“EVALUATION OF THE EFFECT OF COCOA SUPPLEMENTATION ON THE BIOCHEMICAL AND CLINICAL PROFILE AND SENSORY-MOTOR PROCESSING OF PERIPHERAL AND AUTONOMIC DIABETIC NEUROPATHY: RANDOMIZED CLINICAL TRIAL”

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SUMMARY

Background: Type 2 diabetes (T2D) is a highly prevalent disease in Mexico and is associated with the development of chronic-degenerative complications such as diabetic neuropathy. The latter manifests as a set of disorders that occur as a consequence of a chronic hyperglycemic state that can induce oxidative stress and inflammation, resulting in damage to the autonomic and peripheral nervous systems. Diabetic neuropathy increases the risk of pain and skin lesions, ulcers, or even amputations, affecting the quality of life of those who suffer from it. In Mexico, it has been reported that this complication occurs in 29% to 90% of patients with diabetes. Cocoa is a food with a high flavonoids content, which are phenolic compounds with antioxidant and anti-inflammatory effects. Additionally, its consumption has been associated with decreased hyperglycemia and insulin resistance, improved mitochondrial function, and, based on the above, a potential effect on diabetic complications has been suggested. This has been demonstrated in *in vivo* and *in vitro* models but not in human populations. Once symptoms of diabetic neuropathy appear, palliative treatments are prescribed, as no pharmacological compounds have yet been shown to reverse the consequences of peripheral and autonomic diabetic neuropathy. Additionally, clinical trials of compounds with antioxidant properties have only conducted subjective evaluations based on questionnaires about perceived improvement in diabetic neuropathy and some biochemical markers or nerve conduction tests, with inconclusive results. **Methodology:** A double-blind, randomized, controlled clinical trial is proposed to evaluate the effect of cocoa supplementation in patients with type 2 diabetes and peripheral and autonomic diabetic neuropathy on a) the biochemical profile, including the assessment of glycemic and lipid profiles, b) the clinical profile through standardized questionnaires, anthropometric measurements, and blood pressure; and c) somatosensory processing through the rate-dependent depression of the H-reflex test. **Hypothesis:** The hypothesis of this study is that cocoa supplementation will have a beneficial effect on the biochemical and clinical profile and somatosensory processing of peripheral and autonomic diabetic neuropathy. **Statistical analysis:** For the evaluation of intra-group variables, a statistical analysis will be performed using repeated measures ANOVA with Tukey *post hoc*, or if applicable, Friedman with Dunn *post hoc*, as well as Student's t-test for dependent groups, or if applicable, Wilcoxon. The intergroup comparison will be made with Student's t-test for

independent samples, or if applicable, with U Mann Whitney, considering $p < 0.05$ as statistical significance and using GraphPad Prism software version 5.

2. THEORETICAL FRAMEWORK

2.1 Type 2 Diabetes

Type 2 diabetes (T2D) refers to a relative insulin deficiency secondary to a progressive dysfunction of its secretion, often with a prior period of insulin resistance (IR). This type of diabetes accounts for 90-95% of all cases. (1) According to the International Diabetes Federation (IDF), in 2019, the global prevalence of diabetes in adults aged 20 to 79 years was 9.3%, and specifically for the North America and Caribbean region, it was 13.3%. (2) In Mexico, according to the 2018 National Health and Nutrition Survey (ENSANUT), 10.3% of adults over 20 years old have a diagnosis of T2DM, corresponding to 8.6 million people. (3) This is relevant as 2013 data estimated that the total cost attributable to DM in Mexico was $7,736 million, and specifically for ISSSTE, $484 million, figures that could become unsustainable in the long term, especially with the increase in the number of cases. (4)

2.2 Pathophysiology

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia. It occurs due to dysfunction in insulin secretion, resistance, or both (2). IR is the inability of the hormone to stimulate peripheral glucose uptake, as well as other signaling pathways in different tissues (5, 6), and develops as a result of fat accumulation in muscle and organs such as the liver and pancreas. In the pancreas, β-cell dysfunction is generated, as well as inflammation of the islets of Langerhans and cellular apoptosis. (7)

2.3 Diabetic peripheral and autonomic neuropathy

When metabolic dysregulation related to hyperglycemia persists, macrovascular and microvascular complications may develop. The former involve cardiovascular diseases, such as those of the large blood vessels, while the latter includes retinopathy, nephropathy and diabetic neuropathy (DN) and are caused by pathological changes in capillaries, which participate in transporting oxygen and nutrients to the body's systems, including the nervous system. (2, 8) Studies in Mexican population have reported that between 29% and 95% of patients living with DM develop DN, which can be observed as early as five years after the

diagnosis of the disease. (9-11) The two most important predictors for the development of DN are the duration of DM and glycated hemoglobin (HbA1c) levels. Additionally, other factors increase the risk of DN, such as smoking, obesity, hypertension, and dyslipidemias (elevated triglycerides and LDL-c, as well as decreased HDL-c), making adequate metabolic control vitally important. (12, 13)

