# TABLE OF CONTENTS

## Editorial

1. Type 1 Diabetes Update at Children With Diabetes Community  
   – Bernard C. Szirth, Jim Stroud, Liliane Deeb, Kim Duong, Loka Thangma Athesvaran, Michael Ferr, Manan Shah, Radhika Ragam, Christine Weng, Hunter Cope, Christopher Khouri and Kelly Soules

## Research

2. Intestinal Parasites in Diabetes Mellitus Patients in the Limbe and Buea Municipalities, Cameroon  
   – Fominyam Boris Tangi, Eric Bertrand Fokam, Njunda Anna Longdoh and Enoh Jude Etheneng

3. Behavioural Indicators as Risk of Diabetes Mellitus: A Community based Study in Manipur  
   – Nilupher Feroz, Meenal Dhall and Satwanti Kapoor

## Mini Review

4. Basic Ketone Engine and Booster Glucose Engine for Energy Production  
   – Shaw Watanabe, Azusa Hirakawa, Seiichiro Aoe, Kazunori Fukuda and Tetsuo Muneta

## Research

5. Unexplained Hypoglycaemia and Large Glycaemic Variability: Skin Lipohypertrophy as a Predictive Sign  
   – Felice Strollo, Giuseppina Guarino, Vincenzo Armentano, Gennaro Clemente, Emilia Martedi, Stefano de Riu, Iole Gaeta, Gerardo Conglano, Antonio Ceriello, Sandro Gentile on behalf of AMD-OSDI Italian Study Group on Injection Techniques
Type 1 Diabetes Update at Children With Diabetes Community

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Of the estimated 415 million people living with diabetes worldwide, 10% have Type 1 Diabetes (T1D) meaning they will remain insulin dependent until a cure is found. Globally, we can observe an increasing trend of about 5% per year in newly diagnosed cases of T1D involving children as well as adults. T1D places vital organs at risk; additionally, it increases the likelihood of contracting other autoimmune diseases. Life expectancy of individuals affected with T1D can be shortened by as much as 12 years. Careful monitoring of diabetes (glucose, blood pressure, and body mass index (BMI)), including early detection of retinal changes, is key to maintaining a long and healthy life.

For over a decade, the week of July 4 has been set aside by a group called “Children with Diabetes” (CWD) that meets in Orlando, Florida, USA (near Disney World) to educate children about various aspects of diabetes. Siblings, parents, and grandparents of children with T1D also participate in this educational, yet fun event. Earlier this month, a total of 2,300 individuals attended this event where, among many other things, families could have their children’s eyes screened at no cost with the latest technology available. A group of 16 screeners (Figure 1) with various medical backgrounds partook in this effort and for 5 days screened 216 individuals ranging in age from 4 years to 76 years (average age, 23 years).

Figure 1: “Children with Diabetes” Screening team 2016, July 2016.
Front row: C. Khouri, B. Szirth, K. Duong, M. Shah, L Deeb, L. Thangma Athesvaran,
Photo credit: Corporate Image Photography.
The screenings included visual acuity measurement, blood pressure monitoring (with an average reading of 103/65 mmHg blood pressure is responsible for 38% of non-vascular damage when not controlled), BMI assessment, pupillary measurements, automated visual acuity measurement, automated intraocular pressure monitoring, ocular coherence tomography (OCT), ocular coherence tomography with angiographic views (OCTA), and non-mydriatic high-resolution 21-megapixel color and autofluorescence digital retinal imaging. Three reading/interpretation stations were set up where screened individuals and parents received both a reading of their results and digital copies of their tests.

The average age at onset of T1D in this group was 9 years, while the duration of T1D was ten years. A valuable set of innovations in T1D consists of the insulin pump (IP) (average number of years using the IP was 7 years) and the continuous glucose monitoring system (CGMS) (average number of years wearing this device was 3.5 years). The CGMS has come a long way and can now display glucose levels via smart phone technology and alert parents when children’s glucose levels are getting low or too high. Glucose levels in our group varied between 57 and 525 mg/dL with an average of 177 mg/dL (average glycated hemoglobin (HbA1c) percentage was 7.7%).

A unique facet for the children at ‘Friends for life’ is that they are surrounded by other children with diabetes, all while playing and visiting theme parks, and literally becoming “Friends for Life”. They are able to share their experiences, fears, and success stories through the years via various social media platforms. We found that this interaction helps them a great deal in adhering to daily regimens that help ensure good control of T1D. The children with diabetes are never alone to face their condition and parents can connect with other parents that may have similar challenges (children undergoing puberty, children leaving home for college, etc).

One notable case involved an adolescent whom we had followed up for 5 years and who had all his blood glucose numbers well under control for diabetes. Suddenly, he decided (at 19 years old) to no longer follow a good control regimen for his T1D. We did not see him at CWD that year and the following year he reluctantly returned (parental pressure) and underwent a screening. His only complaint at the time was that his feet were hurting (likely due to diabetic neuropathy) and that he found it difficult to walk. He requested a handicap parking tag that we did not facilitate for him! When checking his visual acuity, he had dropped one line of vision from previous visits, his blood pressure was 145/95 mmHg, and his HbA1c was >14% (glucose level was 525 mg/dL). Previous retinal examinations had shown healthy retinas. At the time of his visit in 2015, the 22-year-old subject had a combination of 440 blot and flame hemorrhages between the right and left eye (Figure 2) as well as intraretinal microvascular anomalies (IRMAs). OCT imaging showed very early signs of macular edema, while OCTA findings showed an intact foveal net (foveal avascular zone (FAZ)). After an intense 90-minute consultation with our subject and his family, we were able to convince him to resume use of his incontinentia pigmenti (IP). Within 8 months we succeeded in bringing down his HbA1c to 8.1% (from >14% and his blood pressure to 110/75 mmHg (controlled medication). Eight months after returning to a controlled regimen (in 2016), the subject had only 2 small blot hemorrhages, no IRMAs, and no flame hemorrhages (Figure 3) and his feet no longer hurt.

The beneficial effect of well-controlled T1D cannot be stressed enough, as these individuals face the likelihood of living a fulfilling life over the course of many decades. The value of having a group such as CWD where children and adults with T1D can live in close kinship, connected through social media, cannot be overemphasized. This is as important as their individual health statistics; the availability of a group support for the hundreds of children that live everyday with diabetes makes all the difference in the world while we all await the release of the artificial pancreas.
ACKNOWLEDGEMENTS

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Intestinal Parasites in Diabetes Mellitus Patients in the Limbe and Buea Municipalities, Cameroon

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ABSTRACT

Background: Intestinal parasites are an important cause of morbidity and mortality and a public health problem especially in tropical developing countries including Cameroon. The two main types of intestinal parasites are helminths and protozoa which are important causes of infections in immunocompromised individuals. Diabetes Mellitus (DM) is a metabolic non-communicable disease in which a person has high blood glucose. DM is an increasing problem in our community today and diabetics have been reported to be immunocompromised. The purpose of this study was to determine the prevalence and type of intestinal parasites in DM patients living in Buea and Limbe municipalities.

Methods: This hospital based cross sectional study involved a total 150 diabetic patients and 85 non-diabetic individuals which served as control group. Questionnaires were administered to the Diabetic patients only. Fresh stool specimens were collected and processed using Direct Microscopy, Formalin-Ether Concentration, Stoll’s technique and Modified Ziehl Nielsen staining techniques. Data was analyzed using Microsoft Office Excel 2013 and Statistical Package for Social Sciences (SPSS) version 20.

Results: The overall prevalence of intestinal parasites among diabetics was 10%. The parasites detected in diabetics included Entamoebahistolytica 10(6.7%), Blastocystishominis 4(2.7%) Ascarislumbricoides 1(0.67%), Hookworm 1(0.67%) and Cryptosporidium parvum 1(0.67%). An overall prevalence of 23.5% of intestinal parasites was observed in the control group with detected parasites Entamoebahistolytica 18(21.2%), Ascarislumbricoides 2(2.4%) and Blastocystishominis 1(1.2%). The prevalence in both groups (10% vs. 23.5%, p=0.0052) were statistically significantly different. DM status was significantly associated with the prevalence of intestinal parasites or acquiring intestinal parasitic infection (OR: 0.36 Confidence Interval (CI) =0.17-0.75; p=0.0051)).

Conclusion: The prevalence of intestinal parasites in diabetics is 10%. The most prevalent type of intestinal parasite in diabetics is Entamoebahistolytica which is same with non-diabetics. The more types of intestinal parasites in diabetics and along with the detection of C. parvum indicates a weakened immune system in diabetics. A protective association exists between Diabetes mellitus and Intestinal Parasitic Infections (IPIs). Diabetic patients should be screened routinely for intestinal parasites especially protozoans and treated for their overall well-being.

KEYWORDS: Protozoa; Helminthes; Diabetes mellitus; Prevalence.

INTRODUCTION

Intestinal parasites are an important cause of morbidity and mortality although they usually create non-aggressive diseases and constitute a major public health problem in their transmission from person to person, especially in developing countries where poor sanitary conditions and lack of information result in the contamination of food and water sources with a consequent continuance of parasite cycles. Even in countries where adequate sanitation conditions and education excel, some of these parasites play an important role in causing diseases in specific groups such as immunocompromised individuals and young children. About 340 million people worldwide with varying morbidity and mortality. They affect an estimated 3.5 billion persons and cause clinical signs and symptoms in approximately 450 million. The two main types of intestinal parasites are helminths and protozoa.

Diabetes mellitus (DM) is a metabolic non-communicable disease in which a person has high blood glucose, either because the body does not produce enough insulin or because cells do not respond to the insulin that is produced. There are two types of diabetes mellitus: Type 1, Insulin-Dependent Diabetes Mellitus (IDDM); Type 2, Non-Insulin-Dependent Diabetes Mellitus (NIDDM). This high blood sugar produces the classical symptoms of polyuria, polydipsia and polyphagia. Diabetes is one of the most frequent metabolic diseases and is widely distributed in various populations. Sub-Saharan Africa faces the world’s highest increase in type 2 Diabetes occasioned by adaptation to western lifestyles and genetic pre-dispositions. Diabetic patients have been reported to be immunocompromised. Intestinal parasites have gained increasing attention as important opportunistic pathogens responsible for clinically important infections in immunosuppressed patients.

The paucity of information on the prevalence and type of pathogenic intestinal parasitic infection in diabetic patients in Cameroon prompted this study and will provide useful up-to-date data for parasitic infection in diabetic patients.

