Are insulin analogues an unavoidable necessity for the treatment of type 2 diabetes in developing countries? The case of Jordan

D. Hyassat,[1] N. Al Shekarchi,[1] H. Jaddou,[2] M. Liswi,[1] M. El-Khateeb[1] and K.M. Ajlouni[1]

هل تعتبر نظائر الإنسولين ضرورة لا مفر منها لمعالجة السكري من النمط 2 في البلدان النامية؟ حالة الأردن

دانا حياصات، نزهت الشكرجي، هاشم جدوع، محمد لصوي، محمد الخطيب، كامل العجلوني

الخلاصة: بصرف النظر عن الفوائد المعروفة عن نظائر الإنسولين من حيث ضبط سكر الدم فإنها تعتبر باهظة الثمن بالنسبة للمرضى في البلدان النامية. وقد هدفت هذه الدراسة التي أجريَت في الأردن إلى مقارنة الفاعلية والمضاعفات بين الإنسولين البشري مسبق المزج (BHI30) ونظائر الإنسولين مسبق المزج (BIAsp30) لدى المرضى المصابين بالسكري من النمط 2. ففي دراسة أترابية استعادية – من أكتوبر/ تشرين الأول 2012 إلى مارس/ آذار 2013 – تمت مقارنة نتائج قياسات (HbA1c والوزن ونقص سكر الدم وتضخم الدهون عند بداية الدراسة وبعد 6 أشهر من معالجة 628 مريضاً. فنتج عن استخدام BHI30 انخفاض في HbA1c أكبر بكثير مما نتج عن BIAsp30. وبقي هذا الفارق في HbA1c كبيراً بعد ضبط تأثيرات العمر والجنس ومدة الإصابة بالسكري ومؤشر كتلة الجسم ونقص سكر الدم (كان معامل– بيتا 0.18 لصالح BHI30). وزيادة قليلة للوزن والانخفاض الطفيف لسكر الدم باستعمال BHI30 أعلى بكثير مما كان عليه باستعمال BIAsp30 الفرق ذا دلالة إحصائية. لقد حقق BHI30 تخفيضاً أفضل لـ HbA1c مقارنة مع BIAsp30، مع تكلفة أقل، وزيادة قليلة للوزن، بالإضافة إلى زيادة في إبلاغ المرضى عن الانخفاض الطفيف في سكر الدم.

ABSTRACT Despite their reported benefits in terms of glycaemic control, insulin analogues are expensive for patients in developing countries. This study in Jordan aimed to compare the effectiveness and adverse events of premixed human insulin (BHI30) versus premixed insulin analogue (BIAsp30) in patients with type 2 diabetes. In a retrospective cohort study from October 2012 to March 2013, outcomes (HbA1c, weight, hypoglycaemia and lipohypertrophy) were compared at baseline and 6 months after treatment in 628 patients. BHI30 produced a significantly greater reduction in HbA1c than did BIAsp30. This difference in HbA1c remained significant after controlling for the effects of age, sex, duration of diabetes, body mass index and hypoglycaemia (β-coefficient was –0.18 in favour of BHI30). Weight gain and mild hypoglycaemia was significantly higher with BHI30 than with BIAsp30. BHI30 achieved better reduction in HbA1c compared with BIAsp30, with less cost, slightly more weight gain and greater reported mild hypoglycaemia.

Les analogues de l'insuline sont-ils une nécessité inévitable pour le traitement du diabète de type 2 dans les pays en développement ? Le cas de la Jordanie