DN is a group of disorders that present signs and/or symptoms of peripheral nerve dysfunction with various clinical manifestations in both peripheral and autonomic neuropathy. (8, 14) Diabetic peripheral neuropathy (DPN) can be divided into distal symmetric peripheral neuropathy and focal and multifocal asymmetric neuropathy, which occur as a consequence of the progressive loss of nerve fibers and the resulting instability. (15) Symptoms of DPN depend on the type of fibers affected, the most common being numbness and tingling in the case of large fibers, and allodynia – pain caused by stimuli that are not usually painful-, hyperalgesia, paresthesia and pain, in the case of small fibers (16), symmetric sensory loss or glove or stocking sensory loss in the feet, above the ankles and in the hands, is also evident during the clinical examination. (12, 15)

The pathogenesis of DPN is not well defined and the pain often does not respond to first-line analgesics or requires doses that may cause adverse side effects. (17) Neuropathic pain occurs in approximately 30% of patients living with diabetes (18) and occurs as a consequence of sensory abnormalities, changes in the primary afferent nerves and central sensitization. (19) On the other hand, decreased pain sensation can result in an increased risk of skin lesions, infections and deformities leading to the development of Charcot foot, ulcers or even amputations. (14)

Regarding autonomic neuropathy (ADN), this refers to damage to the nerves that control internal organs, causing alterations in various systems such as the cardiovascular, gastrointestinal and urogenital systems, and thus, the development of signs and symptoms such as arrhythmias, tachycardia at rest, nausea, vomiting, constipation, urinary incontinence, erectile dysfunction and decreased sexual libido. (20)

2.4 Pathophysiology of DN

Oxidative stress has been described as one of the mechanisms by which DN develops, resulting from an imbalance between the production of reactive oxygen species (ROS) and

endogenous antioxidant systems (12) and the state of inflammation. (21) Hyperglycemia can induce oxidative stress by causing the accumulation of glucose and glycolysis intermediates, thereby altering the electron transport chain, reducing H2O and ATP production, and increasing the production of superoxide (O2-). (12)

Additionally, hyperglycemia can stimulate macrophages to secrete proinflammatory cytokines such as tumor necrosis factor-alpha (TNFα), and, in these patients, there is an increase in the expression of C-reactive protein (CRP) and Interleukin-6 (IL-6). (21) These mechanisms are related since the increase in ROS perpetuates the state of inflammation, just as proinflammatory cytokines contribute to the increase in ROS.

Hyperglycemia causes the metabolic pathways to be used to regulate the polyol pathways and the hexosamine pathway, which generates an increase in ROS and inflammation, and consequently, mitochondrial damage and dysfunction in the nervous system. (12, 22)

About the polyol pathway, when there is hyperactivity of this pathway, sorbitol production increases by activating the aldose reductase enzyme system. This can alter the NADPH/NADP ratio and decrease reduced glutathione and oxidized glutathione, causing oxidative stress. (22, 23) NADPH is derived from the pentose pathway and generates glutathione peroxidase from glutathione, and the consumption of this NADPH is what is associated with the development of oxidative stress. (12) Sorbitol formed as a result of the polyol pathway is oxidized to fructose by the enzyme sorbitol dehydrogenase, which uses NAD+ as a cofactor. Excess fructose promotes glycation and NADPH depletion, exacerbating intracellular oxidative stress. Furthermore, in preclinical studies in diabetic rats, the accumulation of sorbitol and fructose in peripheral nerves has been observed. (12) Sorbitol is not able to cross cell membranes, so it accumulates in cells, generating hyperosmolarity and a concomitant flow of taurine, myoinositol and adenosine. This causes inhibition of ATP synthesis, decreasing the activity of Na+/K+ ATPase and protein kinase C (PKC), an alteration in axonal transport and structural degradation of the nerves. (24)

On the other hand, excess glucose causes glycation of structural and functional proteins and produces advanced glycation end products (AGEs), which interact with specific receptors to modify gene expression and intracellular signaling pathways that promote the release of proinflammatory cytokines and ROS. (25) AGEs also decrease axonal transport in neurons,

leading to their degeneration. Likewise, they bind to their receptor (RAGE – receptor for advanced glycation end products), and, by activating it, trigger inflammatory pathways that contribute to oxidative stress. (24)

Hyperglycemia mainly affects those cells that have a limited capacity to regulate glucose uptake, such as vascular cells, Schwann cells and neurons of the peripheral and central nervous system, directly damaging the cells or affecting cell function, leading to degeneration of peripheral nerves, (12) in particular, it has been described in animal models that when Schwann cells cannot provide the factors necessary for the proper functioning of axons, they begin to degenerate, in addition, alterations in the soma of dorsal root ganglion neurons could also contribute to the degeneration of peripheral nerve fibers. (25)

Progressive DN involves the retraction and death of peripheral terminal sensory axons, with a relative preservation of the soma. It is believed that the entire neuron, both the soma and the axon, is affected by DM, however, it is not yet clear whether the first to be damaged are the peripheral axons and their associated Schwann cells or the soma of the dorsal root neurons that support the axons. Schwann cells are affected by persistent hyperglycemia, which causes significant damage to the axon. (25)

