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Effect of flax seeds on lipids: Insulin and ghrelin in type 2 diabetic women with metabolic syndrome

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Abstract

Introduction: Metabolic syndrome and diabetes are emerging diseases nowadays. so adjuvant therapy which is without harmful side effects has become the subject of various researches. Its beneficial effect has been proven however its place in the diet of obese subject remains to be dicovered. The subject of our work is to study the effects of flax seeds on lipids. insulin and ghrelin in women with type 2 diabetes with metabolic syndrome.

Method: We followed a population of 50 women with type 2 diabetes with metabolic syndrome who consumed 30g / d of flaxseed for 8 weeks. Then we compared the anthropometric and biochemical parameters at T0. T4 and T8 weeks. for the hormonal parameters we measured only between T0 and T8 because it is an expensive dosage.

Results: Improvement of the various parameters followed after consumption of flax seeds in our study group. This improvement is significantly better after 8 weeks. For glycemia. CT. TG. HDL-C. IMC. TT. PAS. PAD. insulinemia. ghrelinemia and resistinemia (p between 0.00 and 0.01 for all these parameters studied).

Discussion: Because of their high fibers and in SDG content. the flax seeds improve the anthropometric parameters. probably they favor a better glycemic profile by their richness in omega 3. The flax seen its contents rich in ALA shows an effectiveness to reduce the figures of the plasma lipids as well as the improvement of hormonal parameters.

Conclusion: In the end. this study. combined with the literature. allowed us to better understand the importance of the seeds of this plant in the diet of obese subjects with metabolic syndrome. Enriching your diet with these seeds would therefore be an effective means in the fight against obesity and metabolic syndrome.

Keywords: Diabetes mellitus; Lipids; Ghrelin; Insulin; Metabolic syndrome

1. Introduction

For several decades. the world has been experiencing a demographic and epidemiological transition characterized by a change in lifestyle. behavior and urbanization [1]. This transition is marked by the regression of infectious diseases and the increase in metabolic diseases. metabolic syndrome and diabetes are among them[2.3]. Several recent

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epidemiological studies conducted around the world have clearly demonstrated the increased prevalence of diabetes. The International Diabetes Federation (IDF) reported that currently in 2019. 351.7 million people of working age (20-64 years) have diagnosed or undiagnosed diabetes. This number is expected to increase to 417.3 million by 2030 and to 486.1 million by 2045[4]. The greatest increase will occur in regions moving from low to middle income. The prevalence of diabetes worldwide was 9.3% in 2019 and is estimated to reach 10.3% by 2045 [4]. Metabolic syndrome (MS) is a public health problem. It is an entity that brings together several metabolic abnormalities in the same individual. each of which predisposes to cardiovascular risk and/or type 2 diabetes [5]. The frequency of these complications reflects the severity of this syndrome with renewed interest from professionals. Several definitions have been proposed. which makes the estimation of its real prevalence difficult to specify. Nevertheless. the prevalence of MS remains particularly high in the diabetic population. Indeed. people with SM are three times more likely to develop type 2 diabetes [6]. Despite the evolution of the pharmaceutical industry. there is sometimes resistance even to polytherapy and patients remain poorly balanced. so the role of lifestyle and dietary recommendations remains essential and presents a cornerstone in the management of diabetic patients with diabetes. a metabolic syndrome. Nowadays. the pharmaceutical industry is at the peak of its evolution and we manage to manufacture countless chemical drugs while moving away from nature. which exposes the appearance of several drug interactions. risks of overdose see even toxicity without forgetting the exacerbated prices of these products [7]. The need for a "green" therapy with fewer adverse effects to encourage people to re-engage nature to enhance ancestral knowledge of medicinal plants and draw on their benefits in adjuvant therapy.

In our work we propose to study the effect of flaxseed consumption on anthropological and hormonal parameters in diabetic women with metabolic syndrome.

2. Material and methods

It is a longitudinal prospective interventional study carried out during 8 weeks within the framework of a collaboration between the Laboratory of Biochemistry and Techno biology of the Faculty of Sciences of Tunis. the Service "C" of the diseases of Nutrition and Therapeutic Dietetics. theClinical Biochemistry Laboratory of the National Institute of Nutrition. and the National Institute of Public Health. It involved a group of 50 obese women with type 2 diabetes (diabetes under the age of 5) taking oral antidiabetics with metabolic syndrome. recruited from the outpatient department of the National Institute of Nutrition and followed by a specialist in the service. “C” of nutrition diseases and therapeutic dietetics at the Institute of Nutrition. All the patients involved in the study agreed to participate voluntarily in our survey and expressed a serious interest in losing weight after discussion with a team psychologist.