RÉSUMÉ En dépit des avantages rapportés en termes de contrôle de la glycémie, les analogues de l'insuline sont coûteux pour les patients des pays en développement. La présente étude en Jordanie visait à comparer l'efficacité de l'insuline humaine prémélangée (BHI30) à celle de l'analogue de l'insuline prémélangé (BIAsp30) ainsi que les événements indésirables pour deux substances chez des patients atteints de diabète de type 2. Dans une étude de cohorte rétrospective menée d'octobre 2012 à mars 2013, les résultats (l'hémoglobine glycosylée$_{1c}$, le poids, l'hypoglycémie et la lipohypertrophie) ont été comparés au début de l'étude, puis six mois après le traitement chez 628 patients. Le traitement par BHI30 a entraîné une réduction très supérieure de l'HbA$_{1c}$ par rapport au BIAsp30. Cette différence dans le taux d'HbA$_{1c}$ est restée importante après la correction pour les effets de l'âge, du sexe du patient et de la durée du diabète, de l'indice de masse corporelle et de l'hypoglycémie (le coefficient-β était de – 0,18 en faveur du BHI30). La prise de poids et l'hypoglycémie légère étaient nettement supérieures sous BHI30 que sous BIAsp30. Le traitement par BHI30 a permis une réduction plus importante de l'HbA$_{1c}$ par rapport au traitement par BIAsp30, à un coût moindre, avec une prise de poids légèrement supérieure et un taux d'hypoglycémie légère plus important.

[1]National Center for Diabetes, Endocrinology and Genetics, Amman, Jordan (Correspondence to K.M. Ajlouni: ajlouni@ju.edu.jo). [2]Department of Epidemiology and Public Health, Jordan University of Science and Technology, Amman, Jordan.

Received: 06/09/14; accepted: 13/05/15

Introduction

Insulin has proven to be the most effective anti-diabetic agent in the past century. The United Kingdom Prospective Diabetes Study showed that beta-cell failure is progressive; 53% of patients with type 2 diabetes mellitus initially treated with sulfonylurea required insulin therapy after 6 years and about 80% required insulin after 9 years (*1–3*). Previous studies have concluded that the risk of onset and progression of diabetes-related complications such as retinopathy, nephropathy, neuropathy, cardiovascular disease and stroke can be considerably reduced once a sustained reduction of glycosylated haemoglobin (HbA1c) is achieved (*4,5*). These observations show the importance of intensive and strict glycaemic control starting at the time of diagnosis of diabetes.

Although most clinical trials demonstrate at least equivalent efficacy of analogues relative to human insulin, with additional benefits in terms of better postprandial glycaemic control, flexible injection timing and improvement in adherence (*6–10*), many of these trials did not consider the ethnic variations and the difference in eating habits of the study population. For instance, people in the Middle East, including Jordanians, usually have 3 main meals a day, with a relatively high calorie intake at lunch. For this reason conventional insulin administration twice a day—in the morning and evening—will not result in adequate glycaemic control and may indicate the necessity of a third dose before lunch to improve postprandial glucose and HbA1c levels. On the other hand, insulin analogues incur a considerably greater financial cost in comparison with human insulin. This study aimed to show whether insulin analogues could be an unavoidable necessity for the treatment of diabetes mellitus in developing countries such as Jordan.

Jordan is considered one of the smallest economies in the Middle East. The country is very poor in natural resources such as oil, gas and water. The recent waves of migrants and asylum-seekers from neighbouring countries to Jordan have put additional economic and social burdens on Jordan's economy. According to 2012 official figures, the country faces chronic and increasing levels of budget deficit (11.4%), unemployment (12.2%), inflation rate (4.7%) and poverty (*11*). Furthermore, the public debt increases every year and has reached more than 65% of the country's total gross domestic product. The purchasing power of Jordanian citizens is declining. In addition, the average annual income in 2012 was US$ 4850 and therefore two-thirds of diabetic patients in Jordan could not perform even a single daily testing of blood sugar, given that a single pack of the glucose strips costs around US$ 35. This implies that the patient needs around 1 pack every 17 days and 21 packs every year. The cost of these packs constitutes 15% of Jordanians' average annual income. The cost of insulin, whether human or analogue, is another critical factor. As will be shown later in this paper, the cost of BHI30 is around US$ 31 per month and that of BIAsp30 is around US$ 75 per month. The difference in cost between these 2 alternatives is US$ 525 per patient per year, accounting for an additional 10% of a Jordanian citizen's annual income. These figures sound huge, and necessitate seeking other, less expensive, alternatives. We believe that the cost issue is not unique to Jordan, but a major concern for many other low- and middle-income countries.