Another comorbidity associated with the development of DN is dyslipidemia since under normal conditions, for the degradation of fatty acids and their subsequent use as an energy substrate in Schwann cells, they must go through the β-oxidation pathway, which results in a molecule of acetyl coenzyme A (CoA), which subsequently enters the Krebs cycle, forming NADH and FADH2. (25)

NADH and FADH2 are used in oxidative phosphorylation to produce ATP and low levels of ROS, which can be neutralized by the body's endogenous antioxidant enzymes. (25, 26) However, when there is an overload of substrates, as in the case of T2D, the β-oxidation transport system to the Krebs cycle becomes saturated and the acetyl CoA molecules are converted to acylcarnitines. (25) This accumulation of acylcarnitines appears to be toxic to Schwann cells and dorsal root ganglion neurons, so they are released from these cells, inducing axonal degeneration, mitochondrial dysfunction, and a maladaptive stress response in Schwann cells. (25, 27)

Moreover, substrate overload also affects oxidative phosphorylation, decreasing ATP production and increasing ROS levels that cannot be neutralized, leading to mitochondrial dysfunction and metabolic and oxidative damage in these important cells. (25)

2.5 Diagnosis

Patients with DM should be evaluated for diabetic neuropathy at diagnosis and for 5 years thereafter, and an annual review is recommended through a detailed clinical history, physical examination, and clinical tests. (14) The diagnosis of DN is made when other causes that could be causing the signs and symptoms are excluded and can be supported by tools such as questionnaires and tests. (16)

Among the causes of neuropathy that must be excluded to make the diagnosis are toxic or drug-induced neuropathy, alcoholic neuropathy, neuropathy associated with HIV, hypothyroidism, and neuropathy due to vitamin B12 deficiency. Especially, those subjects who take metformin as a pharmacological treatment for T2D are at high risk of developing vitamin B12 deficiency since it decreases the absorption of this vitamin in the terminal ileum and is related to alterations in intrinsic factor levels. (28, 29) The signs and symptoms of vitamin B12 deficiency are very similar to those observed in diabetic neuropathy, and therefore, their differentiation can be complex. Thus, the determination of serum vitamin B12 is suggested to exclude non-diabetic causes of neuropathy. (29)

On the other hand, among the tools that can support the diagnosis of DN are biopsy, which is an invasive test; nerve conduction tests, which are expensive and are only useful to evaluate fast conduction fibers, which are not very practical; and the sensory test with the monofilament. (30)

The Michigan Neuropathy Screening Instrument (MNSI) is a validated tool available in Spanish, which evaluates the presence of diabetic peripheral neuropathy. This instrument can be applied quickly, includes the history of symptoms and physical examination to detect alterations in the foot. It has a sensitivity of 65% and specificity of 83%, and a score of ≥2 has been proposed as a cut-off point for the diagnosis of DPN. (31, 32)

Another tool that allows the severity of DN to be evaluated is the Toronto Clinical Scoring System (TCSS), which evaluates the symptoms, reflexes, and sensitivity by anatomical

regions. According to the score obtained, 3 grades are established: 0-5 points, no DN; 6-8 points, mild DN, 9-11 points, moderate DN and 12-19 points, severe DN. (33, 34)

There is no standard for the evaluation of ADN, however, the clinical history is essential, for example, for the evaluation of gastrointestinal ADN, tests that identify symptoms such as nausea, vomiting, abdominal pain, constipation or diarrhea can be used (35), such as the Bristol scale and the BEST questionnaire.

2.6 RDD of the H-reflex

The Hoffman reflex (H-reflex hereafter) is produced by an electrical pulse and is recorded in a given muscle, and is analogous to the muscle spindle stretch reflex generated by a mechanical stimulus (myotatic reflex). The H-reflex represents the excitability of the alpha motor neuron (αMN) when presynaptic inhibition and the intrinsic excitability of αMNs remain constant. This test allows the evaluation of the response of the nervous system in conditions of damage to the nerve fibers and the somatosensory processing in the spinal cord. (36) Through the H-reflex, it is possible to measure the efficacy of synaptic transmission of afferent fibers (Ia sensory) to αMNs. The evoked afferent response starts at the point of electrical stimulation, resulting in action potentials that travel along the afferent fibers until they synapse in the αMNs, while on the efferent side, the action potentials travel along the fibers until they reach the neuromuscular junction and produce a contraction response that can be recorded in the electromyogram. (36)

An additional test based on the H reflex is the rate-dependent depression (RDD) test, which is a measure of the change in the amplitude of the spinal H reflex during consecutive paired electrical stimulations at different frequencies (<1 Hz). Recently, it has been described that RDD is affected after disinhibition of spinal sensory processing, e.g., in animal models of experimental diabetic neuropathy (18, 37, 38) and in T1D in humans (18), so the paired-pulse H reflex allows the objective and noninvasive detection of alterations in nerves and spinal sensory processing.