2.1. Inclusion criteria

Obese type 2 diabetic patients with diagnosed metabolic syndrome.

- Ages 30 and over.
- Age of diabetes ≤ 5 years
- Under oral antidiabetic treatment.
- With unbalanced glycemic figures during previous consultations.
- Oral consent of the patient after explanation of the objectives of the work.

2.2. Exclusion criteria

Were excluded from this study

- Patients treated with insulin.
- Severe associated pathological states such as renal and hepatic insufficiency…

2.3. Non-inclusion criteria

- Type 1 diabetic.

2.4. Methodology et Course of the study

All participants were interviewed during their presence at a usual consultation and were informed about the basic principles of the study protocol. Flax seeds were distributed to women who voluntarily agreed to participate in our study.The flaxseeds were cleaned. washed and dried then put in the oven for 10 minutes over low heat so that the seeds are easy to bite into. then using an electronic food scale. we dosed **30 g** of flaxseeds that'we put in small sanitary bags

to give to each patient.We have provided the number of sachets needed according to the number of patients and for all study periods. The patients present themselves at the beginning of each week to the dietician of the service "C" to meet the specialist doctor of the service for a routine control and to receive the sachets of flax seeds for the doses of the week to come at a rate of30g per day. The Flax seeds were ingested in the morning before breakfast with a glass of water.

2.5. Data collection

Each patient benefited during this study

2.5.1. From a meticulous interrogation specifying

General characteristics (Name. first name. file number. age. socio-economic level. smoking. telephone number).. Pathological family history (type 2 diabetes. arterial hypertension. dyslipidemia. obesity). Associated pathologies. The duration of diabetes evolution.Level of physical activity.

2.5.2. Anthropometric measurements

The weight (kg). the Height (m).Waist circumference and the BMI calculation defined by the ratio in kg to height in square meters was calculated. BMI= WEIGHT / (Height) ^{2} expressed in kg/m^2 .

The weight was measured on 2 successive occasions and the average of the two measurements was calculated. - The height was measured using a measuring rod graduated in centimeters.We adopted the same measurement procedure as for weight gain. - Waist circumference was measured using a measuring tape. The systolic and diastolic blood pressure were taken twice successively and the means of the two measurements were calculated.

2.5.3. A biological assessment including

A glycemic assessment forthe determinations of fasting glycaemia (mmol/l). a lipid profile including the dosages of total cholesterol. triglycerides and HDL-cholesterol. and the dosage of insulin. resistin and ghrelin.

Cholesterol. triglycerides and glycaemia were assayed by KIT Beckman enzymatic method on the Beckman USA Synchron Cx7 automaton. while HDL-cholesterol values are measured after selective precipitation (Kit Randox UK). Resistinemia was determined by the “Millipore # EZHR-95K” ELISA method with a sensitivity ranging from 0.16 ng/ml to 10 ng/ml”. The Insulinemia was measured by IRMA (Immuno-Radio-Metri-Assay).

The ghrelin assay was performed with a competitive RIA (Radio Immuno Analysis) technique using kits marketed for research by the Linco laboratory (LINCO Research. Inc. Missouri. United States) kit: GHRA-88HKfor active ghrelin with a sensitivity of 7.8 pg/ml.

Blood glucose. cholesterol. triglycerides and HDL-cholesterol assays were performed on three times:

- At the start of the survey
- After 4 weeks from the first intake
- At the 8th week (4 weeks after the second intake).

For cost reasons. the dosages of insulin. resistin and ghrelin were carried out in two stages:

- At the start of the survey
- At the 8th week (4 weeks after the second intake).

2.6. Statistical analysis of results

The data was entered in Excel 2007 and analyzed using the Statistical Package for Social Sciences “SPSS” software in version 19.0. The results were presented asthe mean ± Deviation. The variations of the biochemical. anthropometric and hormonal indicators were analyzed by test methods on paired series using the Student test. The analysis of covariance between the beginning and the end of the study was used to eliminate the effect of a confounding factor. Our starting group serves as a control.The significance level is $p<0.05$.