The objectives of this study were to compare the effectiveness of premixed human insulin (BHI30) with premixed insulin analogue (BIAsp30) on the reduction of HbA1c in patients with type 2 diabetes mellitus; and to identify the extent of certain adverse events related to the use of human and insulin analogues, such as weight gain, hypoglycaemia and lipohypertrophy.

Methods

Sampling

A retrospective cohort study was carried out at the National Centre for Diabetes Endocrinology and Genetics in Amman, Jordan, during the period from 1 October 2012 to 1 March 2013. The study was approved by the Centre's ethics committee. Information was kept strictly confidential and the data were used only for the purposes of this study.

A list of all patients who had their prescriptions of BHI30 or BIAsp30 insulin dispensed from the Centre's pharmacy during the year 2011 was obtained electronically. In our study both BHI30 and BIAsp30 were administered 3 times daily and all patients received metformin treatment as long as the glomerular filtration rate allowed.

The medical files of those patients were reviewed and all patients who were 18 years old or older, had started BHI30 or BIAsp30 insulin at the Centre and had continued on this medication for at least 6 months were eligible to be included in the study. Pregnant women, those in stage 4 and 5 renal failure, with chronic use of steroid medications and poorly compliant patients were excluded from the study. A total of 628 patients (327 on BHI30 and 301 on BIAsp30) were included in the study.

Data collection

The standard of care at our Centre requires regular follow-up visits for diabetic patients every 2–3 months and all patients receive metformin as long as glomerular filtration rate allows. Routine measurements of blood sugar, HbA1c, blood pressure, weight, waist circumference, urine examination for microalbuminurea, foot screening and fundoscopy are carried out on each visit. Patients on insulin therapy are usually asked about the presence of mild,

moderate or severe hypoglycaemia and are screened for lipohypertrophy at insulin injection sites by a diabetic educator nurse. There is no specific rule in prescribing insulin, whether human or analogue; the decision is left to the physician's preference and experience.

Information gathered from the medical records included: baseline data (date of starting BHI30 or BIAsp30 insulin, age, sex, occupation, smoking status, duration of diabetes, weight, height, waist circumference, systolic and diastolic blood pressure, hypertension medication, and HbA1c) and follow-up data at 6 months (weight, HbA1c, and information regarding certain adverse events of insulin treatment such as hypoglycaemia and lipohypertrophy).

Definitions

Diabetes was considered to be controlled if the patient had HbA1C level < 7.0% (*12*). HbA1c was measured using the ion-exchange high performance chromatography method (Bio-Rad Variant II Turbo HbA1c kit). HbA1c difference was the difference between the HbA1c value at the start date and HbA1c value at the end of the 6-month period.

Hypoglycaemia was diagnosed if the patient had experienced the classic symptoms (light headedness, severe hunger, palpitations, excessive sweating) regardless of blood sugar measurement, and if the patient recovered by ingestion of carbohydrates (simple sugars, juices or fruits). Hypoglycaemia was classified into mild (patient can manage it by him/herself), moderate (patient requires assistance from another person to administer carbohydrates or to take other action) and severe (patient needs hospitalization).

Lipohypertrophy was defined as tumour-like swelling of fatty tissue around the insulin injection sites. The presence of lipohypertrophy was determined by inspection and palpation of insulin injection sites by trained nurses in the clinic after initiation of insulin treatment.

Waist circumference was estimated at the end of a normal expiration using a non-stretchable tape held in a horizontal plane around the abdomen at the level of the iliac crest. Waist-to-height ratio was considered normal at ≤ 0.5, and elevated if it was > 0.5 (*13*). Body mass index (BMI) was expressed as the quotient between weight (kg) and height squared (m^2). Patients with BMI of 30 kg/m^2 or more were considered obese (*14*). Weight difference was the difference between weight at the starting date of insulin therapy and the value at the end of the 6-month period.

Hypertension was defined as systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 80 mmHg or if the patient was on antihypertensive drugs (*12*).