2.7 Treatment

Up to 50% of DN can be asymptomatic, and if not recognized and treated in time, it increases the risk of injuries, decreased quality of life, and even mortality, as in the case of cardiovascular ADN. Currently, the management of DN consists of lifestyle modifications to control glycemia

and pharmacological treatment, especially in the case of painful neuropathy. These drugs include tricyclic antidepressants, antiepileptics, opioid agonists, morphine derivatives, selective serotonin reuptake inhibitors, and nonsteroidal anti-inflammatory drugs, which are only palliative and may have adverse side effects and/or limited long-term efficacy. (13, 16)

Therefore, strategies have been sought to mitigate the symptoms associated with DN. For example, the consumption of flavonoids could have a beneficial effect on patients living with DM by controlling the glycemic profile (glycemia and HbA1c), lipid profile (triglycerides, LDL-c, and HDL-c), and possibly reducing blood pressure and inflammation markers (neutrophil/lymphocyte ratio) without developing adverse effects. (39-41)

2.8 Flavonoids

Flavonoids are phenolic compounds present in plant-based products such as fruits and vegetables, especially those with intense colors such as berries and green leaves, red wine, green tea, and cocoa, and are recognized for their antioxidant, analgesic, and anti-inflammatory effects. Some molecular mechanisms underlying these effects include the inhibition of the nuclear factor κB (NF-κB), thereby decreasing the transcription and secretion of inflammatory cytokines. (39, 42)

Cocoa (Theobroma cacao) has a high polyphenol content: 12 to 18% of the seed's dry weight. Flavonols are the most important polyphenols in cocoa, especially characterized by the presence of monomers and isomers of catechin and epicatechin, such as (-)epicatechin and (+)catechin. (42, 43) These polyphenols have shown beneficial effects in overweight subjects on parameters such as body weight, fat mass, and lipid profile by modulating fatty acid synthesis, which are closely related to glucose metabolism. (44) Additionally, polyphenols have been associated with increased nitric oxide synthase activity, which increases nitric oxide levels and reduces blood pressure. (43)

At the neurological level, it has been observed that the consumption of cocoa and its flavonoids can reduce the risk of neurodegenerative diseases, especially those derived from oxidative stress such as Alzheimer's and Parkinson's disease; reduce depression due to its tryptophan content and its consequent conversion to serotonin; provide protective effects against neurotoxicity induced by β amyloid protein and against cognitive decline associated with aging and protect nerves from injury and inflammation. (45, 46)

On the other hand, the consumption of catechins has been associated with a decrease in hyperglycemia and insulin resistance through the modulation of proinflammatory cytokines such as IL-1β, IL-6 and TNFα, and the activation of signaling pathways that allow maintaining an adequate function of the mitochondrial respiratory chain, and thus, protecting the cell islets and improving insulin resistance. (43) In preclinical studies, the possible decrease of neuropathic pain through different mechanisms has been reported, one of the most important being the modulation of the inflammatory response. (47)

In a study in rats with streptozotocin-induced diabetes, doses of 25 and 50 mg/kg of catechins were administered intraperitoneally for 28 days, observing attenuation of diabetic autonomic neuropathy through the improvement of antioxidant enzymes in the vagus nerve, in addition to the decrease of malondialdehyde (MDA), a marker of oxidative stress. (39, 48) It is therefore relevant to objectively and quantitatively evaluate the effect of cocoa supplementation on the biochemical and clinical profile and sensorimotor processing of diabetic peripheral and autonomic neuropathy in adults with T2D.

3. JUSTIFICATION

T2D increases the risk of developing macrovascular and microvascular complications, which lead to a significant portion of the national budget being allocated to the treatment of these chronic-degenerative conditions. In 2013, it was estimated that the total cost attributable to diabetes in Mexico was USD 7.736 billion, with USD 484 million specifically for the ISSSTE (Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado), figures that could become unsustainable in the long term. (4)

DN is a common complication of T2D that affects the quality of life of those who suffer from it. Currently, the management of diabetic neuropathy includes lifestyle modifications, glycemic control, and pharmacological treatment, which focuses on reducing pain and symptoms (13); however, the medications used are palliative and have adverse or undesirable side effects.

The consumption of foods rich in flavonoids has been linked to the regulation of processes such as oxidative stress and metabolic and inflammatory control. Therefore, supplementation with cocoa, which is rich in flavonoids, could reduce neuropathic damage and improve the quality of life of those affected, thereby reducing the costs associated with the treatment of diabetes and its complications. (39, 43) For these reasons, the objective and quantitative

evaluation of DN is crucial to establish potential beneficial effects in patients. This study proposes implementing the RDD of the H-reflex test as an indicator of alterations in sensory processing at the spinal cord level and nerve fibers, according to previous results in animal models of experimental diabetic neuropathy and in humans with T1D. (17, 18) Additionally, this research will be complemented by the application of quality-of-life instruments, and clinical evaluation. Therefore, the research question for this study is: What is the effect of cocoa supplementation on the biochemical and clinical profile and the somatosensory processing of peripheral and autonomic diabetic neuropathy?