MATERIALS AND METHODS

Study Area

The study was conducted in Buea and Limbe, two important cities in the Fako division and South West (SW) region of Cameroon. Buea is located about 800 m above sea level in Mount Cameroon. Buea, the capital of the SW region is located on the eastern slopes of Mount Cameroon and has a population of over 200,000. It lies at latitudes 4.09 °N and longitudes 9.13 °E and has a total surface area of 870 km². Limbe formally known as Victoria is a natural resource coastal city situated at 4.00 °N and 9.11 °E. It covers a surface area of 549 km² and situated near the Atlantic Ocean. Limbe has an equatorial climate and is dominated by the tropical equatorial rainforest with tall trees.

Study Population

The study focused on diabetic patients who live and visit diabetic units in hospitals and clinics in the study area. The diabetic patients were controlled patients who visited the diabetic unit for routine checkup. Non-diabetic individuals in the study area served as a control group.

Study Design

A hospital based cross-sectional study was carried out from March to June 2015 to determine the prevalence of intestinal parasites in diabetic patients. Non-diabetics served as a control group.

Ethical Considerations

The ethical approval of the study was sought and obtained from the Ethical Review committee of the Faculty of Health Science Institutional Review Board (FHS-IRB) of the University of Buea. Administrative clearance was also sought and obtained from the South West regional delegation for public health and from the director of the Buea Regional Hospital annex and the Limbe Regional hospital after presentation of detailed study objectives and procedures. Written informed consent was gotten from each recruited study participant from 21 years and above with participation being voluntary.

Data Management and Analysis

During data collection completed questionnaires were checked regularly to rectify any discrepancy, logical errors or missing values. Participants data obtained were entered into a log book, and later keyed into a computer using Microsoft excel 2013 and verified for the possibility of entering errors. Data were coded, entered and analyzed using Statistical Package for Social Sciences (SPSS) version 20. Continuous variables were described using mean and standard deviation, and categorical variables using their frequency and percentage. Chi-square test was used to test level of significance at p-value <0.05 considered statistically significant.

Data Collection

After obtaining consents from the participants a structured questionnaire was administered by trained personnel to collect clinical information and socio-demographic characteristics.

Specimen Collection and Laboratory Procedures

Stool specimen was collected from each participant in a dry, clean, leak proof, tight lid plastic container containing a small spoon labelled with an identification number.

Stool samples were analyzed by direct microscopy.
followed by the formol-ether concentration technique for microscopical detection of intestinal parasites. The modified ziehlens method was used to detect intestinal coccidians Cryptosporidium parvum, Isospora belli and Cyclosporacayetanensis. Stoll’s technique was used for the quantification of helminth eggs.13

A drop of blood was gotten from non-diabetic participants using a lancet and analyzed using a glucometer for random blood sugar to confirm they are actually not diabetic. They were also asked if they experienced any of the signs and symptoms of diabetes mellitus. Non-diabetic participants with a random blood sugar of 200 mg/dl or greater and had common signs and symptoms of diabetes was considered diabetic and referred to a diabetologists.

RESULTS

A total of 235 participants were recruited. 150 diabetic patients and 85 non-diabetic individuals (control group) with data on socio-demographic characteristics collected from diabetic patients only.

Among the 150 diabetic participants, 105(70%) were females while 45(30%) were males. Their age ranged from 27-90 years with a mean age of 56.1 years (Standard Deviation (SD) =11.8)). Most of the participants were between the age group of 41-60 years. The mean age of participants in the control group was 29.28 years (SD=7.7) and their ages ranged from 21-63 years. There were 35(41.2%) females and 50(58.2%) males.

Intestinal parasites were diagnosed in 15 out of 150(10%) participants in the diabetic group with some having mixed or co-infections. Figure 1 shows that five different intestinal parasites were identified from the study participants with three protozoans (E. histolytica, B. hominis and Cryptosporidium parvum) and two helminthes (Ascaris and hookworm) identified. Figure 1 also show that E. histolytica 10(6.7%) was the pre-dominant parasite identified from stool of the study participants followed by B. hominis 4(2.7%). One (0.67%) each of A. lumbricoides, Hookworm and Cryptosporidium parvum was also identified from study participants. Females showed a higher prevalence with parasitic infections 11(10.5%) than males 4(8.9%). The prevalence of parasitic infections was higher from the urban areas 10(11.0%) than from the rural areas 5(8.5%). The age group 41-60 years showed the highest prevalence 11(13.3%).

In the control group, 20 stool samples were positive for intestinal parasites 10(20%) males, 10(28.6%) females, with the following prevalence: E. histolytica 18(21.2%), A. lumbricoides 2(2.4%), and B. hominis 1(1.2%) as shown in Figure 2. An overall prevalence of 23.5% of intestinal parasites was observed in the control group. Polyparasitism was observed in only one control patient. A. lumbricoides was found only in the male sex.

Intestinal Protozoa

Three different intestinal protozoans were identified from the diabetic study participants. 13(8.6%) of diabetes mellitus participants were infected with intestinal protozoans with E. histolytica being the most prevalent 10(6.7%) followed by B. hominis 4(2.7%) while the intestinal coccidian Cryptosporidium parvum 1(0.7%) was the least prevalent. Two different intestinal protozoans were identified from the non-diabetic individuals which are E. histolytica 18(21.2%) and B. hominis 1(1.2%).

9 females (8.5%) were infected with intestinal protozoan and 4 males (8.9%). The prevalence of intestinal protozoans was higher in males than in females. E. histolytica was predominant amongst the females and had the only coccidian parasite C. parvum.

In the control group, 10 females and 9 males were infected with intestinal protozoa with E. histolytica being the predominant intestinal protozoa found in more females than males.

![Figure 1: Intestinal parasites in diabetes mellitus patients.](image-url)
Male gender had the only *B. hominis* identified.

Participants from the Urban areas 9(9.9%) were more infected with intestinal protozoans than those from the rural areas 4(6.7%). *E. histolytica* is the predominant intestinal protozoan which was more prevalent in the urban areas than rural areas. *B. hominis* mono-parasitism was observed only in rural areas while a co-infection of *E. histolytica* and *B. hominis* was identified only in urban areas.

Peak values of intestinal protozoa were obtained in age group 41-60 years. All three intestinal protozoans were identified in the age group 41-60 years with *E. histolytica* dominating and only *E. histolytica* was identified in the age group 61-80 years.

### Intestinal Helminths

A total of two different intestinal helminthes was identified among diabetic participants with a prevalence of 2(1.3%). The helminthes were *A. lumbricoides* and hookworm and they were one each amongst diabetic patients with a prevalence of 0.7% per helminth.

Only one intestinal helminth was identified among non-diabetic individuals and that was *Ascaris lumbricoides* with a prevalence of 2(2.4%).

The infection intensity of helminthes eggs was light infections. Classifications were done as specified by the World Health Organization (WHO) classification scheme shown in Table 1. Both Ascaris and hookworm had light infection intensities. *Ascaris lumbricoides* had an egg count of 300 eggs per gram of faeces while hookworm had an egg count of 100 eggs per gram of faeces. Ascaris had a higher egg count than hookworm.

The Ascaris in the control group had an egg count of 100 eggs per gram of faeces in both non-diabetic individuals hence light infection intensities.

### Single and Mixed Parasitic Infections in Diabetic Patients

Multi-parasitism existed only among the protozoa species with two pathogenic protozoa co-infection and a pathogenic and non-pathogenic protozoa co-infection. Helminthes had only single infection or mono-parasitism. Table 2 shows that single infections 9(6%) were more prevalent than mixed infections 6(4%). *E. histolytica* recorded the highest single and multiple infections. Non-pathogenic *E. coli* existed only as co-infections with other parasites or protozoa. Furthermore, there were no cases of helminthes/helminthes co-infection.

### Prevalence of Intestinal Parasitic Infections in Diabetes Mellitus Patients vs. Non-Diabetes Mellitus Individuals

Comparing the prevalence of intestinal parasites in the diabetic group versus prevalence in the non-diabetic group (10% vs. 23.5%) it was seen that the prevalence was statistically significant with a *p*-value less than 0.05.

### Association and Relationship between DM Status and Intestinal Parasitism

Table 3 shows that the association between DM status and infection with parasites is statistically very significant. A protective association exist between Diabetes mellitus and

<table>
<thead>
<tr>
<th>Helminthes Species</th>
<th>Light(Epg)</th>
<th>Moderate(Epg)</th>
<th>Heavy(Epg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ascaris lumbricoides</em></td>
<td>1-4,999</td>
<td>5000-49,999</td>
<td>&gt;50,000</td>
</tr>
<tr>
<td>Hookworm</td>
<td>1-1999</td>
<td>2000-3,999</td>
<td>≥4,000</td>
</tr>
</tbody>
</table>

Table 1: WHO classification scheme for intestinal helminthes intensities.
Intestinal parasitic infections. Diabetes protects against getting intestinal parasitic infections.

Table 3 shows an odd ratio of 0.36 which indicates a decreased risk for diabetes mellitus patients to acquire intestinal parasitic infections.

**DISCUSSION**

The prevalence rates of intestinal parasites, including opportunistic protozoa, in Africa vary from study to study depending on the diagnostic technique used and the study population. An overall prevalence of 10% of intestinal parasitic infection was observed among diabetic patients which is lower than the prevalence reported in the south western part of Nigeria (18.7%)[8] and South east Turkey (47%). Geographical location may account for this difference as the Olusegun et al study was carried out in Nigeria and the Nazligul et al study was carried out in Sanliurfa province which was an endemic zone for intestinal parasites.

Five different intestinal parasites were identified from the study participants with three protozoa (E.histolytica, B.hominis and Cryptosporidium parvum) and two helminthes (Ascaris and hookworm) identified. This differs from the study carried out in Nigeria where three different intestinal parasites were identified and Hookworm being the most prevalent while E. histolytica is the least prevalent. It also differs from the study carried out in South east Anatolia or Turkey where same number of different parasites where identified but the only protozoa were E. histolytica, Giardia lamblia and helminthes were Ascaris, Trichuristrichuria and Taenia. Ascaris was the most prevalent intestinal parasite. Helminthes were the least prevalent intestinal parasite in this study due to the massive drug administration of anti-helminthics by the government of Cameroon recently to help eradicate intestinal helminthes in the nation but intestinal protozoa still pose a threat.

This investigation reveals that there are more female participants who were also more infected (10.5%) with intestinal parasites than males (8.9%). This can be explained by the fact that there is a high prevalence of diabetes mellitus occurring in females than in males and this is similar to the study in Nigeria and Sanliurfa province. Also females are more engaged in farming and domestic work which exposes them to these intestinal parasites. Also there were parasitic infections in the age groups 41-60 years (13.3%) and 61-80 years (7.8%). In the other age groups there were no parasitic infections discovered. This could be due to the weakened immune system that comes with ageing coupled with diabetes mellitus status. Results were similar to those obtained in Nigeria where high prevalence was obtained in the age group 51-60 years followed by 41-50 years and 61-70 years.