3. Results

The baseline characteristics of the subjects studied at the start of our study (T0) are shown in Table I

Table 1 Characteristics of the participants at the start of the study

	Minimum	Maximum	Mean	Standard Deviation
Age (years)	40	55	47.24	4.38
BMI (Kg/m^2)	30.06	47.80	35.03	4.60
Waist size (cm)	94	128	104.80	9.38
Systolic Blood Pressure (mmHg)	130	170	142.70	10.41
Diastolic Blood Pressure (mmHg)	80	96	50	4.06
Insulinemia (µUI/L)	8.69	24.56	14.41	4.44
Resistin(ng/ml)	5.70	19.55	11.31	3.13
Ghrelin (pg/ml)	10.60	30	21.14	5.43
Fasting blood glucose (mmol/l)	5.81	9.27	7.48	1.20
Total cholesterol (mmol/L)	4.15	7.87	5.24	1.02
Triglycerides (mmol/L)	1.16	2.30	1.84	0.19
Hdl-Cholesterol (mmol/L)	0.44	1.21	0.76	0.20

The figures below illustrate the correlation of BMI with biochemical and hormonal parameters

3.1. Correlations of BMI with biochemical parameters

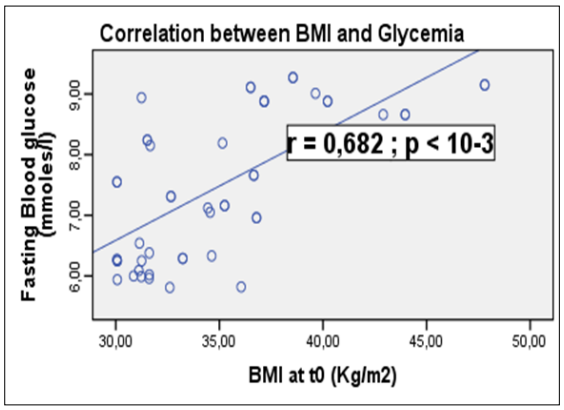

Figure 1 Correlation between BMI and Glycemia at t0

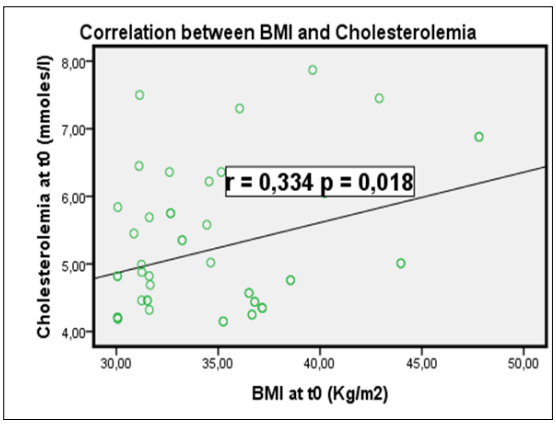

Figure 2 Correlation between BMI and cholesterolemia at to

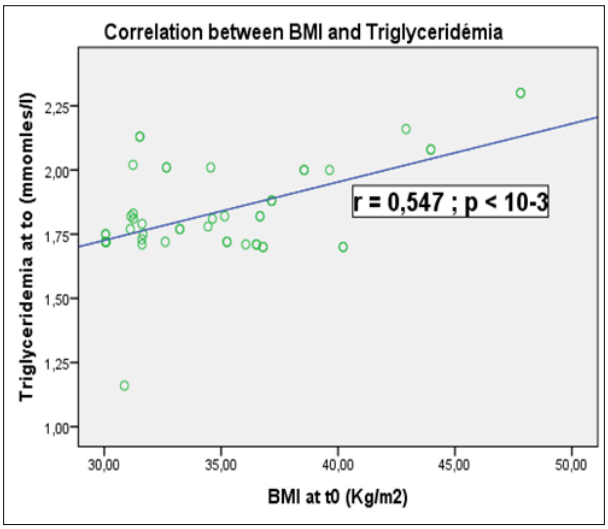

Figure 3 Correlation between BMI and Triglyceridemia at to

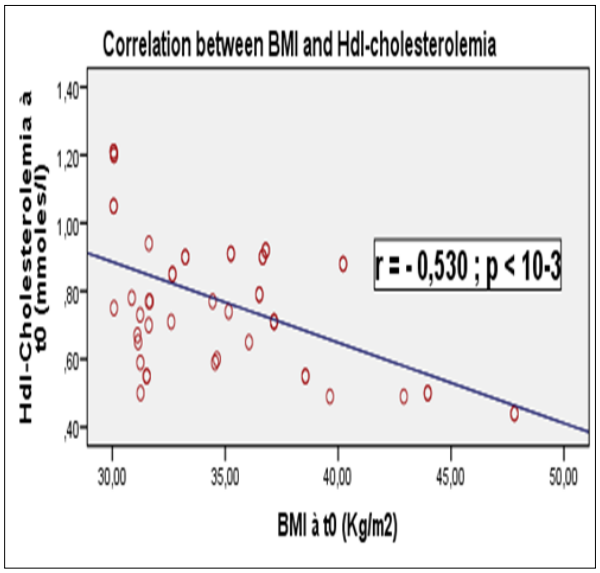

Figure 4 Correlation between BMI and Hdl-Cholesterol at t0

Table 2 Correlations of BMI with insulin. resistin and ghrelin at t0

		BMI at t0	**Insulinemia at to**	**Resistin at to**	**Ghrelin at t0**
BMI at t0	r of Pearson	1	**0.346**	**0.726**	**- .443**
	Sig. (two-sided).		0.014*	0.000**	0.001**
	N	50	50	50	50
Insulinemia at to	r of Pearson	.346	1	.141	-.432
	Sig. (two-sided).	0.014*		.329	0.002**
	N	50	50	50	50

Resistin à to	r of Pearson	0.726	0.141	1	-0.412
	Sig (two-sided).	0.000**	0.329		0.003**
	N	50	50	50	50
Ghrelin à t0	r of Pearson	-0.443	-0.432	-0.412	1