3.1 Relevance

DM has a prevalence of 10.3% in Mexico, and of this percentage, between 29 and 95% develop diabetic neuropathy (9), which causes pain, sensory and autonomic abnormalities and therefore decreases the quality of life of those who suffer from it. Studies have demonstrated the beneficial effect of polyphenols on neuropathic damage; however, to date their effect has not been studied objectively and quantitatively. This study considers the evaluation of three important aspects in the evaluation of the effect of cocoa consumption in patients with DN: the biochemical and clinical profile and somatosensory processing. In this sense, this project is comprehensive and innovative, and the results obtained could serve as indicators of the possible mechanisms by which flavonoids contribute to the improvement of metabolic control and therefore to the reduction of symptoms of peripheral and autonomic DN.

3.2 Feasibility

In order to carry out this research project, work will be carried out at the facilities of the Anáhuac México Norte University, at the Kidney Specialty Center (State of Mexico) and at the Nueva Oxtotitlán clinic (Toluca), where the tests will be carried out with the patients. The equipment to carry out the electrophysiology tests (electrical recording and stimulation) is already available, as well as the cocoa capsule supplement, so the study can be carried out without setbacks. Cocoa supplementation does not affect the patients' dietary regimen or compromise their regular medication. In addition, the RDD of the H reflex test does not produce adverse effects, is painless and noninvasive.

4. STATEMENT OF THE PROBLEM

T2D is associated with the development of chronic-degenerative complications such as diabetic peripheral and autonomic neuropathy. This manifests itself as a set of disorders that occur as a consequence of a chronic state of hyperglycemia that favors a state of inflammation and oxidative stress, resulting in damage to the peripheral and autonomic nervous system.

This hyperglycemia contributes to the increase in the inflammatory state and the production of reactive oxygen species, as well as to the increase in oxidative phosphorylation and overload of the electron transport chain. Among the cells that are most affected are the Schwann cells, which, when damaged, cause alterations in the functioning of the axons and nerve fibers.

Flavonoids are phenolic compounds present in foods such as cocoa, and whose effects are recognized as antioxidant and anti-inflammatory mechanisms. Additionally, in preclinical studies, their consumption has been associated with a decrease in hyperglycemia and insulin resistance, improvement in mitochondrial function and attenuation of autonomic diabetic neuropathy, so they could be very useful in these patients.

The Hoffman reflex objectively and noninvasively assesses the response of the somatosensory system. A study in subjects with T1D reported that the H reflex evoked by paired pulses in the lower extremities may be an indicator of dysfunction in somatosensory processing at the spinal level. To date, few studies have evaluated the dysfunction of sensory and autonomic fibers simultaneously in DN, so this study would provide valuable data since it additionally includes the evaluation of markers related to oxidative stress and inflammation, in addition to considering the evaluation of symptoms reported by subjects with peripheral and autonomic DN during 3 months of cocoa supplementation through a double-blind, randomized controlled clinical trial.

5. RESEARCH QUESTION

What is the effect of cocoa supplementation on the biochemical and clinical profile and somatosensory processing of diabetic peripheral and autonomic neuropathy?

6. HYPOTHESIS

Cocoa supplementation has a beneficial impact on the biochemical and clinical profile and somatosensory processing of diabetic peripheral and autonomic neuropathy.

7. GENERAL OBJECTIVE

To evaluate the effect of cocoa supplementation on the biochemical and clinical profile, and somatosensory processing of diabetic peripheral and autonomic neuropathy during 3 months of treatment.

7.1 SPECIFIC OBJECTIVES

Biochemical profile:

- To evaluate the effect of cocoa supplementation on the glycemic profile (HbA1c and fasting blood glucose).

- To evaluate the effect of cocoa supplementation on lipid profile (triglycerides, HDL-c, LDL-c and TG/HDL).

- To evaluate the effect of cocoa supplementation on markers of inflammation (neutrophil/lymphocyte ratio).

Clinical profile:

- To evaluate the effect of cocoa supplementation on the “Toronto Clinical Scoring System” score.

- To evaluate the effect of cocoa supplementation on anthropometry (weight, waist and abdominal circumference, waist to height ratio).

- To evaluate the effect of cocoa supplementation on blood pressure.

- To evaluate the effect of cocoa supplementation on quality of life.

- To evaluate the effect of cocoa supplementation on gastrointestinal symptoms.

Somatosensory processing:

- To evaluate the effect of cocoa supplementation on sensorimotor processing in the spinal cord using the RDD of the H reflex test.

8. METHODOLOGY

8.1 Study Type: Explanatory

8.2 Design: Double-blind, randomized controlled clinical trial.

8.3 Study population: Adult subjects aged 40-60 years diagnosed with type 2 diabetes mellitus and diabetic neuropathy who attend, by open call, to Anáhuac México Norte University, the Kidney Specialty Center in the State of Mexico, and the Nueva Oxtotitlán clinic (Toluca).