Metabolic abnormalities were defined according to the American Diabetes Association 2011 guidelines (*12*) as follows: total serum cholesterol ≥ 200 mg/dL (5.17 mmol/L), serum low-density lipoprotein cholesterol ≥ 100 mg/dL (2.59 mmol/L), serum triglyceride ≥ 150 mg/dL (1.70 mmol/L), serum high-density lipoprotein cholesterol ≤ 40 mg/dL (1.03 mmol/L) in men, and ≤ 50 mg/dL (1.29 mmol/L) in women, or if the patient was already on antidyslipidaemic agents.

Smoking was classified into non-smoker (never smoked), past smoker (used to smoke but stopped smoking) and current smoker (smoked cigarettes daily or occasionally) (*15*).

Statistical analysis

Analysis was carried out using *SPSS*, version 17. A *P*-value ≤ 0.05 was considered statistically significant. Frequency and percentage distribution was used for categorical variables, and means and standard deviations (SD) for continuous variables. Independent *t*-test was used to test for a significant difference of mean HbA1c difference and mean weight change in patients taking premixed human (BHI30) versus premixed insulin analogue (BIAsp30). Pearson chi-squared test was used to determine the significant difference of hypoglycaemia, lipohypertrophy and HbA1c control in patients taking premixed human (BHI30) versus premixed insulin analogue (BIAsp30). Multiple linear regression analysis was performed to examine the net effect of the mentioned types of insulin on mean difference of HbA1c after controlling for the effect of potential confounders.

Results

Participants' characteristics

A total of 628 patients with type 2 diabetes were studied: 301 on BIAsp30 and 327 on BHI30. As indicated in Tables 1 and 2, the 2 groups were comparable on most of the sociodemographic and health characteristics; however, the mean baseline HbA1c value was significantly higher among BHI30 users (Table 2).

Comparison of HbA1c and weight change, hypoglycaemia and lipohypertrophy between insulin groups

After 6 months of treatment with BHI30 insulin, the mean HbA1c dropped from 10.7% (SD 1.8%) to 8.6% (SD 1.6%), a decrease of 2.1% (SD 2.1%), while with BIAsp30 treatment HbA1c dropped from 9.7% (SD 1.7%) to 8.5% (SD 1.5%), a decrease of 1.2% (SD 1.7%). This difference between the BHI30 and BIAsp30 groups in terms of the decrease of mean HbA1c level was statistically significant ($P < 0.001$) (Table 3).

The baseline mean weight in the BHI30 was 83.7 (SD 16.5) kg and this increased to 86.8 (SD 15.6) kg after 6 months, a mean increase of 3.1 (SD 4.3) kg. In the BIAsp30-treated group the mean weight increased from 85.4 (SD

Table 1 Baseline distribution of anthropometric and clinical characteristics of patients with diabetes mellitus taking insulin analogue (BIAsp30n) or human insulin (BHI30)

Characteristic	BIAsp30n group (n = 301)		BHI30 group (n = 327)		P-value (χ^2-test)
	No.	%	No.	%	
Age (years)					
< 50	72	48.6	76	51.4	0.122
50–70	198	46.0	232	54.0	
> 70	29	61.7	18	38.3	
Sex					
Female	151	46.7	172	53.3	0.523
Male	150	49.3	154	50.7	
Occupation					
Unemployed	135	46.2	157	53.8	0.213
Employed	72	41.6	101	58.4	
Retired	58	52.3	53	47.7	
Duration of diabetes mellitus (years)					
< 5	36	50.0	36	50.0	0.391
5–9	72	46.8	82	53.2	
10–14	75	42.9	100	57.1	
15–19	44	48.9	46	51.1	
≥ 20	71	54.2	60	45.8	
HbA1c controlled					
Yes	7	87.5	1	12.5	0.031
No	294	47.4	326	52.6	
Hypertension present					
Yes	240	47.5	265	52.5	0.763
No	60	49.2	62	50.8	
Dyslipidaemia present					
Yes	273	47.5	302	52.5	0.054
No	22	64.7	12	35.3	
Smoking status					
Not smoker	79	44.1	100	55.9	0.748
Past smoker	27	50.0	27	50.0	
Current smoker	32	45.1	39	54.9	

SD = standard deviation; BMI = body mass index; HbA1c = glycosylated haemoglobin.

