic approach can predict candidate targets for immune responses to SARS-CoV-2. Cell Host Microbe, https://doi.org/10.1016/j.chom.2020.03.002.

[10] Wrapp, D., Wang, N., Corbett, K.S., Goldsmith, J.A., Hsieh, C.L., Abiona, O., Graham, B.S., and McLellan, J.S. (2020). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science. https://doi.org/10.1126/science.abb2507.

[11] Wrapp, D. and McLellan, J.S., 2019. The 3.1-angstrom cryo-electron microscopy structure of the porcine epidemic diarrhea virus spike protein in the prefusion conformation. Journal of virology, 93(23). m DOI: 10.1128/JVI.00923-19

[12] Chi, X., Yan, R., Zhang, J., Zhang, G., Zhang, Y.,

  DOI: https://doi.org/10.30564/jer.v3i1.2884

Hao, M., Zhang, Z., Fan, P., Dong, Y., Yang, Y. and Chen, Z., 2020. A neutralizing human antibody binds to the N-terminal domain of the Spike protein of SARS-CoV-2. Science, 369(6504), pp.650-655. DOI: 10.1126/science.abc6952

[13] Litman GW, Rast JP, Shamblott MJ, Haire RN, Hulst M, Roess W, Litman RT, Hinds-Frey KR, Zilch A, Amemiya CT (January 1993). "Phylogenetic diversification of immunoglobulin genes and the antibody repertoire". Molecular Biology and Evolution. 10 (1): 60–72. DOI:10.1093/oxfordjournals.molbev.a040000

[14] Wu, F., Wang, A., Liu, M., Wang, Q., Chen, J., Xia, S., Ling, Y., Zhang, Y., Xun, J., Lu, L. and Jiang, S., Neutralizing antibody responses to SARS-CoV-2 in a COVID-19 recovered patient cohort and their implications. medRxiv, 2020–033020047365 (2020). DOI: 10.1101/2020.03. 30.20047365. Accessed 2020-04-29. https://doi.org/10.1101/2020.03.30.20047365(2020).

[15] Lo, A.W., Tang, N.L. and To, K.F., 2006. How the SARS coronavirus causes disease: host or organism?. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland, 208(2), pp.142-151. https://doi.org/10.1002/path.1897

[16] Chen, P., Nirula, A., Heller, B., Gottlieb, R.L., Boscia, J., Morris, J., Huhn, G., Cardona, J., Mocherla, B., Stosor, V. and Shawa, I., 2020. SARS-CoV-2 neutralizing antibody LY-CoV555 in outpatients with Covid-19. New England Journal of Medicine. DOI: 10.1056/NEJMoa2029849

[17] Gaunt, E.R., Hardie, A., Claas, E.C., Simmonds, P. and Templeton, K.E., 2010. Epidemiology and clinical presentations of the four human coronaviruses 229E, HKU1, NL63, and OC43 detected over 3 years using a novel multiplex real-time PCR method. Journal of clinical microbiology, 48(8), pp.2940-2947. DOI: 10.1128/JCM.00636-10

[18] Weiss, S.R. and Navas-Martin, S., 2005. Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiology and molecular biology reviews, 69(4), pp.635-664. DOI: 10.1128/MMBR.69.4.635-664.2005

[19] Amanat, F., Stadlbauer, D., Strohmeier, S., Nguyen, T.H., Chromikova, V., McMahon, M., Jiang, K., Arunkumar, G.A., Jurczyszak, D., Polanco, J. and Bermudez-Gonzalez, M., 2020. A serological assay to detect SARS-CoV-2 seroconversion in humans. Nature medicine, pp.1-4. https://doi.org/10.1038/s41591-020-0913-5

[20] Tang, F., Quan, Y., Xin, Z.T., Wrammert, J., Ma, M.J., Lv, H., Wang, T.B., Yang, H., Richardus, J.H., Liu, W. and Cao, W.C., 2011. Lack of peripheral memory B cell responses in recovered patients with severe acute respiratory syndrome: a six-year follow-up study. The Journal of Immunology, 186(12), pp.7264-7268. DOI: https://doi.org/10.4049/jimmunol.0903490