There were more parasitic infections in the urban areas (10%) than rural areas (8.5%) probably due to the migration from the rural to urban areas of diabetic patients to meet their children so that they can be well taken care of and taken to the hospital regularly for checkup. E. histolytica was predominant in the urban areas probably through fruit and food (vegetables)
handlers who sell fruits and food by the road side in the urban towns than in the rural areas. This food and fruits are mostly bought by diabetic patients especially after fasting due to blood sugar control from the nearest vendor and they are also advised to eat vegetables. This same study shows a high prevalence of *E. histolytica* in the general non-diabetic population which serves as a reservoir host for transmission of *E. histolytica*. Intestinal parasites were more prevalent among business people and civil servants probably due to their busy schedules which makes it difficult for them to visit the doctor regularly. Also they spend most of their time out of their homes hence possibly feeding out of the house from places where sanitary conditions are questionable.

The prevalence of intestinal parasites in the control group or non-diabetic individuals (23.5%) was higher in this study and statistically different or lower than the prevalence in the diabetic group (10% vs. 23.5%, *p*=0.0052). This was similar with the findings of Nazligul et al who found out that intestinal parasite prevalence in the diabetic group was found to be significantly lower than in the control subject group (47 vs. 55%, *P*=0.05), This can be explained by the greater number of physician visits incurred by diabetic patients than the non-diabetic patients where diabetic patients consult frequently and are treated for possible intestinal parasitic infections. This differs from the study by Olusegun et al where non-diabetics had no intestinal parasites probably due to the low prevalence of intestinal parasites in that region. The presence of *Cryptosporidium* only in the diabetics reinforces the theory of diabetics being immunologically weaker than non-diabetics.

DM status was significantly associated with the prevalence of intestinal parasites or acquiring intestinal parasitic infection (OR: 0.36 CI=0.17-0.75; *P*=0.0051). Being diabetic was not a risk factor for acquiring intestinal parasitic infection but instead decreases your chances of acquiring the parasites due to a higher prevalence of intestinal parasites in the control group. This differs from the findings of Olusegun et al where being diabetic was a risk factor for acquiring intestinal parasites. This is due to the fact that the standard of living in this communities are better, sanitation is better, diabetic patients visit the doctor regularly, consult, follow the doctor’s order diligently and are well taken care of by their family members since most diabetic patients are old.

CONCLUSION

The prevalence of IPIs (Intestinal Parasitic Infections) among Diabetes mellitus patients in Limbe and Buea communities obtained was 10.0%. The parasites detected in diabetics included *Entamoeba histolytica*, Blastocystis hominis, *Ascaris lumbricoides*, Hookworm and *Cryptosporidium parvum*. Three protozoans and two helminthes. The most prevalent type of intestinal parasite in diabetics is *Entamoeba histolytica* which is same with non-diabetics. The more types of intestinal parasites in diabetics and along with the detection of *C. parvum* indicates a weakened immune system in diabetics. Our study suggests a protective association exist between Diabetes mellitus and Intestinal parasitic infections. Diabetic patients should be screened routinely for intestinal parasites especially protozoans and treated for their overall wellbeing.

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AUTHORS’ CONTRIBUTIONS

FBT, NAL and EBF planned the study and designed the protocols. NAL and EBF supervised the study including collection of stool samples, data from the questionnaire interviews and management of collected data. FBT supervised the laboratory work and administered questionnaires to participants. FBT and EJE carried out the data analysis and interpretation. FBT prepared the first draft of the manuscript and all the authors revised the manuscript critically.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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Behavioural Indicators as Risk of Diabetes Mellitus: A Community based Study in Manipur

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ABSTRACT

Background: Diabetes Mellitus (DM) has become a major health issue all over the world. Lifestyle factors may affect the health of the patients with diabetes directly or indirectly. Family history of diabetes was given importance in various studies of this aspect of metabolic syndrome.

Aim: The present study was conducted to find out the effect of lifestyle indicators and family history of diabetes among the diabetic Muslim population of Manipur.

Materials and Methods: Cross-sectional method was used for the study in which individuals of both sexes in the age group from 20-45 years. The respondents were taken from two districts in Manipur. Information was gathered by using a structured proforma.

Results: Chi-square test showed significant p-values for stress level, family history, physical activity and Quality of Life (QoL) of the participants. All these lifestyle indicators including breakfast habit and family history of diabetes were found to be significant except quality of life on multinomial logistic regression analysis.

Conclusion: Lifestyle had greatly influenced on the life of the diabetic Muslim people of Manipur in which it needed to give more awareness to them.

KEYWORDS: Diabetes mellitus; Family history; Lifestyle; Manipur.

ABBREVIATIONS: QoL: Quality of life; DM: Diabetes Mellitus; CMHA: Canadian Mental Health Association; WHO: World Health Organization; BMI: Body Mass Index; CDC: Centre for Disease Control and Prevention; HRQL: Health Related Quality of Life.

INTRODUCTION

Diabetes mellitus, a metabolic disease is increasing rapidly in almost all regions of the world. India stands at the topmost position in the world with the highest number of people with diabetes mellitus of about 31.7 million in the year 2000 followed by China with 20.8 million in second and the United States 17.7 million in the third place.\(^1\) The maximum increase of the prevalence of diabetes in India will contribute largely to the global increase from 171 million in 2000 to 366 million in 2030.\(^1\) India is experiencing an alarming increase in the incidence and prevalence of type 2 diabetes mellitus (T2DM)\(^2\) both in rural\(^3\) and urban areas\(^4\) with higher prevalence in South than in North India.\(^5\) A higher risk of diabetes has been reported from few Southern and North-eastern states while several northern and central states were at lower risk after adjusting for individual characteristics and place of residence.\(^6\)

Family history of diabetes is considered as a positive factor if either or both the parents have diabetes.\(^7\) Two to three times higher risk of developing glucose intolerance is associated with those individuals who have family history of diabetes. It has been recognized that family history of type 2 diabetes is one of the important risk factor of the disease.\(^8,9\) Individuals who have a family history of diabetes can have two to six times the risk of type 2 diabetes compared with individuals with no family history of the disease.\(^8,10\) The causes of type 2 diabetes are quite complex, family medical history provides valuable genomic information. Hence, this informa-
tion represents the combination of inherited genetic susceptibilities and shared environmental and behavioural factors.\textsuperscript{11}

Physical inactivity is also another major behavioural risk factor of type 2 diabetes. Sedentary habits of the individuals developed higher prevalence of the disease.\textsuperscript{12} Quality of life of people with diabetes is seriously threatened.\textsuperscript{13} The present study was therefore conducted to examine whether these lifestyle indicators affected the diabetic Muslim population of Manipur.

**MATERIALS AND METHODS**

All the participants studied were from Muslim community of Imphal-East district and Thoubal district and were under medical supervision. A cross-sectional research method was used. Among a total of 400 participants, 200 were diabetics and 200 were non-diabetics of both sexes. The purpose of the study and techniques to be used were explained to each participant. Only those participants who gave written consent were included for the study. Ethical permission was taken from Institutional Ethical Committee (IEC) prior to the fieldwork. Direct interview method was used. Detailed information of the participants was collected using standardized proforma. Stress level of the participants was assessed by using standardized questionnaires given by Canadian Mental Health Association (CMHA).\textsuperscript{14} Total stress level was calculated and classified according to its cut-off points (14-22=considerably above average, 10-13=above average, 9-0=average). QoL was assessed by using the World Health Organization (WHO) Quality of life-BREF (WHOQL-BREF) questionnaire.\textsuperscript{15} Statistical analysis of all the data collected were analysed by using 17.0 version of SPSS. Cross tabulations were carried out to find out the frequencies, percentages and chi-square values. Risk factors of the variables were determined by using multinomial logistic regression.

**RESULTS**

The basic characteristics of the population under study are displayed in Table 1. The numbers of subjects were 200 each for both males and females. Mean values of age, height and weight were more for the patients with type 2 diabetes. Table 2 shows cross tabulation of different stress levels among patients with type 2 diabetes and non-diabetic participants. All the stress level percentages were higher among patients with type 2 diabetes as compared to non-diabetic participants. Chi-square value was found to be statistically significant at $p<0.001$. Distribution of patients with type 2 diabetes according to family history of diabetes has been displayed in Table 3. Maximum number of diabetic subjects (60%) was found to have a family history of type 2 diabetes mellitus and 4.5% subjects had type 1 family history of this disease. But the corresponding values were comparatively less among non-diabetic participants. Statistical significance was found for this factor at $p<0.001$.

Table 4 displays the cross tabulation of physical activity status. It was found from the study that more number of patients with type 2 diabetes were physically inactive as compared to non-diabetics. Most of the non-diabetes were found to be physically more active (98%). However, 77% patients with type 2 diabetes were active for physical activity. Marked differ-

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td>Male (N=200)</td>
</tr>
<tr>
<td>Diabetic</td>
<td>Non-diabetic</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41.2±4.16</td>
</tr>
<tr>
<td>Height (centimetre)</td>
<td>160.1±5.10</td>
</tr>
<tr>
<td>Weight (kilogram)</td>
<td>63.3±8.78</td>
</tr>
</tbody>
</table>

Table 1: Distribution of participants under different characteristics.

<table>
<thead>
<tr>
<th>Stress level</th>
<th>Diabetic N(%)</th>
<th>Non-diabetic N(%)</th>
<th>Total no.(%)</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Considerably above average</td>
<td>80(40.0%)</td>
<td>24(12.0%)</td>
<td>104(26.0%)</td>
<td>61.7***</td>
</tr>
<tr>
<td>Above average</td>
<td>49(24.5%)</td>
<td>29(14.5%)</td>
<td>78(19.5%)</td>
<td>61.7***</td>
</tr>
<tr>
<td>Average</td>
<td>71(35.5%)</td>
<td>147(73.5%)</td>
<td>218(54.5%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>200(100.0%)</td>
<td>200(100.0%)</td>
<td>400(100.0%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Cross tabulation of different stress levels.

<table>
<thead>
<tr>
<th>Family history of diabetes</th>
<th>Diabetic N(%)</th>
<th>Non-diabetic N(%)</th>
<th>Total no.(%)</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>9(4.5%)</td>
<td>1(0.5%)</td>
<td>10(2.5%)</td>
<td>78.2***</td>
</tr>
<tr>
<td>Type 2</td>
<td>120(60.0%)</td>
<td>41(20.5%)</td>
<td>161(40.3%)</td>
<td></td>
</tr>
<tr>
<td>Not applicable</td>
<td>71(35.5%)</td>
<td>158(79.0%)</td>
<td>229(57.3%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>200(100.0%)</td>
<td>200(100.0%)</td>
<td>400(100.0%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Cross tabulation of family history of diabetes.
ence with statistically significance ($p<0.001$) was also observed for physical activity status among patients with type 2 diabetes and non-diabetic participants. In the present study, maximum subjects had their breakfast regularly (93% diabetic and 98% non-diabetic). Five percent diabetic and 1% non-diabetic took breakfast irregularly. Two percent diabetic and 1% non-diabetic were not taking breakfast at all. The differences in the various categories were found statistically non-significant (Table 5).