17.4) kg to 87.5 (SD 17.3) kg, a mean increase of 21. (SD 3.8) kg (Table 3). The mean difference in weight change between the 2 groups [1.0 (SD 0.3) kg]was statistically significant (P = 0.002) (Table 3).

During the treatment period, the percentage of patients who reported hypoglycaemia with BHI30 treatment was 29.7% compared with only 17.4% in the BIAsp30-treated group (P < 0.001) (Table 4). However, further analysis of the data indicated that only mild hypoglycaemia was significantly higher among the BHI30 group than the BIAsp30 group (P < 0.001), while there were no significant differences in moderate or severe hypoglycaemic attacks between the 2 groups (P = 0.71).

Lipohypertrophy was detected in 40.0% of patients treated with BHI30 compared with 31.9% of patients treated with BIAsp30, a difference which was not statistically significant (P = 0.28).

Further analysis of data was performed using multiple linear regressions to test for significant difference in the mean difference of HbA1c between the 2 groups after controlling for the effect of sex, age, duration of diabetes mellitus, BMI and hypoglycaemia. As indicated in Table 5, shifting patients from BHI30 to BIAsp30 was expected to reduce the mean difference in HbA1c level by 0.18 unit after 6 months of treatment (P < 0.001).

Table 2 Baseline mean values of key variables of patients with diabetes mellitus taking insulin analogue (BIAsp30n) or human insulin (BHI30)

Variable	BIAsp30n group (n = 301)	BHI30 group (n = 327)	P-value (t-test)
	Mean (SD)	Mean (SD)	
Age (years)	56.9 (11.2)	56.1 (9.2)	0.396
Duration of diabetes mellitus (years)	12.8 (7.5)	12.1 (6.9)	0.194
HbA1c level (%)	9.8 (1.7)	10.8 (1.8)	< 0.001
BMI (kg/m^2)	32.5 (6.6)	31.6 (6.1)	0.079

HbA1c = glycosylated haemoglobin; BMI = body mass index; SD = standard deviation.

Table 3 Mean glycosylated haemoglobin (HbA1c) and weight difference at baseline and after 6 months of treatment for patients with diabetes mellitus taking insulin analogue (BIAsp30n) or human insulin (BHI30)

Variable	BHI30 group (n = 327)	BIAsp30 group (n = 301)	P-value (t-test)
	Mean (SD)	Mean (SD)	
HbA1c level (%)			
Baseline	10.7 (1.8)	9.7 (1.7)	
6 months	8.6 (1.6)	8.5 (1.5)	
Change	–2.1 (2.1)	–1.2 (1.7)	0.001
Weight (kg)			
Baseline	83.7 (16.5)	85.4 (17.4)	
6 months	86.8 (15.6)	87.5 (17.3)	
Change	+3.1 (4.3)	+2.1 (3.8)	0.002

SD = standard deviation.

Discussion

The major findings in the present study were that premixed human insulin (BHI30) was superior to premixed insulin analogue (BIAsp30) in improving glycaemic control as indicated by the improvement in HbA1c level in patients with inadequately controlled diabetes. This finding differs from those reported in clinical trials conducted by Boehm et al. (*6*), Boehm et al. (*7*) and Abrahamian et al. (*8*), who reported no significant difference in mean HbA1c levels between premixed human insulin versus premixed insulin analogue and found better postprandial glycaemic control with premixed insulin analogue. This inconsistency is perhaps related to differences in the study designs, ethnic variations, lifestyle differences and/or to differences in the clinical characteristics of the study groups.