[21] Wagner, A. and Weinberger, B., 2020. Vaccines to Prevent Infectious Diseases in the Older Population: Immunological Challenges and Future Perspectives. Frontiers in Immunology, 11, p.717. https://doi.org/10.3389/fimmu.2020.00717

[22] Braun, J., Loyal, L., Frentsch, M., Wendisch, D., Georg, P., Kurth, F., Hippenstiel, S., Dingeldey, M., Kruse, B., Fauchere, F. and Baysal, E., 2020. SARS-CoV-2-reactive T cells in healthy donors and patients with COVID-19. Nature, pp.1-5. doi: https://doi.org/10.1101/2020.04.17.20061440

[23] Grifoni, A., Weiskopf, D., Ramirez, S.I., Mateus, J., Dan, J.M., Moderbacher, C.R., Rawlings, S.A., Sutherland, A., Premkumar, L., Jadi, R.S. and Marrama, D., 2020. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. Cell. https://doi.org/10.1016/j.cell.2020.05.015

[24] Prompetchara, E., Ketloy, C. and Palaga, T., 2020. Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic. Asian Pac J Allergy Immunol, 38(1), pp.1-9. http://apjai-journal.org/wp-content/uploads/2020/03/1.pdf?fbclid=IwAR1kVlFnoc-6mobqEy7FDz_93fX__AaY8ZgE4TUwtjzh-h8oVqgs82rgf3QoU

[25] Faure, E., Poissy, J., Goffard, A., Fournier, C., Kipnis, E., Titecat, M., Bortolotti, P., Martinez, L., Dubucquoi, S., Dessein, R. and Gosset, P., 2014. Distinct immune response in two MERS-CoV-infected patients: can we go from bench to bedside?. PloS one, 9(2), p.e88716. https://doi.org/10.1371/journal.pone.0088716

[26] Killerby, M.E., Biggs, H.M., Haynes, A., Dahl, R.M., Mustaquim, D., Gerber, S.I. and Watson, J.T., 2018. Human coronavirus circulation in the United States 2014–2017. Journal of Clinical Virology, 101, pp.52-56. https://doi.org/10.1016/j.jcv.2018.01.019

[27] Maruggi, G., Zhang, C., Li, J., Ulmer, J.B. and Yu, D., 2019. mRNA as a transformative technology for vaccine development to control infectious diseases. Molecular Therapy, 27(4), pp.757-772. https://doi.org/10.1016/j.ymthe.2019.01.020

[28] Connors, M., Graham, B.S., Lane, H.C. and Fauci, A.S., SARS-CoV-2 Vaccines: Much Accomplished, Much to Learn. Annals of Internal Medicine. https://doi.org/10.7326/M21-0111

[29] Chen, W.H., Strych, U., Hotez, P.J. and Bottazzi, M.E., 2020. The SARS-CoV-2 vaccine pipeline: an

overview. Current tropical medicine reports, pp.1-4. https://doi.org/10.1007/s40475-020-00201-6

[30] Vartak, A. and Sucheck, S.J., 2016. Recent advances in subunit vaccine carriers. Vaccines, 4(2), p.12. https://doi.org/10.3390/vaccines4020012

[31] Gao, Q., Bao, L., Mao, H., Wang, L., Xu, K., Yang, M., Li, Y., Zhu, L., Wang, N., Lv, Z. and Gao, H., 2020. Development of an inactivated vaccine candidate for SARS-CoV-2. Science, 369(6499), pp.77-81. DOI: 10.1126/science.abc1932

[32] Li, L., Guo, P., Zhang, X., Yu, Z., Zhang, W. and Sun, H., 2020. SARS-CoV-2 vaccine candidates in rapid development. Human vaccines & immunotherapeutics, pp.1-10. https://doi.org/10.1080/21645515.2020.1804777