The median of the total score of QoL was calculated and it was found to be 77. It was categorized as <77 as low quality of life and ≥77 as good quality of life. It was found from the scores of quality of life of the participants (Table 6) that 54.5% of them had low QoL out of which proportionately larger number were diabetic (66.5%). Only 42.5% non-diabetics had low quality of life. Good quality of life was comparatively more in number among non-diabetics (33.5% diabetic and 57.5% non-diabetic). The difference in distribution of participants in the quality of life categories was statistically significant ($p<0.001$).

Multinomial logistic regression of lifestyle indicators and family history of diabetes was found out to the risk factors for each category as given in Table 7. The patients under the categories of considerably above average level of stress and above average level of stress were 6.9 times and 3.8 times more risk of having diabetes respectively. Patients with family history of

<table>
<thead>
<tr>
<th>Physical activity status</th>
<th>Diabetic N(%)</th>
<th>Non-diabetic N(%)</th>
<th>Total no.(%)</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactive</td>
<td>46(23.0%)</td>
<td>4(2.0%)</td>
<td>50(12.5%)</td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>154(77.0%)</td>
<td>196(98.0%)</td>
<td>350(87.5%)</td>
<td>40.3***</td>
</tr>
<tr>
<td>Total</td>
<td>200(100.0%)</td>
<td>200(100.0%)</td>
<td>400(100.0%)</td>
<td></td>
</tr>
</tbody>
</table>

N: Number of participants.

$**p<0.001.$

Table 4: Cross tabulation of physical activity status.

<table>
<thead>
<tr>
<th>Breakfast consuming pattern</th>
<th>Diabetic N(%)</th>
<th>Non-diabetic N(%)</th>
<th>Total no.(%)</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irregular</td>
<td>10(5.0%)</td>
<td>2(1.0%)</td>
<td>12(3.0%)</td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>4(2.0%)</td>
<td>2(1.0%)</td>
<td>6(1.5%)</td>
<td>6.3</td>
</tr>
<tr>
<td>Regular</td>
<td>186(93.0%)</td>
<td>196(98.0%)</td>
<td>382(95.5%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>200(100.0%)</td>
<td>200(100.0%)</td>
<td>400(100.0%)</td>
<td></td>
</tr>
</tbody>
</table>

N: Number of participants.

$**p<0.001.$

Table 5: Cross tabulation of breakfast consumption pattern.

<table>
<thead>
<tr>
<th>Quality of life categories</th>
<th>Diabetic N(%)</th>
<th>Non-diabetic N(%)</th>
<th>Total no.(%)</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low quality of life</td>
<td>133(66.5%)</td>
<td>85(42.5%)</td>
<td>218(54.5%)</td>
<td></td>
</tr>
<tr>
<td>Good quality of life</td>
<td>67(33.5%)</td>
<td>115(57.5%)</td>
<td>182(45.5%)</td>
<td>23.2***</td>
</tr>
<tr>
<td>Total</td>
<td>200(100.0%)</td>
<td>200(100.0%)</td>
<td>400(100.0%)</td>
<td></td>
</tr>
</tbody>
</table>

N: Number of participants.

$**p<0.001.$

Table 6: Cross tabulation of quality of life categories.

<table>
<thead>
<tr>
<th>Lifestyle indicators</th>
<th>Categories</th>
<th>Exp(B)</th>
<th>CI(95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress level</td>
<td>Considerably above average</td>
<td>6.9</td>
<td>(3.6, 13.3)</td>
</tr>
<tr>
<td></td>
<td>Above average</td>
<td>3.8</td>
<td>(2.0, 7.4)</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>0*</td>
<td></td>
</tr>
<tr>
<td>Family history of diabetes</td>
<td>Type 1 diabetes mellitus</td>
<td>20.5</td>
<td>(2.2, 186.7)</td>
</tr>
<tr>
<td></td>
<td>Type 2 diabetes mellitus</td>
<td>7.1</td>
<td>(4.2, 12.1)</td>
</tr>
<tr>
<td></td>
<td>Not applicable</td>
<td>0*</td>
<td></td>
</tr>
<tr>
<td>Physical activity</td>
<td>Inactive</td>
<td>13.2</td>
<td>(4.2, 41.6)</td>
</tr>
<tr>
<td></td>
<td>Active</td>
<td>0*</td>
<td></td>
</tr>
<tr>
<td>Breakfast</td>
<td>Irregular</td>
<td>6.5</td>
<td>(0.9, 43.9)</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>8.1</td>
<td>(1.4, 45.5)</td>
</tr>
<tr>
<td></td>
<td>Regular</td>
<td>0*</td>
<td></td>
</tr>
<tr>
<td>Quality of life</td>
<td>Low quality of life</td>
<td>1.5</td>
<td>(0.9, 2.5)</td>
</tr>
<tr>
<td></td>
<td>Good quality of life</td>
<td>0*</td>
<td></td>
</tr>
</tbody>
</table>

Note
CI: Confidence Interval.
0*: Reference (normal).
Diabetic: Dependent category.
Non-diabetic: Reference category.

Table 7: Multinomial logistic regression of various lifestyle indicators.
type 1 and type 2 diabetes mellitus were 20.5 times and 7.1 times more likely to have risk of diabetes respectively. Those patients who were less active in physical activity were 13.2 times more at risk to develop diabetes compared to the active group. Irregular breakfast consumers and none breakfast consumers were 6.5 times and 8.1 times more at risk of developing diabetes respectively. Patients with low quality of life had 1.5 times more risk to suffer from diabetes.

DISCUSSION

Stress level, physical inactivity, breakfast consumption pattern, quality of life, etc. do indicate the health status of an individual. The present study was conducted to determine whether these indicators affected the health of the diabetic and non-diabetic Muslim population of Manipur. It was observed from the present study that most of the patients with diabetes were found to have stress and it was identified as one of the risk factor of diabetes. This was consistent with the results of earlier studies and was suggestive of direct or indirect negative impact of stress on blood glucose level through the release of stress hormones or by disrupting self-care practices.

Indian population were commonly seen to have familial aggregation of diabetes with a high prevalence among very close relatives and was transmitted vertically through more than two generations. A study conducted among the South Indian population showed the development of diabetes to be earlier among the subjects with family history of diabetes as compared to subjects without family history. Further, it was demonstrated by which study that glucose intolerant subjects with family history were 7 years younger than subjects without a family history of diabetes.

In a self-reported study among US adults, family history of diabetes was shown to be a significant predictor of diabetes. The study estimated that those adults with a family history of diabetes on their parents or siblings had four times more risk of having diabetes than adults without a family history of the disease, after adjusting for gender, age, race, and body mass index (BMI).

The risk of type 2 diabetes was six times higher among the women with family history of diabetes as compared with individuals without a family history of the disease. Furthermore, the study conducted by Centre for Disease Control and Prevention (CDC) demonstrated that the risk of having diabetes among adults with two diabetic parents was more than twice.

In the present study, it was clearly found that patients with type 2 diabetes had 60% type 1 and 4.5% type 2 family histories of diabetes respectively. However, the risk of developing diabetes was more among those who had type 1 diabetes family history. Similarly, in a recent clinic based study among patients with diabetes of Western Indian population, it was reported that 57.7% had positive family history out of which 37.0% had single parent with diabetes, 10.5% had both parents with diabetes and 26.7% had near relatives suffering with diabetes which showed high familial aggregation of T2DM in the Western Indian population.

Sedentary lifestyle adversely affected the health of the people that might contribute to the increase in body weight, a major risk factor of diabetes. Maintaining exercise regularly and active physical work could contribute in improving the health of the diabetic patient. Regular physical exercise whether aerobic or resistance was proved to be effective in the reduction of degree of obesity and the incidence of metabolic results such as type 2 diabetic individuals. Wing et al. suggested walking as a form of exercise and prescribed an increment of exercise. In the present study, it was found that the percentage of physically inactive individuals was more among diabetics as compared to non-diabetics and the difference between the two was also statistically significant. Moreover, it was marked that the physically inactive persons were 13 times more likely to be at risk of diabetes. This finding was consistent with the study by Dowse et al. that there was an association between physical inactivity and risk of non-insulin dependent diabetes mellitus and impaired glucose tolerance.

The present study among the Muslims of Manipur showed relative risk between irregular or absence of breakfast consumption and diabetes. Maximum percentage of patients with diabetes had regular breakfast and very few them did not consume breakfast at all. The risk of diabetes was found 8.1% on patients not taking breakfast and 6.5% among regular consumers. This result was consistent with a large prospective study by Mekary et al. that the risk of type 2 diabetes might be decreased among men by breakfast consumption. Breakfast omission was associated with an increased risk of type 2 diabetes mellitus in men even after adjustment for BMI which needed further studies to elucidate this association in women and in other ethnic and racial groups and to conduct an in-depth analysis of specific breakfast foods.

Marked significant difference in the QoL between patients with diabetes and non-diabetic participants was revealed in the present study. However, in multinomial logistic regression analysis, it showed less difference and statistically non-significant. It could be due to social conditions and lifestyle of the people which adversely affected their physical and psychological domains. Similarly, this type of difference was found in Health Related Quality of Life (HRQoL) of both genders in a study conducted in Iran. Eljedi et al. in their study on the diabetic patient living in refugee camps in the Gaza strip analyzed HRQoL in comparison with gender and age matched non-diabetic controls from the same camps. They reported that all the domains of the WHOQOL-BREF was negatively affected by diabetes and its complications that had greatest effects on the physical health and psychological domains but the effects was weaker for the social relationships and environmental domains. Further, interactions between gender and disease status between diabetic patients and
non-diabetic were also strong. However, this finding could not
be explained fully because the situation of the female patient
was worse which showed the evidence for gender inequalities.28

Another study29 found the deterioration of the QoL of
patients with type 2 DM by the presence of depression. This
finding resulted on the conclusion that the QoL of the subject
could be made better by treating depression.

CONCLUSION

The family history of diabetes as well as lifestyle indicators such
as stress level, physical activity, breakfast consuming pattern
and quality of life among Muslim males and females of Mani-
pur played crucial role in the life of patients with diabetes. The
present study demonstrated these parameters as risk factors of
this metabolic disease. Thus, there is a need to make efforts for
improving the lifestyle which might help in the reduction of this
disease.