	Sig (two-sided).	0.001**	0.002**	0.003**	
	N	50	50	50	50
*.The correlation is significant at the 0.05 level (two-sided).					
*.The correlation is significant at the 0.05 level (two-sided).					

3.2. Mean deviations of values of anthropometric. biochemical and hormonal parameters

Table 3 Change in values of biochemical. anthropometric. insulin. resistin and ghrelin parameters after 4(t4) and 8(t8) weeks of Lin consumption

Matched differences			**p**
	Mean	**Standard deviation**	
(Cholesteloremia t0) - (cholesterolemia t4)	0.63	0.12	**< 10^{-3}**
(Cholesteloremia t0) - (cholesterolemia t8)	1.09	0.21	**< 10^{-3}**
(blood sugar t0) - (blood sugar t4)	1.22	0. 74	**< 10^{-3}**
(blood sugar t0) - (blood sugar t8)	1.97	0.76	**< 10^{-3}**
(Triglycridemia t0) - (Triglycridemia t4)	0.20	0.02	**< 10^{-3}**
(Triglycridemia t0) - (Triglycridemia t8)	0.37	0.04	**< 10^{-3}**
(Hdl-cholesterol t0) - (Hdl-cholesterol t4)	- 0.21	0.18	**< 10^{-3}**
(Hdl-cholesterol t0) - (Hdl-cholesterol t8)	- 0.11	0.16	**< 10^{-3}**
(BMI t0) - (BMI t4)	5.93	1.3	**< 10^{-3}**
(BMI t0) - (BMI t8)	10.00	1.7	**< 10^{-3}**
(Waist size t0) - (Waist size t4)	11.74	7.1	**< 10^{-3}**
(Waist size t0) - (Waist size t8)	24.77	6.9	**< 10^{-3}**
(Insulinemia t0) - (Insulinemia t8)	5.29	3.2	**< 10^{-3}**
(Resistin to) - (Resistin t8)	3.58	1.2	**< 10^{-3}**
(Ghrelin t0) - (Ghrelin t8)	- 1.34	2.98	**< 10^{-3}**

Table 3 shows an improvement in the values of the anthropometric. biochemical and hormonal parameters after 4 and 8 weeks of consuming flax.These variations are greater after 8 weeks.The figures below illustrate these variations well.

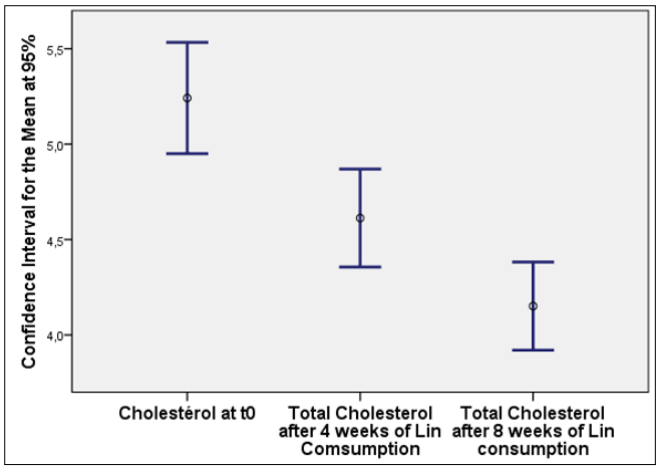

Figure 5 Variation values of cholesterolemia after 4 and 8 weeks of Lin consumption