[33] Ng, W.H., Liu, X. and Mahalingam, S., 2020. Development of vaccines for SARS-CoV-2. F1000Research, 9. doi: 10.12688/f1000research.25998.1

[34] Chen, Y. and Li, L., 2020. SARS-CoV-2: virus dynamics and host response. The Lancet Infectious Diseases, 20(5), pp.515-516. DOI:https://doi.org/10.1016/S1473-3099(20)30235-8

[35] Wrapp, D., Wang, N., Corbett, K.S., Goldsmith, J.A., Hsieh, C.L., Abiona, O., Graham, B.S. and McLellan, J.S., 2020. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science, 367(6483), pp.1260-1263. DOI: 10.1126/science.abb2507

[36] Daniloski, Z., Guo, X. and Sanjana, N.E., 2020. The D614G mutation in SARS-CoV-2 Spike increases transduction of multiple human cell types. BioRxiv. DOI: https://doi.org/10.1101/2020.06.14.151357

[37] Korber, B., Fischer, W.M., Gnanakaran, S., Yoon, H., Theiler, J., Abfalterer, W., Hengartner, N., Giorgi, E.E., Bhattacharya, T., Foley, B. and Hastie, K.M., 2020. Tracking changes in SARS-CoV-2 Spike: evidence that D614G increases infectivity of the COVID-19 virus. Cell, 182(4), pp.812-827. https://doi.org/10.1016/j.cell.2020.06.043

[38] Callaway, E., 2020. Making sense of coronavirus mutations. Nature, pp.174-177. https://search.bvsalud.org/global-literature-on-novel-coronavirus-2019-ncov/resource/en/covidwho-752538, MEDLINE | ID: covidwho-752538

[39] van Dorp, L., Acman, M., Richard, D., Shaw, L.P., Ford, C.E., Ormond, L., Owen, C.J., Pang, J., Tan, C.C., Boshier, F.A. and Ortiz, A.T., 2020. Emergence of genomic diversity and recurrent mutations in SARS-CoV-2. Infection, Genetics and Evolution, 83, p.104351. https://doi.org/10.1016/j.meegid.2020.104351

[40] Subissi, L., Posthuma, C.C., Collet, A., Zevenhoven-Dobbe, J.C., Gorbalenya, A.E., Decroly, E., Snijder, E.J., Canard, B. and Imbert, I., 2014. One severe acute respiratory syndrome coronavirus protein complex integrates processive RNA polymerase and exonuclease activities. Proceedings of the National Academy of Sciences, 111(37), pp.E3900-E3909.

[41] Bartolini, B., Rueca, M., Gruber, C.E.M., Messina, F., Giombini, E., Ippolito, G., Capobianchi, M.R. and Di Caro, A., 2020. The newly introduced SARS-CoV-2 variant A222V is rapidly spreading in Lazio region, Italy. medRxiv. DOI: https://doi.org/10.1101/2020.11.28.20237016

[42] Zhang, B.Z., Hu, Y.F., Chen, L.L., Yau, T., Tong, Y.G., Hu, J.C., Cai, J.P., Chan, K.H., Dou, Y., Deng, J. and Wang, X.L., 2020. Mining of epitopes on spike protein of SARS-CoV-2 from COVID-19 patients. Cell research, 30(8), pp.702-704. https://doi.org/10.1038/s41422-020-0366-x

[43] He, S. and Wong, S.W., 2021. Statistical challenges in the analysis of sequence and structure data for the COVID-19 spike protein. arXiv preprint arXiv:2101.02304. https://arxiv.org/abs/2101.02304

[44] Duong, D., 2021. What's important to know about the new COVID-19 variants?.DOI: https://doi.org/10.1503/cmaj.1095915 de Alcañiz, J.G.G., Lopez-Rodas, V. and Costas, E., 2021. Sword of Damocles or choosing well. Population genetics sheds light into the future of the COVID-19 pandemic and SARS-CoV-2 new mutant strains. medRxiv. doi: https://doi.org/10.1101/2021.01.16.21249924