8.4 Sample Size Calculation:

$$N = \frac{2(Z\alpha + Z\beta^2)s^2}{d^2} = N = \frac{2(1.96 + 0.84^2)(0.84)^2}{(0.776)^2} = N = \frac{(15.68)(0.7056)}{(0.6021)}$$

A total of 18 subjects per group were obtained, considering the following data:

s= Baseline standard deviation: 0.84 (obtained from a study in which probucol and methylcobalamin are used in subjects with DN and the TCSS is used to assess the effect of the intervention.) (49)

d= Proposed difference: 0.776 (refers to the expected difference → 7.5% decrease with respect to the control group)

Zα= 1.96 (value in tables, corresponding to a significance level of 95% for two tails)

Zβ= 0.84 (value in tables, corresponding to a power of 80%)

8.5 Selection criteria

Inclusion Criteria	***Non-inclusion Criteria***
- Adults aged 40-60 years with a diagnosis of type 2 diabetes mellitus (T2D) and diabetic neuropathy - Minimum duration of T2DM diagnosis: 5 years. - MNSI (Michigan Neuropathy Screening Instrument) score ≥ 2. - Both genders. - Must sign the informed consent form	- Subjects with known sensitivity or hyperreactivity to cocoa or any of its components. - Pregnant women. - Subjects with neurological diseases, such as multiple sclerosis, epilepsy, or previous cerebrovascular events. - Subjects with implanted electronic devices, e.g., pacemakers. - Subjects with peripheral nerve damage in the lower extremities caused by trauma.

	- Subjects with a diagnosis of disc herniation below T10. - History/diagnosis of herpes zoster. - Serological diagnosis of rheumatoid arthritis or systemic lupus erythematosus. HIV/AIDS. - Venous insufficiency (C2-C6 class, asymptomatic/symptomatic according to Nicolaides CEAP classification) in lower limbs. - Clinical hypothyroidism. - Use of orthopedic equipment (e.g., prosthetics, splints, wheelchair, crutches). - Cancer diagnosis, use of oncological drugs and/or radiotherapy, or history of cancer or tumors treated with oncological drugs or radiotherapy. - Smoking, considered positive in those with a smoking index greater than 10. - History of diabetic foot (Wagner Scale 3-5) or amputation. - Presence of ulcers and open and/or extensive wounds (>2 cm) - Allodynia - Sciatic nerve root compression - Gout/Hyperuricemia
Elimination Criteria	
- Subjects who modify their pharmacological treatment during the study. - Subjects who do not attend one of the intermediate consultations - Subjects who withdraw informed consent or decide not to continue participating in the	

study

- Subjects with less than 80% adherence to cocoa supplementation
- Subjects who miss more than one consultation or do not attend the final consultation.

8.6 Study Variables

Type	Variable	Conceptual Definition	Operational Definition	Indicator (unity)	Scale
Independent	Cocoa supplementation	Cocoa powder capsules	4 cocoa powder capsules providing 50 mg of flavonoids (12.5 mg per capsule), administering two capsules in the morning and two capsules at night, every day, for three months. (Appendix 2)	Present (supplement) / Absent (placebo)	Qualitative nominal dichotomous
Dependent	Peripheral diabetic neuropathy	Presence of signs and/or symptoms of peripheral nerve dysfunction in people with diabetes, after excluding other causes. (50)	It is obtained after applying the MNSI questionnaire regarding symptoms (filled out by the patient and with a maximum score of 13 points) and the physical examination (evaluated by the researcher) with a maximum score of 13 points. (Appendix 3) (51)	Total sum of points obtained from the application of the MNSI questionnaire No unit	Discrete quantitative
			Toronto Clinical Scoring System: is a clinical evaluation system carried out by the researcher, assigning a score to the symptoms, reflexes and sensory tests. (33, 34) (Appendix 4)	6-8 points: mild DN 9-11 points: moderate DN 12-19 points: severe DN Total sum of points obtained from the application of the TCSS No unit	Qualitative ordinal Discrete quantitative
			H reflex test: This is performed by electrical stimulation, placing the active electrode on the Achilles tendon, the positive electrode above the gastrocnemius and the reference electrode at the height of the gastrocnemius heads. Stimulation is carried out behind the knee (anatomical site of the tibial nerve). The electrical stimulus consists of the application of 10 pulses (1 every 10 seconds) and the maximum intensity applied is according to the sensitivity and tolerance of each individual. (18, 36)	Quotient R2/R1 µV	Discrete quantitative