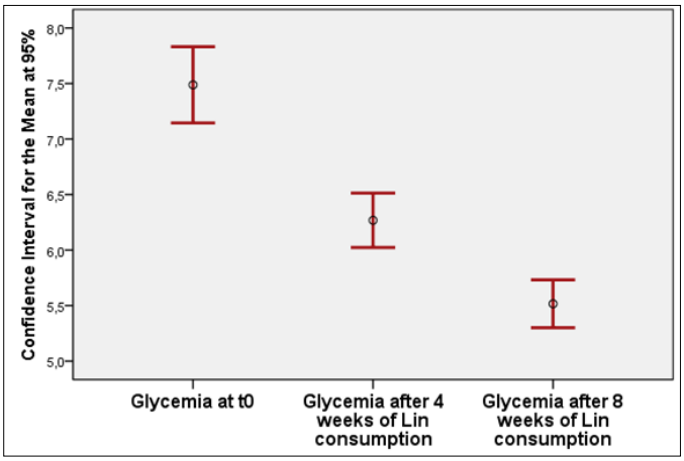

Figure 6 Variation values of Blood sugar after 4 and 8 weeks of Lin consumption

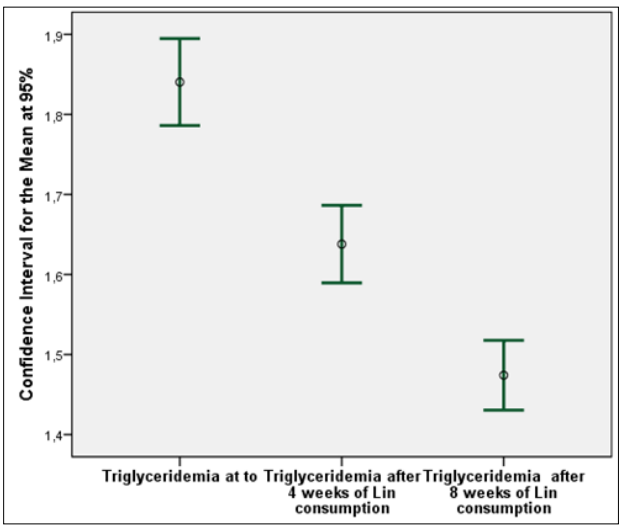

Figure 7 Variation values of Triglyceridemia after 4 and 8 weeks of Lin consumption

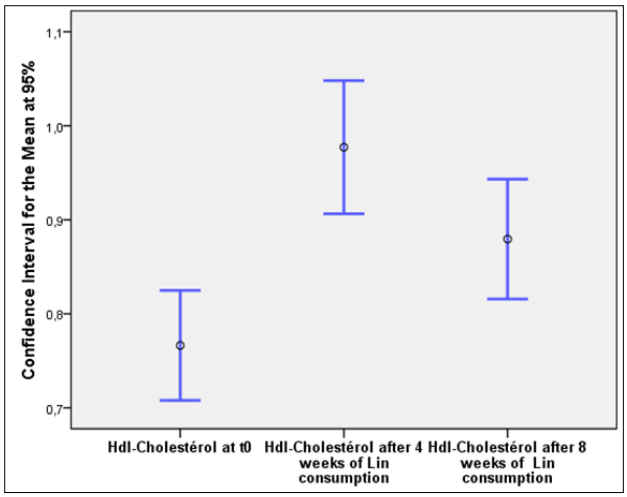

Figure 8 Variation values of Hdl-cholesterol after 4 and 8 weeks of Lin consumption