ACKNOWLEDGEMENTS

The authors are thankful to all the participants of the Muslim
community of Imphal-East district and Thoubal district for their
full cooperation in the present study. Nilupher Feroz is grate-
ful to the University Grants Commission for financial assistance
during the study under the Non-NET fellowship scheme.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

CONSENT

The participants have been properly, made aware about the ob-
jectives, relevance and purpose of the research study. The partic-
ipants have been told that the anthropological information col-
llected from them will be utilized for the real purpose of research
and academic activities. They are also made to understand that
no money will be charged from them for any of the tests, and
they can withdraw from the study at any time, but will keep get-
ting counselling benefits till the duration of the project.

All the participants consciously gave their consent to participate
in the above research study.

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cessed April 26, 2016


Mini Review

Basic Ketone Engine and Booster Glucose Engine for Energy Production

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ABSTRACT

Recently, hyperketonemia induced by fasting or ketogenic diet calls attention because of the possibilities for various clinical applications. Animal species comparisons and biochemical data show that all fetuses can develop by using ketogenic energy through a pathway which seems to have been maintained. We hypothesized that 3-β-hydroxybutyrate (β-HB) could be the fuel for the basic engine that produces energy in all terrestrial species. However, ATP production from glucose-pyruvic acid pathway seems to be added as a dominant system in human. We hypothesize the establishment of TCA cycle in mitochondria and enough oxygen supply since two billion years ago would be the key events to promote this change. The efficacy of ATP production from β-oxidation product is 10 ATP molecules, while it is 12.5 molecules from pyruvic acid. So, evolution should select glucose burning system as a booster engine for energy production. The liver and kidney are major ketone producing organs which contain abundant glycogen particles. So, a close relationship between ketone and glucose burning system may be present. This explains why certain level of glucose is steadily maintained even in the hyperketogenic state. Ketogenic diets efficiently treat with gestational diabetes. Placenta is the ketogenic tissue which reflects high concentration of ketones in umbilical cord blood. In addition to the role of energy source, β-HB shows various pharmacological effects on disease prevention, such as cardiovascular disease, Alzheimer’s disease, epilepsy, etc. Inhibition of histone deacetylase, stimulation of FOXO, resistance to oxidative stress, protection of mitochondria, stimulation of adiponectin release, suppression of inflammasome, etc. are included. The contribution of intestinal microbiota to ketone body production should open a new field of medicine. Altogether, the above data suggest that longer-term human studies are necessary to exclude risks of unbalanced diet and to confirm the best combination of fuels for energy production, disease prevention and medical treatment.

KEYWORDS: Ketone; β-hydroxy butyrate; TCA cycle; Histon; Gestational diabetes; Fast; Endocrine; Metabolism; Pharmacology.


DIABETIC KETOACIDOSIS

Diabetic ketoacidosis (DKA) is an acute, major, life-threatening complication of diabetes which occurs in patients with either type 1 or type 2 diabetes.1 This condition is a complex disordered metabolic state characterized by hyperglycemia, ketoacidosis, and ketonuria. Insulin deficiency leads to the release of free fatty acids from adipose tissue, hepatic fatty...
acid oxidation, and the formation of ketone bodies, such as acetoacetate, 3-β-hydroxybutyrate (β-HB) and acetone (Figure 1). The observed pro-inflammatory and pro-coagulant states in hyperglycemic crises and hypoglycemia may be the result of adaptive responses to acute stress, and not hyperglycaemia or hypoglycaemia per se.1

Usually, ketones are dealt with a risk factor of worse prognosis by ADA and other diabetes supporting organization.

FASTING AND HYPERKETONEMIA

In the fasted state, gluconeogenesis is diminished; the flow of substrates entering the citric acid cycle drops, and ketone production are turned on. Cahill2,3 studied the glucose metabolism of people who let themselves fast for 40 days. He reported that in the starving human adult, β-HB and aceto-acetate are produced in the liver from long-chain fatty acids and β-HB could be the energy source in the brain and other tissues. A rise of β-HB blood concentration to approximately 6 mM was characteristic. The estimated glucose production at 5-6 wk of starvation was reduced to approximately 86 g/24 hr. Of this amount the liver contributes about one-half and the kidney the remainder. Approximately all of the lactate, pyruvate, glycerol, and amino acid carbons which are removed by the liver and kidney are converted into glucose, as evidenced by substrate balances across these organs.

Cahill’s research was integrative rather than reductionism, but he opened the unique insight on the metabolic adaptation of humans to starvation. During starvation, extremely low insulin levels facilitate acyl-CoA entry into mitochondria, producing excess amounts of acetyl-CoA that cannot be metabolized in the Krebs cycle and are diverted towards the synthesis of ketone bodies (Figure 1).4 In 1960s, it was widely held that the brain did not oxidize ketone bodies for the production of energy. Cahill was one of the few clinical investigators at that time who believed that during starvation there was not enough nitrogen in the urine to account for the alleged amount of glucose that the brain was thought to need for normal function.5 Glucose, β-HB, and acetocetate appeared to be used for energy sources of the brain of these people, as only ketone bodies increased without symptoms of ketoacidosis.5

The tissues that produce β-HB include the liver, kidney and the brain astrocytes.6 As the brain consumes about 20% of its energy from glucose, similar amounts of ketones should be substituted to glucose to keep the brain functioning in the fasting state (Figure 2).

Koda’s fasting and dietary therapy has proven effective for many intractable diseases in Japan.7 In an extreme case the blood concentration of β-HB remained above 3 mM without any clinical and laboratory symptoms.8 So, β-HB can substitute glucose as an energy source. It seems to stimulate the human nervous and endocrine systems, and to increase self-healing ability.

GESTATIONAL DIABETES

Gestational diabetes is not a rare complication of pregnancy. During pregnancy, the placenta produces high levels of various hormones. Almost all of them impair the action of insulin in maternal cells, raising the blood sugar level. Controlling the blood glucose can prevent birth complications and keep the
baby healthy, so insulin therapy is usually tried in gestational diabetes.⁹

As the fetus grows, the placenta produces more and more insulin-blocking hormones. In gestational diabetes, placental hormones induce a rise in blood glucose up to a level that can affect the growth and welfare of the baby. Gestational diabetes is particularly severe in obese pregnant women. In such cases, insulin therapy is often ineffective, and doctors recommend abortion if elevated blood glucose levels fail to be under control.

American Diabetes Association (ADA) does not recommend a very low-carbohydrate diet where the uptake of carbohydrate is lower than 130 g per day. Muneta et al⁹ recently reported the cases of 16 gestational diabetic patients who had normal deliveries after a very low-carbohydrate diet (less than 5 g per diet). The blood level of ketone bodies and free fatty acids increased consistently, with a respiratory quotient (CO₂ eliminated/O₂ consumed) of 0.72, which means main energy comes from ketone bodies under eucaloric condition.¹⁰

Most patients were obese and lost body weight with the MEC ketogenic diet (100 g each of meat, eggs, cheese and leaves of green-yellow vegetables), blood glucose levels returned within normal range within a few weeks, and all deliveries were under control.

Maternal starvation in late gestation lowers insulin, and lipolysis supervenes. The continued glucose drain by the conceptus aids in converting the maternal liver to the ketogenic organ, and ketone bodies produced from incoming fatty acids cross the placenta to be utilized by the fetus. Muneta¹¹ found high β-HB level (1 to 8 mM) among mothers, or in the umbilical cord blood, and also in the placental tissue fluid (Table 1).

High β-HB levels in the placenta have occasionally been reported by veterinarians, but most of them deals with domestic animals, where ketonemia is classically a sign of problematic delivery.¹²

**DUAL ENGINE FOR ENERGY PRODUCTION**

The β-HB and acetoacetate (AcAc) support mammalian survival during states of energy deficit by serving as alternative sources of ATP.²⁻⁴ Mitochondrial 3-hydroxy-3-methylglutaryl-CoA

![Figure 2: Glucose and ketone formation at fast or severe carbohydrate restriction. Hepatocytes, renal tubular cells and intestinal epithelial cells and astrocytes are able to carry out ketogenesis in prolonged fasting and ketogenic diets. After glucose is provided by gluconeogenesis in the liver and kidney (red arrow), ketone formation substituted (yellow). Astrocyte is considered to produce ketones up to 20% to support brain function.](image)

<table>
<thead>
<tr>
<th>Material</th>
<th>β-hydroxybutyrate</th>
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<td>Peripheral blood at 1 month</td>
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**Table 1:** Concentration of β-hydroxybutyrate of placenta and new born peripheral blood.
synthase and HMGCS2 is the rate limiting enzyme of the ketogenic pathway (Figure 1).\textsuperscript{13-15} Succinyl CoA acetoacetate-CoA transferase (SCDT) is necessary to convert β-HB to acetoacetate in the peripheral tissue cells. SCDT is lacking in hepatic cells, so, the liver is a major ketogenic organ but cannot use it. Animal species comparisons and biochemical data show that all fetuses can develop by using ketogenic energy through a pathway which seems to have been maintained throughout evolution.\textsuperscript{16} We thus hypothesized that β-HB could be a fuel for the basic engine that produces energy in all terrestrial species. However, ATP production from glucose-pyruvic acid pathway seems to become a dominant system in human. Why glucose has become the major fuel in the body? We hypothesize the establishment of TCA cycle in mitochondria and enough oxygen supply since 2 billion years ago would be the key reason. The efficacy of ATP production from β-oxidation product is 10 ATP molecules, while it is 12.5 molecules from pyruvic acid.\textsuperscript{17} So, more efficient burning system has developed by using various glucose transporters in various organs throughout evolution. We may call it a booster engine for energy production. Endosymbiosis with mitochondria, which should support the burning system with oxygen, is a miracle signs of life.

The liver and kidney contain abundant glycogen particles. These organs are also ketone producing organs, so it may represent a close relationship between ketone and glucose burning system (Figure 2). This explains why certain level of glucose is steadily maintained in the blood even in the hyperketonemic person. Some people could not raise ketone levels in the blood concentration as expected even by the ketogenic diet. A metabolic homeostasis would be different in these people, and needs further study.

**THERAPEUTIC USE OF KETONE DIET**

**Epilepsy, Autistic Behavior and Childhood Obesity**

Fasting and ketogenic diet was first introduced for the treatment of epilepsy by HR Geyelin and RM Wilder’s group in 1920’s.\textsuperscript{18} Over 250 medical centers worldwide offer ketogenic diets to children with epilepsy.\textsuperscript{19} However, access to these therapies has remained extremely limited for adults until recent years.\textsuperscript{20} From observations in 229 adults (age range of 18-86 years) attending the Adult Epilepsy Diet Center, ketogenic diet therapies appeared effective and safe in the long-term in adults.