[45] Liu, S., Shen, J., Fang, S., Li, K., Liu, J., Yang, L., Hu, C.D. and Wan, J., 2020. Genetic spectrum and distinct evolution patterns of SARS-CoV-2. Frontiers in Microbiology, 11, p.2390. https://doi.org/10.3389/fmicb.2020.593548

[46] Arif, T. 2021. The 501.V2 and B.1.1.7 variants of coronavirus disease 2019 (COVID-19): A new time-bomb in the making? Infection Control & Hospital Epidemiology, 1-2. DOI:10.1017/ice.2020.1434

[47] Cheng, M.H., Krieger, J.M., Kaynak, B., Arditi, M.A. and Bahar, I., 2021. Impact of South African 501. V2 Variant on SARS-CoV-2 Spike Infectivity and Neutralization: A Structure-based Computational Assessment. bioRxiv. DOI: https://doi.org/10.1101/2021.01.10.426143

[48] 51Lopez-Rincon, A., Perez-Romero, C., Tonda, A., Mendoza-Maldonado, L., Claassen, E., Garssen, J. and Kraneveld, A.D., 2021. Design of Specific Primer Sets for the Detection of B. 1.1. 7, B. 1.351 and P. 1 SARS-CoV-2 Variants using Deep Learning. bioRxiv. DOI: https://doi.org/10.1101/2021.01.20.427043

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ARTICLE

Evaluation of Genetics of Obesity and MC4R Deficiency: A Gene-oriented Approach to Obesity

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ABSTRACT

Obesity is a multifactorial and complex health problem that is affected by several factors including genetic, environmental, social, behavioural, and biological aspects. Even though the influence of other environmental and behavioural factors such as sedentary lifestyle, high-calorie nutritional intake, and the inadequate expenditure of energy are acknowledged as important aspects that cause obesity, the issue of inheritance is indisputable. The study aims to investigate the effects of inheritance on obesity and examine how understanding and detecting genetic reasons behind obesity may benefit the treatment and prevention of the obesity epidemic. The relationship between common gene variants and obesity is now being studied through the emergence of GWAS. It is undeniable that genetic mutations and gene deficiencies particularly MC4R deficiency are significant factors. The process of detecting genes that create a tendency to obesity is currently being studied. It may be possible to prevent and treat obesity with the detection of certain genes.

1. Introduction

Obesity is a public health concern worldwide. Obesity cases show a significant increase in both developed and developing countries. The disease is now considered an epidemic since it threatens public health in a global sense. Not only obesity influence societies in terms of health, but also social and economic terms [1]. Obesity is known to be a factor that induces several other diseases such as stroke, hypertension, reflux, several cancer types, liver cirrhosis, T2D, depression, etc. Therefore, it is argued that obesity decreases average lifetime [2 3 4]. Exercise has many beneficial effects, leads to less telomere attrition and may diminish the risk of cancer, these two outcomes are possible resulted by a reduction in oxidative stress and chronic inflammation [5]. Hippocrates was the first to recognize the need for a balanced diet and exercise and the fact that different age has different needs [6]. Hippocrates' innovative spirit laid the foundations of modern medicine and the wellbeing movement: Exercise Is Medicine (EIM) ®, which has been inspired-adopted by scientific institutions such as the American College of Sports Medicine (ACSM), the American Medical Association (AMA) and Harvard Medical School [7].

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Obesity is a multifactorial and complex health problem. Even though a wide number of studies indicate that genetics played a role for 40 to 70% of obese patients by provoking weight gain, it cannot be suggested that genetics only cause this disease [8 9]. Obesity is affected by several factors including genetic, environmental, social, behavioural, and biological aspects. In this sense, it is important to emphasize that one factor only does not directly lead to obesity. For instance, it is possible for an individual who has a genetic tendency to obesity, to stay healthy and have a normal BMI by adjusting their physical and nutritional habits [10]. In fact, in a study, Xiang et al. compared those who carry a genetic variant that may cause obesity and the control group without the variant. The study indicated that following the right nutrition program and with adequate physical activity, a person with the genetic variant can reach a normal weight and live a healthy life [11]. For this reason, while it is severely important to examine the impact of inheritance on obesity, it is also important to understand the interaction of the genes with the environment and other aspects.