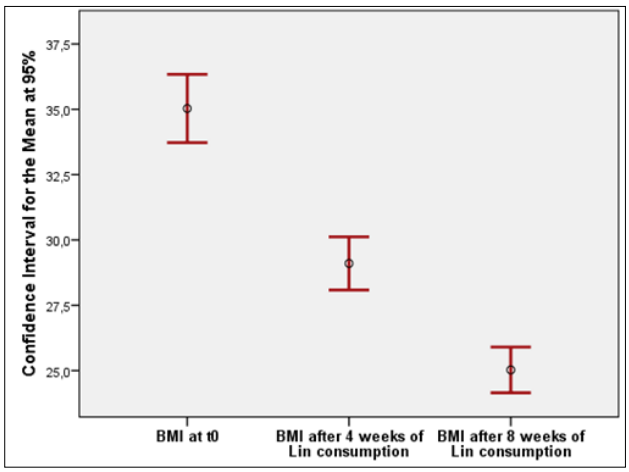

Figure 9 Variation values of BMI after 4 and 8 weeks of Lin consumption

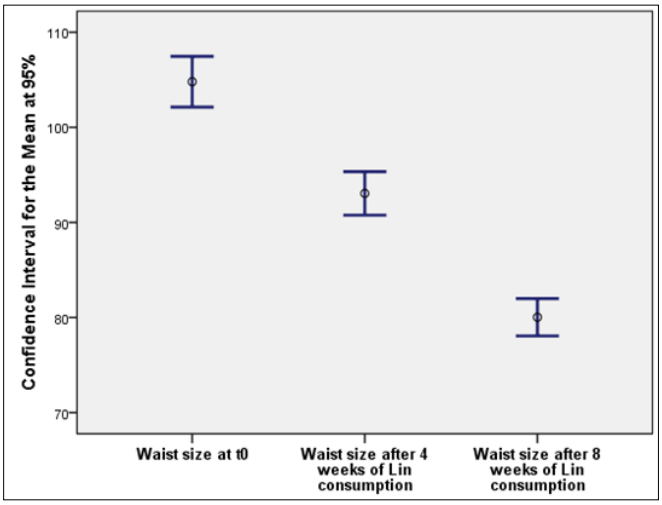

Figure 10 Variation values of Waist Size after 4 and 8 weeks of Lin consumption

3.3. Relative variation of the different anthropometric and biochemical parameters between t0 and t8

We present in table 4 the relative variation of the different parameters studied between t0 and t8 using the formula

$$\frac{(\text{Value to}) - (\text{value t8})}{(\text{Value to})} \text{ x } 100$$

Table 4 Relative variation of the different parameters studied between t0 and t8

Parameters measured and dosed	Mean relative variation
BMI	28.46 ± 1.98
Waist Size	23.43 ± 5.22
Blood Sugar	25.73 ± 6.91
Total Cholesterol	20.80 ± 0.00
Triglyceridemia	19.90 ± 0.00
Hdl- Cholesterol	-17.82 ± 27.72
Insulin	34.65 ± 10.75
Resistin	31.47 ± 6.55
Ghrelin	- 8.97 ± 3.02

It appears from these results that there is a satisfactory relative variation concerning all the parameters studied between the beginning and the end of our study.

4. Discussion

Our work is a clinical interventional study which examines the effect of flaxseed consumption on the various biochemical and hormonal factors of the metabolic syndrome. in type 2 diabetic women. At the end of this study. the consumption of 30g of flaxseed for 8 weeks induces a significant decrease in weight. blood sugar. insulinemia. resistin. CT and TG which are compensated by an increase in HDL-C.and ghrelin.Our study shows a decrease in BMI and WC values with a statistically significant difference (p=0.00) this decrease is greater after 8 weeks of flax consumption in fact the average BMI goes from 5.92 ± 1.29 kgm-1 after 4 weeks at 10.00±1.68 kg m-1 after 8 weeks. which agrees with results found in previous studies such as that of M. Mohammadi-Sartang and Z. Mazloom in 2017 where they show by analyzing45 scientific articles that the consumption of flax reduces weight with significant values (p between 0.000 and 0.008) and that the ideal dose to have this effect is 30 g per day.

The World Health Organization (WHO) estimates that about 80% of the world's population still relies on the use of medicinal plants as a first treatment [8] among the oldest plants traditionally used is flax [9]. Flaxseed is composed of 45% oils. 20% protein. 30% dietary fiber. 7.7% moisture and 4% ash [10]. Clinical studies have used flax as a dietary supplement in several clinical trials to investigate its effect on lipid profile. blood pressure measurements. and blood glucose levels [11]. total cholesterol and circulating LDL-Cholesterol [11]. improving the biochemical and metabolic profile in type 2 diabetics thanks to their hypoglycemic action [12] as well as their effect on blood pressure measurements [11]. Like any product. flax is not free from adverse effects. several studies have been conducted with the aim of examining the safety of its therapeutic use. According to clinical studies. flax is considered a safe product in 87% of cases and the adverse effects reported from its use are headaches and flatulence [13].