Dependent	Autonomic diabetic neuropathy	Damage to the nerves that control internal organs, causing alterations in various systems such as the cardiovascular, gastrointestinal and urogenital systems. (20)	- BEST questionnaire: Includes: 1) severity of intestinal symptoms; 2) impact of symptoms on the ability to enjoy everyday things; 3) patient perception of the condition; and 4) impact of symptoms on mood and humor. Scale from 0 to 100 according to the subject's responses. (Appendix 5) (52) -Bristol scale: It is made up of 6 categories that include an image and an explanation, ranging from 1 to 7, with 1 being hard, separated pieces that pass with difficulty and 7 being watery stools. (Appendix 6) (53)	No unit No unit	Continuous quantitative Discrete quantitative
Dependent	Weight	Total body mass of an individual (54)	Weight of an individual in kg determined by the scale. The measurement is carried out without shoes and with the minimum possible clothing. The subject must be placed in the center and remain motionless during the measurement. (54)	kg	Continuous quantitative
Dependent	Waist circumference	Body measurement used as an indicator of central adiposity, as well as cardiovascular risk (55)	The tape is placed in a horizontal plane around the waist, taking the midaxillary line as a reference, locating the midpoint between the lower costal margin and the highest lateral edge of the iliac crest. (55)	cm	Continuous quantitative
Dependent	Waist-to-height ratio (WtH)	Index resulting from dividing the waist circumference by the height. (74)	It is obtained by dividing the waist circumference in cm by the height in cm, with a result of ≥ 0.5 indicating an increased risk for cardiometabolic disease. (74)	No unit	Continuous quantitative
Dependent	Abdominal circumference	Body measurement that directly correlates with visceral fat. (54)	The top of the hip bone and the top of the right iliac crest are located and the tape measure is placed horizontally around the abdomen, at the level of the iliac crest, at the end of a normal expiration. (55)	cm	Continuous quantitative

Dependent	Arterial Pressure	Hydrostatic force of blood on arterial walls resulting from the pumping function of the heart, blood volume, resistance of the arteries to flow, and diameter of the arterial bed. (56)	A sphygmomanometer is used to obtain blood pressure using the technique specified in the Clinical Practice Guidelines for the diagnosis and treatment of arterial hypertension in the primary care level. (57)	mmHg	Continuous quantitative
Dependent	Neutrophil/ lymphocyte ratio	Index resulting from the division of the serum concentration of neutrophils by the serum concentration of lymphocytes. (75)	It is obtained by dividing the serum concentration of the absolute number of neutrophils by the serum concentration of the absolute number of lymphocytes. (75)	No unit	Continuous quantitative
Dependent	Glycemia	Blood glucose concentration (58)	Obtained from laboratory study by spectrophotometry of blood sample, with a fasting period of 8 hours. (59)	mg/dL	Continuous quantitative
Dependent	Glycated hemoglobin (HbA1c)	Value of the fraction of hemoglobin that has glucose attached (58)	Analyzed by high-performance liquid chromatography (HPLC), without a specific fasting period for this test. (62)	%	Continuous quantitative
Dependent	Triglyceridemia	Blood triglyceride concentration (58)	Obtained from a laboratory study by spectrophotometry of the lipid profile of a blood sample, with a 12-hour fasting period. (59)	mg/dL	Continuous quantitative
Dependent	High density cholesterol (HDL-c)	HDL-c concentration in blood.	Obtained from a laboratory study by spectrophotometry of the lipid profile of a blood sample, with a 12-hour fasting period. (59)	mg/dL	Continuous quantitative
Dependent	Low density cholesterol (LDL-c)	LDL-c concentration in blood.	Obtained from a laboratory study using spectrophotometry of the lipid profile of a blood sample, with a fasting period of 12 hours. (59)	mg/dL	Continuous quantitative
Dependent	Triglycerides/ HDL-c ratio (TG/HDL)	Index resulting from the division of the serum concentration of triglycerides by the serum concentration of HDL (63)	It is performed after dividing the serum triglyceride concentration in mg/dL by the serum HDL concentration in mg/dL.(63)	No unit	Continuous quantitative
Dependent	Diabetes 39 questionnaire	Questionnaire designed to measure quality of life in patients with type 1 and type 2 diabetes. (64)	Questionnaire containing 39 closed items grouped into 5 sections: energy and mobility, diabetes control, anxiety-worry, social burden and sexual functioning. Subjects rate their perception of their quality of life	No unit	Discrete quantitative

			in a global manner, with a range from 1 (minimal) to 7 (extremely severe), and the scores obtained are transformed into a scale of 0 to 100. (Appendix 7) (64)		
Confounding	Hypoglycemic drugs	Drugs that lower or regulate glucose. (65)	The use of hypoglycemic drugs will be considered when the patient is taking any drug from the following groups: Sulfonylureas (glibenclamide), biguanides (metformin), glinides, thiazolidinediones or glitazones, GLP-1 analogues, DPP4 inhibitors (sitagliptin) and alpha glucosidase inhibitors (acarbose). (66)	Present/ Absent	Qualitative nominal dichotomous

8.7 STRATEGIC PLAN

Through an open invitation, subjects living with T2D who meet the inclusion criteria will be invited to be assessed and tested at Anáhuac México Norte University, the Kidney Specialty Center, and the Nueva Oxtotitlán Clinic (Toluca), as appropriate. The invitation will be extended, explaining the procedures, duration, and ethical issues; if the subject decides to participate, he or she will be asked to sign the informed consent letter (Annex 1). Once the subject enters the protocol, he or she will be randomized (using a random number table previously generated in a spreadsheet) to the experimental group (diet + capsules with cocoa) or the control group (diet + capsules with methylcellulose).

The study will last three months and patients will be seen on four occasions: at the beginning (baseline measurement), at the first and second month (review appointments) and at the third month (final measurement).