The potential benefits of ketogenic diets are considerable. However, the effects of carbohydrate-depleted (ketogenic) diets on the metabolic parameters of children have been insufficiently assessed.\textsuperscript{21}

To compare the efficacy and metabolic impact of ketogenic and hypocaloric diets in obese children and adolescents, fifty-eight obese subjects were assigned to one of two diets for 6 months. In both group participants significantly reduced their weight, fat mass, waist circumference, fasting insulin, and HOMA-IR, but the differences were greater in the ketogenic group. Only the ketogenic group had increasing in high molecular weight adiponectin.

Ketogenic diets may be used as a additional or alternative therapy in autistic behavior.\textsuperscript{22}

**β-HB and Prevention of Diabetic Complication**

**Myocardial infarction:** Cardiovascular events are common in diabetic patients. After infarct by the thrombosis, resuscitated blood flow by thrombolysis often damages remained tissue. Short-term fasting reduces the extent of myocardial infarction and incidence of reperfusion arrhythmias in rats.\textsuperscript{23} In experiments on cardiac ischemic tolerance in rats, short-term fasting increased the concentration of β-HB compared to controls. In addition, fasting limited the infarct size (48.5% of the area at risk) compared to 74.3% controls; the total number of premature ventricular complexes (12.5) was reduced compared to 194.9 controls; and the duration of ventricular tachycardia (0.6 s vs. 18.8 s) occurring at early reperfusion.

To investigate the role of high concentrations of β-HB in preventing heart damage after prolonged fasting, infarct size and the incidence of apoptosis caused by ischemia-reperfusion were determined in the Wistar rats.\textsuperscript{24} Apoptosis in the sub-endocardial region was significantly reduced in fasting. In addition, the levels of ATP in the fasting DL-β-HB treated group were significantly higher compared with control groups after 30 min of ischemia and 120 min of reperfusion.

**Alzheimer and other nervous disease:** The Hisayama study\textsuperscript{25} started in 1961 is a prospective cohort study characterized by all dead people being autopsied. The prevalence of all-causes, dementia and Alzheimer’s disease (AD) significantly increased over time. Diabetes-related factors, such as fasting glucose, 2-hours post-load plasma glucose, fasting insulin, and homeostasis model assessment of insulin resistance (HOMA-IR) were measured in 1988.\textsuperscript{26} The results suggest that hyperinsulinemia and hyperglycemia caused by insulin resistance accelerate Alzheimer’s disease in combination with the effects of APOE epsilon.

Excess weight, especially abdominal obesity, can cause or exacerbate cardiovascular and metabolic diseases. Obesity is also a proven risk factor for AD.

Various studies have demonstrated the beneficial effects of a ketogenic diet in weight reduction and in modifying the disease activity in neurodegenerative disorders, including AD.\textsuperscript{27,28} Compared with obese rats fed a control diet, obese rats fed a ketone diet showed significant weight loss, improvements in lipid profiles and insulin resistance, and up-regulation of adiponectin mRNA expression in adipose tissues. In addition,
the ketone diet triggered significant down-regulation of the expression of brain amyloid protein precursor, apolipoprotein E and caspase-3n, and improved brain oxidative stress responses. These findings suggest that a ketone diet has anti-obesity and neuro-protective effects.

Caprylic acid triglyceride is registered as a therapeutic food supplement apparently effective for the treatment of Alzheimer’s disease in the United States. Caprylic acid is a middle chain fatty acid (molecular formula C₈H₁₆O₂ with eight carbon atoms). The middle chain fatty acid can penetrate blood brain barrier, so it becomes substrate of beta oxidation, which increases the production of the derived ketone body in astrocytes.

Evidence suggests that energy production in the nervous system increases in parallel with symptomatic improvement. In addition, the protection of nerve cells involves adjustments in gene expression, as well as multi-inflammatory, anti-oxidation, and anti-apoptosis mechanisms (later description). β-HB also improves cognitive functions by increasing brain perfusion.

Aging is associated with an increased susceptibility to hypoxic or ischemic damages and a decline in behavioral functions which may be due to attenuated adaptive and/or defense responses. Diet-induced ketosis could improve the behavioral performance of aged rats. For example, old Fischer rats were fed either standard or ketogenic (KG) diets for 3 weeks, and then exposed to hypobaric hypoxia. Cognitive function was measured using the T-maze and object recognition tests. KG diet significantly increased blood ketone levels in both young and old rats. In the aged rats, the KG diet improved cognitive performance under normoxic and hypoxic conditions. Capillary density and HIF-1α levels were elevated in the aged ketonic group, independently of hypoxic challenge. These data suggest that diet-induced ketosis may be beneficial in the treatment of neurodegenerative conditions.

**Pharmaceutical Mechanisms of β-HB Action**

**Inhibition of histone deacetylase:** Shimizu et al report that the ketone body β-HB is an endogenous and specific inhibitor of class I histone deacetylases (HDACs). Histone acetylation is a prominent epigenetic modification of the central nervous system that is unequivocally associated with an increase in the rate of gene transcription. Histone acetylation generally favors long-term memory. Histone acetylation is also amenable to pharmacological interventions, predominantly the use of histone deacetylase (HDAC) inhibitors (Figure 3). It has therefore spurred considerable interest as a putative target of cognitive enhancement.

Because of the ubiquitous presence of histone acetylation, HDAC inhibitors have great potential not only to treat cognitive impairment resulting from neuro-degenerative disorders, but also to serve as cognitive enhancers for the healthy ones. Gräff and Zsai reviewed the state of the art of HDAC inhibitors used as cognitive treatments or cognitive enhancers. They describe epigenetic priming as a new model for their mode of action, caution against their unsupervised usage, despite their overall great promise.

Recent evidence indicates that the inhibition of histone deacetylase (HDAC) protects the heart against myocardial injury and stimulates endogenous angiomyogenesis, even in the diabetic heart. Sodium butyrate (1%), a specific HDAC inhibitor, was added daily to the drinking water in streptozocin induced diabetic mice to inhibit HDAC activity. HDAC inhibition resulted in a significant functional improvement in STZ-injected diabetic mice. Likewise, HDAC inhibition attenuates cardiac hypertrophy, as evidenced by reduction of heart/tibia ratio and in areas of cardiomyocyte distribution. This was associated with reduced interstitial fibrosis, a decrease in capase-3 activity and apoptotic histochemical staining, but also with increased angiogenesis in diabetic myocardium. Notably, glucose

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**Figure 3:** Epigenetic activity as inhibitor of histone deacetylases leads to various effects. Activity as an inhibitor of histone deacetylases leads to various epigenetic modulations, associated with global histone hyperacetylation, induction of stress response gene FOXO leads to reactive oxygen species detoxification, apoptosis, etc.
transporters (GLUT) 1 and 4 were up-regulated following HDAC inhibition, which was accompanied with increases of GLUT1 acetylation and p38 phosphorylation. Furthermore, myocardial superoxide dismutase, an important antioxidant, was elevated following HDAC inhibition in the diabetic mice.

Class I HDAC inhibition elicits the protection of contractile function following ischemia reperfusion. The study highlights the need for the development of new strategies that target specific HDAC isozymes in cardiac insufficiency.36

In the heart, the enhancement of lysine acetylation or SUMOylation using HDAC inhibitors or SUMO-1 gene transfer respectively, has been shown to be cardio-protective.37 The treatment of cardiomyocytes and cardiac fibroblasts with pharmacological inhibitors of HDAC catalytic activity robustly increased the conjugation of SUMO-1 with several high molecular weight proteins in both cardiac cell types. The use of a battery of selective HDAC inhibitors and short hairpin RNAs demonstrated that HDAC2 is the primary HDAC isoform that controls cardiac protein SUMOylation.

**Stimulation of FOXO:** The fast controls growth hormones, insulin and IGF-1 serves as signals in the transduction system and causes the acetylation of histones in the promoter domain of the FOXO3 gene, inducing the expression of FOXO3.38 It induces resistance to stress by raising the transcription activity of FOXO.

A transcription factor called FOXO3 is activated at the time of starvation. FOXO3 is able to raise the resistance to oxidation stress and starvation stress. FOXO is a transcription factor belonging to the subgroup “O” of the Forkhead family, interacting DNA-binding domain FOX (Forkhead box) by abbreviation of “Forkhead box O”.

In addition, β-HB acts in GPR109A developing in a fat cell and macrophage, which leads to improve arteriosclerosis (Figure 4). The action increases the expression of antioxidant enzymes, such as SOD or catalase, protecting myocardium against oxidation injury.

**Resistance to oxidative stress:** Concentrations of acetyl-coenzyme A and nicotinamide adenine dinucleotide (NAD (+)) affect histone acetylation and thereby couple cellular metabolic status and transcriptional regulation. The administration of exogenous β-HB, or fasting or calorie restriction, two conditions associated with increased β-HB abundance, all increased global histone acetylation in mouse tissues.36,37 The inhibition of HDAC by β-HB was correlated with pleiotropic changes in transcription, including transcription of genes encoding oxidative stress resistance factors FOXO3A and MT2. The treatment of cells with β-HB increased histone acetylation at the FOXO3A and Mt2 promoters, and both genes were activated by selective depletion of HDAC1 and HDAC2. Consistent with increased FOXO3A and MT2 activity, the treatment of mice with β-HB conferred substantial protection against oxidative stress.

**Protection of mitochondria:** It is known that the improvement of the oxidative phosphorylation within mitochondria is important for the cell protection of nerve cells from injury and the reinforcement of cognitive functions. In neuronal energy metabolism, mitochondrial function is important for the treatment of dementia, such as Alzheimer’s disease and other

![Figure 4: Signal transduction by β-HB by cell surface receptors and activation of genes.](http://dx.doi.org/10.17140/DROJ-2-125)
neurodegenerative diseases.

Zang et al.\textsuperscript{39} reported that the mechanism whereby β-HB methyl ester (HBME), becoming β-HB of the ketone body by ingestion in the body, protects mitochondria.

Alzheimer’s disease (AD) is caused by multiple mechanisms, including a decrease in the cellular utilization of glucose, and mitochondrial alterations in brain cells. HBME inhibits cell apoptosis under conditions of glucose deprivation, and it rescues the activities of mitochondrial respiratory chain complexes that are impaired in AD patients. HBME stabilizes the mitochondrial membrane potential. AD mice treated with HBME demonstrated that HBME has a positive \textit{in vivo} pharmaceutical effect to improve the spatial learning and working memory of mice. A reduction in amyloid-β deposition in mouse brains after intra-gastric administration of HBME was also observed.

When the concentration of blood β-HB was raised between 0.6-1.5 mM in the mice by giving fast and direct β-HB, it was confirmed that the acetylation of histones increases in multiple organs, including the kidney.