Flaxseed is therefore considered to be of negligible risk [14]. Allergy to flax is rare [15]. there are very few case reports of anaphylaxis caused by flax since 1930 [16].

In general. fiber-rich foods tend to be more satiating due to their low palatability. Secondly. dietary fibers have the property of increasing the viscosity of the intestinal contents and therefore slowing down digestion. which promotes the feeling of satiety.In addition. these dietary fibers act as a mechanical barrier that decreases the rate of digestion [17].

This significant reduction in weight is due to the richness of flax in secoisolariciresinol diglucoside (SDG) which is the major lignan found in flax and has many biological activities which gives a feeling of satiety and prevents weight gain

[18]. Studies conducted on mice have shown that flax. when given high in omega 3. improves glycemic profile and decreases fasting blood glucose values [19]. Our study therefore perfectly complements this study and shows a drop in fasting blood sugar in patients by an average of 1.97 ± 0.76 mmol / L after 8 weeks of consumption of 30g of flax. Furthermore. the mechanism of blood sugar reduction is not yet defined. but it has been shown that the lignans found in flax. thanks to their powerful antioxidant activity. increase the elimination of glucose through the translocation of GLU-4in the cell membrane and thus decrease basal glucose uptake [20]. To closely assess this glycemic profile. various hormonal parameters were measured. namely insulinemia. resistinemia and ghrelinemia. Indeed. resistin is an adipokine which plays an important role in the regulation of insulin sensitivity and also this hormone exerts a pro-inflammatory effect. In our study. we show a correlation between resistinemia and BMI with Pearson's r = 1.76 and at a high level of significance (p = 0.00).Our results show that after 8 weeks of flax consumption. there is a highly significant decrease (p=0.00) in resistin levels with an average of 3.58±1.25ng/ml.Indeed this hormone is known by its tissue effect of insulin resistance and therefore a consumption of flaxseed can protect against this effect.This agrees with the results of a study by Hossein Shirvani1 and Saleh Rahmati-Ahmadabad (2018) using linseed oil showing a decrease in resistin of a significant value p= 0.02 [21]. Given the negative correlation already demonstrated in our study between resistin and ghrelin which is highly significant (p=0.00 and Pearson's r = -0.41) we can say that a decrease in resistinemia will be accompanied by an increase inghrelinemia.In our study there is an increase in ghrelin values with an average of 1.34±2.98 pg/ml with a high significance level (p=0.003). Our results are in conflict with the tests of M. Kristensen a and F. Savorani carried out on young people for 8 h of flax consumption and which showed that the variation in ghrelin is not significant (p=0.68) **[22].** This could be due among other things to the fact that ghrelin is a recently discovered hormone (1999) and clinical studies on these hormones are still under development but this hormone remains promising for the management of type 2 diabetes [23]. The last hormonal parameter tested in our study is insulinemia. which shows a significant drop in its values after 8 weeks of consumption of 30g of flaxseed. i.e. an average of 5.29±3.22µIU/L (p = 0.00).This variation could be explained among other things. within our study by the strong significant correlation established between the BMI and the insulinemia(r of Pearson = 0.34; p=0.01) from where the fall of BMI at the population ofour study reflected on the values of insulinemia in the direction of their decreases. Regarding the lipid profile. there was a significant drop in CT (p = 0.00) after flax consumption for 4 and 8 weeks with averages of 0.62 ± 0.12 mmol / L and 1.09 ± 0.21 mmol / L respectively. This decrease in CT in our diabetic subjects with metabolic syndrome is greater after 8 weeks of flax consumption. According to our results. we also notice a significant drop in TG (p=0.000) after a period of 8 weeks of consumption of flaxseed. The decrease in CT and TG is complemented by the significant increase in HDL-Cholesterol.

These results are consistent with the work of Atefeh Akrami. MSc (2016) who studied the effect of flaxseed oil on patients with metabolic syndrome and highlighted the drop in CT and TG after 16 weeks of daily consumption of 25 g flaxseed [24].