Table 4 Reported experience of hypoglycaemia during the follow-up period in patients with diabetes mellitus taking insulin analogue (BIAsp30n) or human insulin (BHI30)

Hypoglycaemia	BHI30 group (n = 255) [a]		BIAsp30 group (n = 235) [a]		P-value (χ^2-test)
	No.	%	No.	%	
Yes	76	29.7	41	17.4	< 0.001
No	179	69.9	194	82.6	

[a] *Data about hypoglycaemia were unavailable for 72 patients from the BHI30 group and 66 patients from the BIAsp30 group.*

It is known that glycaemic control, weight change and hypoglycaemic episodes are insulin dose-dependent. The research design in clinical trials requires patients to be randomly assigned to treatment and comparison groups in a controlled environment. The results from this design have high internal validity at the expense of generalizability. In field studies like ours, assignment of patients to treatment and comparison groups was based on physicians' preference after due consideration of the patients' sociodemographic characteristics, clinical status and laboratory results. This is especially true when we know that all the treating physicians in our study were endocrinology specialists. The findings from this study can perhaps be generalized to various sociodemographic groups, especially in Jordan, and possibly to other Arab countries.

Europeans and Arabs are not only ethnically different but they also differ in their lifestyles, especially their eating habits. Arabs usually have 3 main meals with a relatively high energy intake at lunch. For these people, conventional insulin administration twice a day—in the morning and evening—is not physiologically appropriate and results in a lack of insulin at lunchtime, followed by post-lunch and pre-dinner hyperglycaemia. Unlike clinical trials, in our study both BHI30 and BIAsp30 were administered 3 times daily. The results of our study are not in line with the data published from the PRESENT (*9*) and IMPROVE (*10*) studies, which

Table 5 Standardized beta coefficients and levels of significance of the predictor variables on mean difference in glycosylated haemoglobin level of the 2 groups of patients with diabetes mellitus on insulin analogue (BIAsp30n) or human insulin (BHI30)

Model	Standardized coefficients		*P*-value
	β	*t*	
(Constant)		7.876	< 0.001
Premixed	–0.175	–30.766	< 0.001
Female/male	–0.087	–10.796	0.073
Age	–0.039	–0.778	0.437
Duration of diabetes mellitus (years)	–0.110	–20.213	0.027
BMI	–0.239	–40.976	< 0.001
Hypoglycaemia	–0.102	–20.212	0.027

BMI = body mass index.

showed that switching type 2 diabetes patients from premixed human to premixed insulin analogue resulted in significant improvements in glycaemic control (the mean HbA1c reduction from the baseline was 1.84% in the IMPROVE study and 1.58% in the PRESENT study). Inconsistencies could be due to the difference in the study design, as one of the major limitations of previous studies was the absence of a comparison group, while the presence of comparison group in our study gives our finding more strength.

In our study, weight gain and reported hypoglycaemia were significantly higher with premixed human insulin (BHI30) than with premixed insulin analogue (BIAsp30) and this weight gain was expected especially with the improvement in glycaemic control, which was better with BHI30 than BIAsp30.

Study data have indicated higher rates of mild hypoglycaemia among patients receiving premixed human insulin (BHI30) compared with the premixed insulin analogue (BIAsp30), but no significant difference in the rates of moderate and severe hypoglycaemia. This finding is not inconsistent with Boehm et al.'s study, which reported that premixed insulin analogue was associated with a significantly lower incidence of major hypoglycaemia in comparison with premixed human insulin (*6*). Additionally, the IMPROVE study had also reported a reduction in hypoglycaemia upon shifting uncontrolled diabetes mellitus patients from premixed human insulin to premixed insulin analogue (*10*).

On the other hand, Boehm et al. (*7*) and the PRESENT study (*9*) reported no significant difference in weight gain between BHI30- and BIAsp30-treated patients, a finding which is inconsistent with our study results.

In conclusion, premixed human insulin (BHI30) achieved significantly better reduction in HbA1c levels compared with premixed insulin analogue (BIAsp30), with less cost at the expense of slightly more weight gain, and a greater reported mild hypoglycaemia. Insulin cost was calculated according to the baseline insulin dose per month which could be increased after the titration of insulin dose. The initial cost was approximately US$ 31 per month and US$ 75 per month for BHI30 and BIAsp30 respectively. Therefore, in low- and middle-income countries such as Jordan, premixed human insulin may be a good, affordable choice to achieve glycaemic control among diabetic patients in need of insulin.