1.1 Types of Obesity

In a general sense, obesity can be divided into categories based on its etiology. These categories include monogenic obesity, syndromic obesity, and polygenic/common obesity. Monogenic obesity, which is also known as single gene obesity, is not a common but quite severe type of obesity. The reason for monogenic obesity is a single gene mutation or deficiency. Syndromic obesity is considered to be linked to mental retardation and certain abnormities in the development of organs. Polygenic obesity is caused by multiple polymorphic genes. It is a common type of obesity that is known to be seen more frequently in society and increases the risk for other diseases [12].

1.2 Genetic Basis of Obesity

The importance of inheritance in the practice of medicine and community health has gained quite significance. Since inheritance is considered an issue significant to community health, genetics pose a crucial factor for urgent health problems that threaten people's health. Obesity, being of those public health problems, is considered a

Table 1. Single genes are known to be linked to obesity

NAME	GENE	MIM	MODE of INHERITANCE	CHROMOSOMAL POSITION
Leptin	*LEP*	164160	AR	7q32.1
Leptin receptor	*LEPR*	601007	AR	1p31.2
Proopiomelanocortin	*POMC*	176830	AR	2p23.2
Melanocortin 4 receptor	*MC4R*	155541	AD/AR	18q21.32
Single-minded Drosophila Homologue-1	*SIM1*	603128	AD	6q16.3
Nurotrophic Tyrosine Kinase Receptor Type 2	*NTRK2*	600456	AD	9q21.33
Kinase suppressor of Ras2	*KSR2*	610737	AD	12q24.22-q24.23
Carboxypeptidase	*CPE*	114855	AD	4q32.3
Proconvertase 1	*PCSK1*	162150	AR	5q15
Brain Derived Neurotropic factor	*BDNF*	113505	AD	11p14.1
SH2B adaptor protein	*SH2B1*	608937	AD	16p11.2
Tubby, Homogue of Mouse	*TUB*	601197	AR	11p15.4

AD= Autosomal dominant, AR = Autosomal recessive.

For detailed information and references, refer to Online Mendelian Inheritance in Man using the MIM number: https://www.omim.org

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severe risk factor for other diseases such as coronary heart disease (CHD) [14 15].

The fact that obesity has a genetic origin is indisputable. Even though the influence of other environmental and behavioural factors such as sedentary lifestyle, high-calorie nutritional intake, and the inadequate expenditure of energy are acknowledged as important aspects that cause obesity, the issue of inheritance is a considerable factor.

Earliest findings of the association between obesity and inheritance date back to 2007 [16 17]. Along with the improvements in technic and analysis methods, genome-wide association studies (GWAS) started. GWAS is an approach that helps scientists to reveal and discover the genetic reasons behind certain diseases. According to GWAS, common variants altogether can pass on through the family (International HapMap Consortium, 2005) [18]. With this information, scientists were able to detect nearly 80% of common gene variations [19 20].

1.2.1 MC4R Deficiency

The melanocortin-4-receptor (*MC4R*) is encoded by the MC4R gene. It is a G-protein coupled seven-transmembrane receptor G protein-coupled receptor and is proven to be associated with obesity disease. It regulated the nutritional behaviour in the hypothalamus [22]. α-melanocyte-stimulating hormone (α-MSH) binds and activates MC4R and this helps control appetite. Appetite regulation is linked to MC4R [23]. Thus, one of the most common reasons for obesity based on genetics is certain mutations in MC4R. This type of obesity is quite prevalent in societies at a rate of 0.5-6% [24 25 26].

2. Discussion

Many researchers are studying genetics' influence on obesity and twin studies are quite common. These studies suggest that a similar phenotype is observed in the other twin or either of the parents, indicating the inheritability of obesity [27 28 29]. According to several studies conducted on families, twins, and adopted family members, BMI is affected by genetics by 70-80% [30 31 32 33]. Similar findings are seen in studies conducted on societies from various ethnic groups [34].