\textbf{Stimulation of adiponectin release:} Niacin (nicotinic acid) has recently been shown to increase serum adiponectin concentrations in men with the metabolic syndrome. Since niacin appears to exert its effects on lipolysis through receptor (GPR109A)-dependent and -independent pathways, the role of the identified GPR109A receptor in adiponectin secretion is noteworthy.\textsuperscript{40} As niacin administration had no effect on adiponectin and NEFA concentrations in the GPR109A receptor knockout mice, the GPR109A receptor plays an important role in the dual regulation of adiponectin secretion and lipolysis. β-HB has the similar effects on GPR109A receptor.

\textbf{Suppression of inflammasome:} It becomes clear that the inflammasome plays an important role in the onset and the progress of type 2 diabetes, Alzheimer’s disease, arteriosclerosis and inflammatory diseases, in addition to a number of autoimmune diseases. The inflammasome activates inflammatory caspase and cyto-kines of the IL-1 family within a complex of proteins involved in inflammation and apoptosis.\textsuperscript{38} The inflammasome has been seen as a natural immunity system that protects living organisms against alien substances and pathogenic microorganisms.

Prolonged fasting reduces inflammation. However, we do not know what effect on the innate immune response result from ketones, and other alternative metabolic fuels produced during energy deficits. β-HB, contrary to AcAc and the structurally related butyrate and acetate, suppresses the activation of the NLRP3 inflammasome in response to urate crystals, ATP and lipotoxic fatty acids. Mechanistically, β-HB inhibits the NLRP3 inflammasome by preventing K\textsuperscript{(+)} efflux and by reducing ASC oligomerization. The inhibitory effects of β-HB on NLRP3 are not dependent on chirality or starvation-regulated mechanisms like AMP-activated protein kinase (AMPK), reactive oxygen species (ROS), autophagy or glycolytic inhibition. β-HB reduces NLRP3 inflammasome-mediated interleukin IL-1β and IL-18 production in human monocytes. The anti-inflammatory effects of caloric restriction or ketogenic diets may be linked to β-HB-mediated inhibition of the NLRP3 inflammasome.\textsuperscript{41}

\textbf{FUTURE PROBLEM}

A new therapeutic approach could merge for the treatment of cancer.\textsuperscript{42-47} Metformin is usually used for the treatment of type 2 diabetes. Recently, metformin, vitamin D and ketone in combination showed broad-spectrum antitumor activity.\textsuperscript{48} In combination, metformin and vitamin D exhibited synergistic effects on cancer cell proliferation and apoptosis. The underlying anti-tumor mechanisms may involve m-TOR related pathways, which are related to activating expression of cleaved caspase-3, Bax and p-AMPK.

However, many problems remain. Humans have no experience to delete carbohydrate from meal at all, and high protein and lipid intake should be inevitable to compensate total energy expenditure. The balance of these effects should be studied, but notably original ketogenic diet for epilepsy by Russel Wilder in 1920 contained nearly 90% fat. High protein diet is a risk factor of cardiovascular diseases, renal insufficiency and cancer.\textsuperscript{49} So, the balance of risk and benefits should be considered.\textsuperscript{50}

Finally, the interaction with intestinal microbiota should be clarified. We have previously hypothesized that \textit{bifidobacterium} contribute to produce β-HB.\textsuperscript{5} There are many bacteria that can synthesize poly(3-hydroxy butyrate-co-3 hydroxyvalerate) oly-beta hydroxyl butyrate.\textsuperscript{51} These bacteria are capable of using a broad range of carbon sources for their growth and for the production of polyhydroxyalkanoates (PHAs). They can use monosaccharides (glucose and fructose), disaccharides (sucrose), pentoses (xylose and arabinose), various organic acids (acetic acid, propionic acid and octanoic acid) and even the acid pre-treated liquor (APL) of sugarcane trash, a lignocellulosic biomass, for growth and the production of polyhydroxyalkanoates (PHAs).

The contribution of intestinal microbiota to ketone body production should open a new field of medicine.

\textbf{ACKNOWLEDGEMENT}

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\textbf{CONFLICTS OF INTEREST}

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be constructed as a potential conflicts of interest.
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Unexplained Hypoglycaemia and Large Glycaemic Variability: Skin Lipohypertrophy as a Predictive Sign

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ABSTRACT

Rationale and Aims of the Study: Strong efforts to keep DM under control cause frequent hypoglycaemic events (Hypos) with relevant clinical, social and economic consequences. Moreover, long-lasting rebound hyperglycaemia and patients’ poor compliance or exaggerated corrective behaviors cause high glucose variability (GV). Several Hypos are unexplained and might be due to inappropriate insulin administration. In fact, the reported association between lipohypertrophy (LH) and Hypos might depend on poor education. The purpose of this study was to assess whether LH rate might predict Hypos and GV in insulin treated patients.

Methods: This was an observational, retrospective, multicentre study involving 387 DM patients referred to specialized settings for Hypos and/or GV carefully performing self-monitoring blood glucose (SMBG) and filling in a structured Hypo questionnaire.

Results: Twenty-eight percentages had at least one severe and 72% one non-severe episode (average: 3.3 Hypos/week). T1DM, long standing disease and high insulin doses were significantly associated with LH and impressive odds ratio (ORs) were reached by unexplained Hypos/GV (UHGV; \( p < 0.001 \)). The presence of LH predicted about 77.1% of UHGV, which displayed a 4.38 times higher risk when LH was combined with low socio-economic level, loneliness, poor metabolic control, disease complications and inappropriate injection technique (longer/larger and/or reused needles, missed injection site rotation). In multivariate analysis, the latter was still strongly associated with UHGV.

Conclusions: In the presence of UHGV, injection sites should be systematically explored in search of LH areas. Furthermore, appropriate educational activities should be implemented to improve patients’ behavior, including periodical verification of their injection habits.

ACKNOWLEDGEMENTS

*AMD is the Italian acronym for the Association of Diabetes Specialists and OSDI is the Italian acronym for Diabetes Care Health Professionals.

KEYWORDS: Unexplained hypoglycaemia; Hypoglycaemia; Glucose variability; Lipohypertrophy; Diabetes.


INTRODUCTION

The main goal of diabetes treatment is to maintain an adequate metabolic control in order to prevent or delay disease complications. However, when too strong efforts are made to keep glycated haemoglobin (HbA1c) within target levels, patients often are at a high risk for hypoglycaemia. Hypoglycaemic episodes (Hypos), especially severe ones, have a relevant clinical, social and economic impact. From a clinical standpoint, they are characterized by several symptoms ranging from general discomfort to seizures and coma eventually resulting into either sudden cardiac arrhythmic death or lethal brain damage due to long-lasting cerebral tissue glucose deprivation. Repeated severe Hypos are associated with a higher cardiovascular risk, and dementia. Even mild symptomatic hypoglycaemia is associated with an increased risk of cardiovascular events and all-cause hospitalization/mortality.

Hypoglycaemia is also a key driver for direct and indirect costs. In greater detail, up to one third of people with long standing type 1 diabetes mellitus (T1DM) and one fifth of insulin treated people with type 2 diabetes mellitus (T2DM) suffer from one or more severe Hypos every year. This generates a high economic burden in terms of both direct (>3000 € each hospital admission all over Europe) and indirect costs (mostly due to patients or their relatives abstaining from work).

Moreover, from a social point of view, several studies suggest that Hypos may disrupt quality of life (QoL) in terms of driving efficiency, work performance and recreational pursuits and thereby negatively affect patient’s mood and health perception. In addition, Hypos are by far the strongest factors hindering metabolic control in insulin-treated patients as long-lasting hyperglycaemia invariably follows Hypos due to rebound counter-regulatory hormone reaction and to previous frightening experiences causing excessive corrective behaviour and poor adherence to therapy. As a consequence of that high glucose variability (GV) is also often observed. Iatrogenic causes explain nearly all Hypos in people with T1DM. Predictable triggers (i.e. skipped meals, strenuous exercise, insulin overdose, neoplasms and chronic renal or liver disease), as well as, defective glucose counter-regulation and established hypoglycaemia-associated autonomic failure have been extensively described.

On the other hand, many Hypos are difficult to explain on the basis of the above listed factors and might also depend on improper insulin administration. This includes either accidental intramuscular injection through excessively long needles or bad patients’ habits, like shooting insulin into skin nodules. Diabetologists spend too little time in educating patients to perform correct insulin injections and, as a consequence of that, several groups have been describing a close relationship between recurrent hypoglycaemia and lipohypertrophy (LH) so far. In fact, patients and clinicians are not used to systematically and thoroughly investigate upon any possible reasons behind unexplained Hypos.

The main purpose of this study was to assess whether LH occurrence (Figure 1) might predict a high Hypos or GV rate in insulin treated patients and, if so, whether high Hypo rates might depend on inappropriate injection habits like performing insulin shots into LH nodules with long needles.

PATIENTS AND METHODS

This was an observational, retrospective, multicentre study on T1 or T2DM patients referring to five diabetes outpatient centres.

Eligibility criteria were: age ≥18 years, insulin treatment (>2 daily shots) for at least the last 12 months, as well as, frequent unexplained Hypos and/or unjustified GV over the last three months.

Exclusion criteria: Hospitalization, use of insulin syringes, oral hypoglycaemic agents or steroids, neoplastic, liver or kidney diseases, pregnancy.

The study was approved by the Ethics Committee of the five participating centres and all patients agreed to participate by signing their informed consent to the study. The latter was performed according to the Helsinki Declaration.
All enrolled patients filled in the HYPOS-1 questionnaire to collect information on Hypos or GV as previously described.31,32 Briefly, the questionnaire investigated upon socio-demographic and clinical characteristics including age, gender, education level, employment, living status, spouse/caregiver support, number of daily medications (other than blood glucose lowering agents), number of severe hypoglycaemic episodes during the past 12 months, and number of symptomatic Hypos in the past 4 weeks. The above mentioned time intervals were chosen according to the ability of insulin treated subjects to recall such episodes as ascertained in previous studies.23 Study investigators completed a web-based electronic clinical record form (eCRF) where patients were identified by a unique ID number, which was also used as a key linkage to merge their data with questionnaire answers and to anonymously analyse them thereafter.

The evaluation-validation of Hypos and GV was based on the analysis of patients’ self-monitored blood glucose (SMBG) recordings using a twice-a-day “staggered” scheme as depicted in Figure 2. This pattern is characterized by high flexibility, which makes it better tolerated and accepted with consequent greater patient adherence to given instructions, and is strongly supported by specialists, as well, because of its easier and immediate interpretation.24 Patients were also trained to perform additional glucose checks in case of sudden hypoglycaemic symptoms.