Acknowledgements

Funding: None.

Competing interests: None declared.

References

1. United Kingdom Prospective Diabetes Study Group. United Kingdom Prospective Diabetes Study 24: a 6-year, randomized, controlled trial comparing sulfonylurea, insulin, and metformin therapy in patients with newly diagnosed type 2 diabetes that could not be controlled with diet therapy. Ann Intern Med. 1998 Feb 1;128(3):165–75. PMID:9454524
2. Wright A, Burden AC, Paisey RB, Cull CA, Holman RR, Group UKPDS; U.K. Prospective Diabetes Study Group. Sulfonylurea inadequacy: efficacy of addition of insulin over 6 years in patients with type 2 diabetes in the U.K. Prospective Diabetes Study (UKPDS 57). Diabetes Care. 2002 Feb;25(2):330–6. PMID:11815505
3. Turner RC, Cull CA, Frighi V, Holman RR; UK Prospective Diabetes Study (UKPDS) Group. Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies (UKPDS 49). JAMA. 1999 Jun 2;281(21):2005–12. PMID:10359389
4. The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med. 1993 Sep 30;329(14):977–86. PMID:8366922
5. Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med. 2005 Dec 22;353(25):2643–53. PMID:16371630
6. Boehm BO, Home PD, Behrend C, Kamp NM, Lindholm A. Premixed insulin aspart 30 vs. premixed human insulin 30/70 twice daily: a randomized trial in type 1 and type 2 diabetic patients. Diabet Med. 2002 May;19(5):393-9. PMID:12027927
7. Boehm BO, Vaz JA, Brøndsted L, Home PD. Long-term efficacy and safety of biphasic insulin aspart in patients with type 2 diabetes. Eur J Intern Med. 2004 Dec;15(8):496–502. PMID:15668084

8. Abrahamian H, Ludvik B, Schernthaner G, Prager R, Zellenka U, Knudsen L, et al. Improvement of glucose tolerance in type 2 diabetic patients: traditional vs. modern insulin regimens (results from the Austrian Biaspart Study).Horm Metab Res. 2005 Nov;37(11):684–9. PMID:16308837

9. Shestakova M, Sharma SK, Almustafa M, Min KW, Ayad N, Azar ST, et al. Transferring type 2 diabetes patients with uncontrolled glycaemia from biphasic human insulin to biphasic insulin aspart 30: experiences from the PRESENT study. Curr Med Res Opin. 2007 Dec;23(12):3209–14. PMID:18005503

10. Shah S, Benroubi M, Borzi V, Gumprecht J, Kawamori R, Shaban J, et al.; IMPROVE Study Group Expert Panel. Safety and effectiveness of biphasic insulin aspart 30/70 (NovoMix 30) when switching from human premix insulin in patients with type 2 diabetes: subgroup analysis from the 6-month IMPROVE observational study. Int J Clin Pract. 2009 Apr;63(4):574–82. PMID:19210701

11. Department of Statistics [Internet]. The Hashemite Kingdom of Jordan (http://www.dos.gov.jo, accessed 18 June 2015).

12. American Diabetes Association. Standards of medical care in diabetes–2011. Diabetes Care. 2011 Jan;34 Suppl 1:S11–61. PMID:21193625

13. Khader YS, Batieha A, Jaddou H, Batieha Z, El-Khateeb M, Ajlouni K. Anthropometric cutoff values for detecting metabolic abnormalities in Jordanian adults. Diabetes Metab Syndr Obes. 2010;3:395–402. PMID:21437109

14. Physical status: the use and interpretation of anthropometry. Report of a WHO Expert Committee. World Health Organ Tech Rep Ser. 1995;854:1–452. PMID:8594834

15. Králíková E, Himmerová V. Lekaruv pruvodce Ramcovou umluvou o kontrole tabaku. [The doctors' guide to the WHO framework convention on tobacco control]. Cas Lek Cesk. 2004;143(7):496–7. PMID:15373296