According to the studies in the field of epidemiology, as the degree of affinity lowers, so does the risk of obesity. Therefore, it is argued that inheritance is an important factor. In twin studies, it is indicated that dizygotic twins demonstrate a concordance rate by less than half of the monozygotic twins (~ 0.68 vs~0.28) [35 36].

Similarly, in studies conducted on families with adopted children, it was observed that the adopted children's BMI is more proximate to their biological parent compared to the BMI of their legal parents. This significantly emphasizes that environmental factors such as a mutual home environment are still important but the influence of inheritance on obesity is undeniable [37 38].

Farooqi et al. examined the MC4R deficiency or mutations and obesity relationship in a study conducted with families. Endocrine and metabolic analyses were performed on subjects. The study reported that 5.8% of the patients that suffered from obesity showed MC4R mutations. Farooqi et al. indicated in this wide-ranging study that the monogenic type of obesity is associated with MC4R deficiency. However, it is noted that in similar studies conducted on diverse ethnic populations, this rate

Table 2. Comparison of phenotypic features of monogenic forms of obesity

Gene	Obesity	Birth weight	Endocrine abnormalities	Hyperphagia	Inheritance	Chromosome
LEP	Severe	Normal	Low leptin Hypogonadism High thyroid-stimulating hormone High insulin	+	Recessive	7q31.3
LEPR	Severe	?	High leptin Pituitary dysfunction Hypogonadotrophic hypogonadism Hypothalamic hypothyroidism Sympathetic dysfunction High insulin	+	Recessive	1p31
POMC	Severe	Normal	Red hair pigmentation ACTH deficiency, hypocortisolism Lowa-MSH	+	Recessive	2p23.3
PC1	Severe	?	Hypogonadotrophic hypogonadism Hypocortisolism High proinsulin, low insülin Postprandial hypoglycemia High POMC	?	Recessive	5q1.5–2.1
MC4-R	Severe	Normal	Not observed	+	Dominant	18q22
NROB2	Mild	High	Mild hyperinsulinemia	-	Dominant	1p36.1

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was lower [39].

Bonnefond et al. reported that even though obese individuals that have monogenic MC4R mutations are suitable for bariatric operations. These patients lose less weight after the operation compared to people that have common obesity. Bonnefond et al. Lapsen et al. [40 41] Collet et al. conducted a study on patients with MC4R mutations and a control group. While the group carrying the mutation was treated with Setmelanotide which is known to be an MC4R agonist, they lost an average weight of 3,5 kilograms. However, the control group receiving placebo treatment lost 0,85 kilograms within the same period [42].

3. Conclusions

Obesity is a multifactorial epidemic and it is the result of various factors. Genetics plays a crucial role in the occurrence of the disease. The relationship between common gene variants and obesity is now being studied through the emergence of GWAS. Regarding our extensive literature review, it is understood that it is undeniable that genetic mutations and gene deficiencies particularly MC4R deficiency are significant factors. The process of detecting genes that create the tendency to obesity is currently being studied. It may be possible to prevent and treat obesity with the detection of certain genes. Investigation of genetic factors on obesity should be further studied for future and current patients to maintain a healthier life.

References

[1] Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C.et al. (2014). Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet; 384: 766-781.

[2] Bluher, M. (2016). Adipose tissue inflammation: a cause or consequence of obesity-related insulin resistance?.Clin Sci (Lond).; 130: 1603-1614.

[3] Long, E., Beales, I.L. (2014). The role of obesity in oesophageal cancer development.Ther Adv Gastroenterol; 7: 247-268.

[4] Rosso, N., Chavez-Tapia, N.C., Tiribelli, C., Bellentani, S. (2014).Translational approaches: from fatty liver to non-alcoholic steatohepatitis.World J Gastroenterol; 20: 9038-9049.