Along the same lines. Andrea L Edel and Delfin Rodriguez-Leyva tested the effect of consuming 30g of flaxseed in 52 patients over 12 months. plasma lipids were measured at 0.6 and 12 months.These studies show a significant reduction in CT and TG with an increase in HDL-Cholesterol (p < 0.008) **[25].** To complete the research Komal F. Khan MK tested the effect of foods rich in polyunsaturated fatty acids (PUFA). flaxseed oil. given its rich content of ALA. shows an effectiveness in reducing plasma lipid figures. this is explained by the key role ALA plays in activating transcription factors that control nutrient trafficking in lipid metabolism pathways by enhancing AMP-activated protein 5' kinase (AMPK). This AMPK is considered as a major sensor of the cellular energy state. These studies show that given flaxseed oil rich in ALA (Alpha-linolénique Acid) results in a significant decrease in TG. Regarding systolic and diastolic blood pressure. the decrease is more significant after 8 weeks of flax consumption. A bibliographic research to study the effect of the consumption of different flax products (seed. lignan. oil) carried out in 2015 on SAP and DBP shows that with the consumption of flax in the group of flax who consumed it during a duration of 12 weeks there is a drop in blood pressure values. these results agree with those of our study where a significant drop in blood pressure numbers was noted (p = 0.00) **[26].** Indeed the biological mechanism of the hypotensive property of flax is not fully understood. it is assumed that a lignan named SDG (Secoisolaricirsinol di-glucoside) is a phytoestrogen known as an inhibitor of the angiotensin converting enzyme has a strong hypotensive action. another Also important component of flaxseed is ALA which also intervenes in the lowering of blood pressure values by its anti-inflammatory action by reducing the activity of soluble hypoxide hydrolase which is responsible for the loss of vasodilation. Therefore. the hypotensive effect of flax results from the synergy of action of ALA and SDG**[27].** As for the correlations between the biochemical parameters. there is a correlation between BMI. blood sugar and TG with a significant significance threshold (p = 0.00) we also found a significant correlation (p = 0) between CT and TG this is due to the fact that obesity will automatically be associated with a lipid balance disorder and especially an increase in TG and therefore CT. Correlation studies between BMI and hormonal parameters show that BMI correlates significantly with insulinemia. ghrelinemia and resistinemia with significant thresholds (p = 0.01. 0.00 and 0.00) respectively. hence BMI is a good indicator for the hormonal state of the glycemic profile. We can deduce from these correlation results of our study that diabetes is associated with a metabolic

syndrome because obesity tends to raise biochemical values and glycemic figures in subjects. this is proven by studies carried out between 2019 and 2020 by Merlis James to assess the association of these two pathologies (diabetes and SM) in 350 subjects and showed that these two pathologies correlate significantly (p = 0.01) **[28].**

5. Conclusion

Flax is a plant used since antiquity. its intervention in a diet helps diabetic patients with metabolic syndrome to maintain to maintain fasting blood sugar in the normal forks..The metabolic syndrome associated with diabetes is a handicap for people wishing to have a healthy life and increase their life expectancy. It would therefore be wise to look for effective ways to lose weight to improve the quality of life because it has been proven that a modest loss of weight clearly influences the state of health of the diabetic subject with metabolic syndrome and allows in particular to better control blood pressure. blood sugar and lipid profile. In our study. we tried to prove through clinical tests the role of flax seeds in improving these parameters due to their richness in omega-3 unsaturated fatty acids and α-linoleic acid. The collection of biochemical and hormonal anthropometric parameters allowed us to appreciate the improvement in BMI. TT. blood sugar. ghrelinemia. resistinemia. CT. TG and HDL-C between the start of the study and after 8 weeks of consumption of 30 g/d of flaxseed. Indeed. there is a significant decrease in BMI and WC as well as insulinemia. The use of flax seeds in different food products and in the treatment of several human pathologies remains promising. It is therefore important to promote a diet rich in flax in order to ensure the recommended daily intake. To conclude. we accept the hypothesis that the consumption of flaxseed improves the lipid profile and biochemical parameters while reducing weight in diabetic subjects with metabolic syndrome. Further studies with a longer intervention period. larger sample size. and different doses of flaxseed are needed to verify these effects.

Compliance with ethical standards

Disclosure of conflict of interest

All authors declare that no conflict of interest exists for this work.

Statement of ethical approval

All the patients involved in the study agreed to participate voluntarily in our survey and expressed a serious interest in losing weight after discussion with a team psychologist.

Statement of informed consent

Since the patients who agreed to voluntarily participate in the study are chronically ill in the same department where we carried out our research. they gave their consent orally since it was the attending physician who explained the protocol and the objectives to them. of the study.

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