In greater detail:

1. Hypo was defined as the occurrence of one or more symptoms of hypoglycaemia (such as palpitations, tiredness, sweating, hunger, dizziness and tremor) and a confirmed blood glucose (BG) meter reading of ≤70 mg/dL, according to ADA statements.15 Frequent unexplained hypoglycaemia was defined as having a Hypo at least once a week in the absence of any identified precipitating event, such as changes in insulin dosage, diet composition or amount of physical activity. Hypos were further defined as severe (SH; BG<50 mg/dl), and non-severe (NSH; 50th>BG<70 mg/dl).33-35

2. GV was investigated by a validated questionnaire already used in previous studies.28,31,36 As GV has no universally accepted definition, patients were defined as having GV if blood glucose values unpredictably and inexplicably swung from <60 mg/dL to >250 mg/dL on a continuous basis at least three times a week.28,31,36

3. LH was looked for at all insulin injection sites using a previously described validated methodology: when filling in their questionnaires patients also indicated all sites they used16 and the medical staff checked them for the presence of skin lesions according to a structured protocol, as previously described.37,38

Participating centres screened 3710 consecutive ≥18 year old outpatients treated with insulin pens for at least two years and free from any kind of (i) steroid-based treatment regimens, (ii) chronic kidney/liver disease or (iii) cancer. Two thousand three hundred out of them (62%) had experienced at least one episode of either SH or NSH over the last three months. After excluding all cases associated with factors known to cause hypoglycaemia, including dietary errors, inadequate insulin dosage, poor treatment adherence, occasional utilization of drugs endowed with intrinsic hypoglycaemic effects, acute gastrointestinal troubles, only 387 subjects (16.8%) with unexplained GV and/or Hypos were finally included in the study: 294 had both Hypos and GV, 23 only Hypos and 70 only GV. In line with the typical distribution among people with diabetes attending such centre, 80 out of the 387 enrolled subjects had T1DM.

Main clinical participant characteristics are described in Table 1, whereas needle length and gauge are summarized in Table 2. Participants using premixed or NPH insulin (9.3% and 2.8%, respectively) or 12 mm length and 29 gauge needles (1.8% each) turned out to be only a minority and were therefore excluded from subsequent statistical evaluation to avoid any bias due to treatment in homogeneity. No patient used any 33 gauge needles.

**Statistical Analysis**

Continuous variables were reported as mean±standard deviation (SDs) and were compared between groups by student’s t-test for independent samples or analysis of variance (ANOVA) as needed. Non-parametric Mann-Whitney U-test was used when
appropriate. Categorical variables were summarized as rate or percentage and their bivariate associations were evaluated by Chi-square or Fisher exact tests. Based on the fact that a significant association had been already documented in the Hypos-1 study, among the presence of SH/NSH and diabetes complications, type of insulin preparation or treatment with drugs associated with reduced Hypo awareness including angiotensin-converting-enzyme inhibitor (ACE inhibitor) or beta-blockers, we also evaluated the relationship between LH lesions and these factors based on univariate followed by Poisson multivariate model analysis. Patients were grouped and compared according to their LH identification rate characteristics, namely by considering those identified by all health providers (HPs) vs. those identified by none of them. Odds ratios (ORs) for LH were reported along with their 95% confidence intervals (CI). In addition, the relationship between needle length/gauge and the presence of LH was also evaluated according to the same statistical procedure by taking into account school education, employment and marital status or living conditions. The analysis were carried out using STATA software, version 12 (Stata Corp, College Station, TX, USA) and p-values <0.05 were accepted a priori as statistically significant.

RESULTS

General patient characteristics are described in Table 1. Twenty-eight percentages subjects had faced at least one SH, and 72% one NSH in the last four weeks, with an average of 3.3 episodes per week. Table 2 describes needle utilization rate as referred to length and gauge and shows that lengths >4-5 mm and gauges <32 were largely preferred. Table 3 clearly describes the relationship between unexplained GV/Hypos and LH (p<0.0001), characterized by a significantly high OR (7.18) for GV/Hypos in case of LH.

Tables 4 and 5 describe the results of univariate analysis by comparing subjects with LH to those without LH regardless of general/demographic parameters, insulin treatment features, injection site rotation habits, as well as, needle reuse rate, utilization of drugs associated with reduced hypoglycaemia awareness, employment, school education, living conditions and marital status. In greater detail, 40% patients used the same needle at least twice and 44.2% at least three times; only 15.8% subjects changed needles at each injection. Tables clearly show that needle reuse and failure to rotate injection sites associated to a high LH risk.

T1DM, long standing disease and high insulin requirements significantly associated to LH, and very high ORs were attained by unexplained Hypos (4.43), GV (5.23) or both (7.18). The risk of diabetes complications was significantly higher in subjects with LH (retinopathy 4.12; nephropathy 4.19; lower limb complications 4.48; sensory-motor neuropathy 5.18, and autonomic neuropathy 3.72) (Figures 3 and 4). Any use of drugs associated with reduced hypoglycaemia awareness turned out to be irrelevant.

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</tbody>
</table>

Table 1: Characteristics of insulin pen using patients.

<table>
<thead>
<tr>
<th>length (mm)</th>
<th>Subjects n. (%)</th>
<th>Gauge</th>
<th>Subjects n. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>58 (14.9)</td>
<td>33</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>61 (15.8)</td>
<td>32</td>
<td>58 (14.5)</td>
</tr>
<tr>
<td>6</td>
<td>115 (29.7)</td>
<td>31</td>
<td>176 (45.5)</td>
</tr>
<tr>
<td>8</td>
<td>146 (37.7)</td>
<td>30</td>
<td>146 (37.7)</td>
</tr>
<tr>
<td>12</td>
<td>7 (1.8)</td>
<td>29</td>
<td>7 (1.8)</td>
</tr>
</tbody>
</table>

Table 2: Needle length and gauge.
Table 3: Association between unexplained glycaemic variability (GV)/Hypoglycaemic episodes (Hypos) and lipohypertrophy (LH); n and (%) are displayed within cells (χ² test, p<0.0001).

<table>
<thead>
<tr>
<th>LH</th>
<th>GV/Hypos</th>
<th>Yes n=298 (77.1%)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>yes</td>
<td>287 (74.2%)</td>
<td>1.20 (0.88-1.64)</td>
<td>0.243</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>7 (1.8%)</td>
<td>0.90 (0.81-1.00)</td>
<td>0.051</td>
</tr>
<tr>
<td>total</td>
<td></td>
<td>294 (94%)</td>
<td>0.99 (0.96-1.02)</td>
<td>0.492</td>
</tr>
</tbody>
</table>

Table 4: Univariate analysis of clinical parameters with respect to lipohypertrophy in insulin pen using patients.

From 387 subjects with hypoglycaemia and/or glycaemic variability, 23 were Hypos- and 70 were GV-. Because of the low number of subjects using premix or NPH insulin, these were not included in the statistical evaluation.

Table 5: Univariate analysis of needle length and gauge with respect to lipohypertrophy in insulin pen using patients.
With reference to the socio-economic status, the level of education did not affect the association between Hypos and LH. The same applied to living conditions: in fact no significant association was found between Hypos and LH in those who lived alone, or were unmarried/separated/divorced.

Finally, as shown in Figures 5 and 6, the risk of developing LH was associated with needle characteristics: both longer and larger needles were related to a higher LH risk ($p<0.001$). Needle reuse and missed injection site rotation were also LH risk factors strongly associated with unexplained Hypos /GV.

After multivariate analysis (Table 6) short-acting and basal insulin analogues kept highly associated with increased LH risk in T1DM patients with unjustified Hypos /GV.

When pooling socio-economic and family statuses under the term of living conditions and putting together all diabetes complications, we could also confirm both complex parameters to be strongly associated to LH and GV/Hypos. Likewise, needle length/gauge, needle reuse and missed injection site rotation kept their significant role as risk factors for LH in the presence of unexplained GV/Hypos. This was not the case, instead, with either disease duration or daily insulin dosage.

DISCUSSION

Hypoglycaemia is an acute complication increasing diabetes morbidity and mortality. It is also responsible for a heavy—still not fully defined-economic burden. However it is by far the strongest limiting factor in terms of tight glycaemic control in insulin-treated patients. Hypoglycaemia-associated autonomic neuropathy, as well as his diseases, can certainly play a role.
nomic failure.\textsuperscript{21-23}

Nevertheless, apparently inexplicable hypoglycaemic episodes may be related to incorrect insulin injection techniques including the use of long needles—eventually allowing insulin injection into the subcutaneous muscle tissue—or patients’ incorrect habit to inject insulin into LH nodules.\textsuperscript{25-27}

In the series of patients in the Hypos-1 study\textsuperscript{32,33} a high rate of SHs, NSHs and GV was reported (28%, 70% and 56%, respectively). Accordingly, our data documented an overall 62% Hypos rate, 16.8% in fact being inexplicable and showing a strong correlation with the presence of LH, as well as, with all known associated factors.

The profile of people with diabetes displaying inexplicable Hypos/GV included the presence of LH nodules, as well as, poor metabolic control, high micro-/macrovascular complication rates, longer/larger needles, missed injection site rotation, low socio-economic level and solitary life. The risk for LH to be deemed as responsible for unexplained Hypos or GV rose to 4.38 in the case of simultaneously occurring above mentioned factors and LH.

Conversely, the presence of LH was able to predict about 77.1% of unexplained Hypos/GV. Indeed, factors known to be associated with LH were also present at a high rate in subjects with such phenomena.

However, the remaining 22.9% people without any LH nodules experienced unexplained Hypo/GV too. It might depend on the fact that identifying subtle skin lesions is very difficult so that “no identified LH” is not equivalent at all to “absence of any LH”. In fact, we feel like stressing what was already pointed out by other authors\textsuperscript{28,31,36}: education on injection techniques is still inadequate and diabetes specialists and dedicated nurses should make their best to prevent patients from causing themselves any skin damage through wrong habits.

Our conclusion then is that, in the presence of unexplained Hypos/GV, injection sites should be systematically and thoroughly explored in search of LH areas. Furthermore, appropriate educational activities should be implemented to improve patients’ behaviour, including periodical investigation on their injection habits.

A recent meta-analysis of population based studies\textsuperscript{42} including 46 publications (n=532, 542) found Hypos to occur quite frequently among people on insulin: mild/moderate events were in fact about 50% with an incidence of 23 episodes per person-year while severe episodes were about 21% with an incidence of 1 event per person-year.

Should we ideally select people with unexplained Hypos from the extremely large number of patients mentioned above, we would deal with an impressive cohort: in that case the systematic utilization of a suitable LH identification protocol followed by patient rehabilitation would be expected to dramatically reduce Hypo prevalence, incidence and health consequences, as well as, the costs related to this frightening, yet neglected, complication of insulin treatment.

Further studies are warranted to assess whether educational activities like those reported in our study may result into a decreased burden of diabetes as for unexplained hypoglycaemia.

**CONFLICTS OF INTEREST**

The authors declare that they have no conflicts of interest.

**REFERENCES**

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