[5] Nomikos NN, Nikolaidis PT, Sousa CV, Papalois AE, Rosemann T, Knechtle B. Exercise, Telomeres, and Cancer: "The Exercise-Telomere Hypothesis". Front Physiol. 2018;9:1798. Published 2018 Dec 18. DOI:10.3389/fphys.2018.01798.

[6] Nomikos, N., Trompoukis, C., Lamprou, C., & Nomikos, G. (2016). The Role of Exercise in Hippocratic Medicine. American Journal of Sports Science and Medicine, 4(4), 115-119.

[7] Nomikos, N., Lamprou C. (2021). Proceedings of 47 Panhellenic Medical Congress. The reports about dietary and exercise in the texts of Hippocrates.

[8] Chung, W.K., Leibel, R.L. (2008). Considerations regarding the genetics of obesity. Obesity (Silver Spring). Suppl 3:S33-9.

[9] Wood, A.C. (2018). Appetitive Traits: Genetic Contributions to Pediatric Eating Behaviors. In: Pediatric Food Preferences and Eating Behaviors. Elsevier; 127-146.

[10] Scuteri, A., Sanna, S., Chen, W.M., et al. (2007). Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. PLoS Genet, 3:e115.

[11] Xiang, L., Wu, H., Pan, A., et al. (2016). FTO genotype and weight loss in diet and lifestyle interventions: a systematic review and meta-analysis, 2. Am J Clin Nutr. ;103(4):1162-1170.

[12] Herrera B.M., Lindgren C.M. (2010). The Genetics of Obesity. Curr Diab Rep 10:498–505 DOI 10.1007/s11892-010-0153-z.

[13] Thaker V. V. (2017). Genetic And Epigenetic Causes Of Obesıty. Adolescent medicine: state of the art reviews, 28(2), 379-405.

[14] Mangum, B.P., Mangum, T.L. (2018). Gene-environment interactions and the genetic epidemiology of obesity: correlates for preventative medicine. J Obes Metab Disord.;1(1):25-28.

[15] Torres, K. Y. (2010). Chronic Disease Epidemiology and Control, 3rd Edition. Preventing Chronic Disease, 8(1), A25.

[16] Scuteri, A., Sanna, S., Chen, W.M., et al. (2007). Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. PLoS Genet, 3:e115.

[17] Frayling, T.M., Timpson, N.J., Weedon, M.N., et al. (2007). A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. Science, 316:889-894.

[18] International HapMap Consortium (2005). A haplotype map of the human genome. Nature, 437:1299-1320.

[19] Barrett, J.C., Cardon, L.R. (2006). Evaluating coverage of genome-wide association studies. Nat Genet, 38:659-662.

[20] Pe'er, I., de Bakker, P.I., Maller, J., et al. (2006). Evaluating and improving power in whole-genome association studies using fixed marker sets. Nat Genet, 38:663-667.

  DOI: https://doi.org/10.30564/jer.v3i1.2977

[21] LeRoith, D., Taylor, S.I., and Olefsky, J.M. (2004). Diabetes Mellitus: A Fundamental and Clinical Text (3rd Ed.) Lippincott Williams & Wilkins.

[22] Mountjoy, K.G., Wong, J. (1997). Obesity, diabetes and functions for proopiomelanocortin-derived peptides. Mol Cell Endocrinol.;128(1-2):171-7.

[23] Fan, W., Boston, B.A., Kesterson, R.A., Hruby, V.J., Cone, R.D. (1997). Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. Nature. ;385(6612):165-8.

[24] Nowacka-Woszuk, J., Cieslak, J., Skowronska, B., et al. (2011). Missense mutations and polymorphisms of the MC4R gene in Polish obese children and adolescents in relation to the relative body mass index. J Appl Genet.;52(3):319-23.

[25] Wangensteen, T., Kolsgaard, M.L.P., Mattingsdal, M., et al. (2009). Mutations in the melanocortin 4 receptor (MC4R) gene in obese patients in Norway. Exp Clin Endocrinol Diabetes.;117(6):266-73.

[26] Stutzmann, F., Tan, K., Vatin, V., et al. (2008). Prevalence of melanocortin-4 receptor deficiency in Europeans and their age-dependent penetrance in multigenerational pedigrees. Diabetes.;57(9):2511-8.

[27] Elks, C.E., Den Hoed, M., Zhao, J.H., et al. (2012). Variability in the heritability of body mass index: a systematic review and meta-regression. Front Endocrinol.;3:29.

[28] Silventoinen, K., Jelenkovic, A., Sund, R., et al. (2017). Differences in genetic and environmental variation in adult BMI by sex, age, time period, and region: an individual-based pooled analysis of 40 twin cohorts. Am J Clin Nutr.;106(2):457-466.

[29] Llewellyn, C., Wardle, J. (2015). Behavioral susceptibility to obesity: gene– environment interplay in the development of weight. Physiol Behav.;152:494–501.

[30] Allison, D.B., Kaprio, J., Korkeila, M., Koskenvuo, M., Neale, M.C., Hayakawa, K. (1996).The heritability of body mass index among an international sample of monozygotic twins reared apart. Int J Obes Relat Metab Disord.; 20: 501-506.

[31] Katzmarzyk, P.T., Perusse, L., Rao, D.C., Bouchard, C. (2000). Familial risk of overweight and obesity in the Canadian population using the WHO/NIH criteria. Obes Res; 8: 194-197

[32] Koeppen-Schomerus, G., Wardle, J., Plomin, R. (2001). A genetic analysis of weight and overweight in 4-year-old twin pairs. Int J Obes Relat Metab Disord; 25: 838-844.

[33] Pietilainen, K.H., Kaprio, J., Rissanen, A., Winter, T., Rimpela, A., Viken, R.J. et al (1999). Distribution and heritability of BMI in Finnish adolescents aged 16y and 17y: a study of 4884 twins and 2509 singletons. Int J Obes Relat Metab Disord; 23: 107-115.

[34] Fesinmeyer, M.D., North, K.E., Ritchie, M.D., Lim, U., Franceschini, N., Wilkens, L.R. et al. (2013).Genetic risk factors for BMI and obesity in an ethnically diverse population: results from the population architecture using genomics and epidemiology (PAGE) study. Obesity (Silver Spring); 21: 835-846

[35] Poulsen, P., Vaag, A., Kyvik, K., Beck-Nielsen, H. (2001). Genetic versus environmental aetiology of the metabolic syndrome among male and female twins. Diabetologia, 44:537-543.

[36] Wardle, J., Carnell, S., Haworth, C.M., Plomin, R. (2008). Evidence for a strong genetic influence on childhood adiposity despite the force of the obesogenic environment. Am J Clin Nutr, 87:398-404.

[37] Herrera B.M., Lindgren C.M. (2010). The Genetics of Obesity. Curr Diab Rep 10:498–505 DOI 10.1007/s11892-010-0153-z.

[38] Moll, P.P., Burns, T.L., Lauer, R.M. (1991). The genetic and environmental sources of body mass index variability: the Muscatine Ponderosity Family Study. Am J Hum Genet, 49:1243-1255.

[39] Farooqi, I.S., Keogh, J.M., Yeo, G.S., Lank, E.J., Cheetham, T., O'Rahilly, S. (2003). Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. N Engl J Med. 20;348(12):1085-95.

[40] Bonnefond, A. et al., (2016). Eating Behavior, Low-Frequency Functional Mutations in the Melanocortin-4 Receptor (MC4R) Gene, and Outcomes of Bariatric Operations: A 6-Year Prospective Study. Diabetes Care; 39(8): 1384-1392.

[41] Lepsen, E.W., et al. (2018) Patients with Obesity Caused by Melanocortin-4 Receptor Mutations Can Be Treated with a Glucagon-like Peptide-1 Receptor Agonis. Cell Metabolism: 28, 23-32.

[42] Collet, T., et al. (2017). Evaluation of a melanocortin-4 receptor (MC4R) agonist (Setmelanotide) in MC4R deficiency. Molecular Metabolism Volume 6, Issue 10; 1321-1329.

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