Patients will be asked to attend the appointments on an empty stomach, after having urinated and defecated. Weight will be determined according to the Lohman technique, that is, without shoes and with as few clothes as possible, the individual will be placed in the center of the scale and must remain still during the measurement. (54) Waist circumference will be measured according to the proposal of the Ministry of Health of Mexico: placing the tape in a horizontal plane around the waist, taking as reference the mid-axillary line, locating the midpoint between the lower costal margin and the highest lateral edge of the iliac crest (55) and the abdominal circumference: the upper part of the hip bone and the upper part of the right iliac crest are located and the measuring tape is placed in a horizontal plane around the abdomen, at the level of the iliac crest, at the end of a normal expiration. (55) Likewise, blood pressure will be measured using a sphygmomanometer (Nebucor brand, model HL-888JA), according to the Clinical Practice Guide for the Diagnosis and Treatment of Arterial Hypertension in the first level of care (57). These evaluations will be carried out every month, until completing the three months of the duration of the study. In addition, at the beginning of the study the MNSI (Annex 3) will be applied as part of the diagnosis of DN, and both at the beginning and at the end, the TCSS (Annex 4), the questionnaire as the physical examination (51), the BEST questionnaire (Annex 5), the Bristol scale (Annex 6) and the Diabetes 39 quality of life questionnaire (Annex 7) will be applied and laboratory studies will be taken to know the biochemical parameters (triglyceridemia, HDL-c, LDL-c, glycemia,

HbA1c, TG / HDL index and neutrophil/lymphocyte ratio will be determined, as described in the table of variables.

Participants in both groups, being subjects with a cardiometabolic disease, will be nutritionally assisted, that is, all patients will be given a meal plan according to the characteristics of each one of them, calculated by the Mifflin-St. Jeor energy expenditure prediction formula (67) for subjects with obesity, while, according to the Joslin Diabetes Center, a distribution of 40-45% carbohydrates, 30-40% lipids, and 20-30% protein is suggested. (68) Two capsules will be prescribed – cocoa for the experimental group or methylcellulose for the control group – in the morning and two capsules at night.

8.7.1 H-reflex test

The H-reflex test will be performed by electrical stimulation through disposable surface electrodes (3M) connected to a bipolar constant current electrical stimulator (Digitimer DS8R). Electrophysiological signals will be recorded using surface electrodes (3M) connected to the signal acquisition and amplification system (LabChart and PowerLab 8/35, ADInstruments). The signals obtained will be sampled at 10 KHz with a band-pass filter of 0.5-500 Hz. The signals will be stored in a computer for later analysis. Once the recording and stimulation sites have been identified (see below), the subject's skin will be cleaned with an alcohol swab. Micropore® adhesive tape will be used to improve contact between the electrodes and the subject's skin. The electrodes for stimulation will be placed as follows: the active electrode (anode) at the level of the Achilles tendon, the positive electrode (cathode) above the inverted "V" between the calf muscles (gastrocnemius). The reference electrode will then be placed at the height of the heads of the gastrocnemius. The subject will be stimulated behind the knee, where the tibial nerve runs through its anatomical course. To begin the tests, the subject will be asked to indicate with his hand to the researcher when he perceives a tolerable physical sensation due to the applied current. The test will start with an intensity of 0 mA and then pulses will be given every 0.5 mV until the evoked potential (H reflex) is observed in a consistent and clearly identifiable manner based on the latency (35-45 ms). The electrical stimulus consists of the application of 1 square pulse (1 ms duration each pulse) every 10 seconds (10 pulses in total). The maximum intensity of the applied current will be in accordance with the sensitivity and tolerance of the individual in both lower limbs during the tests on the sensory and motor nerves. The applied electrical

pulse should not cause a painful sensation, but it may cause a tingling sensation. The test will be suspended if the individual reports pain or does not wish to continue with the research protocol. For the recording of the H reflex, the active electrode will be placed between the lateral malleolus and the Achilles tendon. Next, the reference electrode will be marked below it and 14 cm will be measured from the active electrode towards the middle of the calf. The "H" reflex test will be performed in two parts. The first part of the protocol consists of determining the curve of stimulus intensity vs. amplitude of the motor responses from the appearance of the "M" wave and the "H" wave, for which the electric current will be increased in steps of 0.5 μA until the appearance of the waves. For this part, only one electric pulse (1 ms duration) will be given every 10 seconds. The intensity of the electric current that will be used for the second part of the protocol will be that whose value on the curve of amplitude of the H wave-electric current intensity reaches 60% of the maximum amplitude. This stimulation value guarantees the reproducibility and minimum variability of this wave, which also avoids muscle contraction that contaminates the electrical recording. The second part is the paired electrical stimulation test in which two electrical pulses (1 ms duration) will be produced at different frequencies between the pulses: 0.1, 1, 5 and 10 Hz. The interval between the paired pulses will be 10 s, until completing 10 series.

Electrophysiological recordings will be analyzed with Clampfit 10.0 software. The latency and amplitude of the H1 and H2 evoked potentials will be determined for each electrical pulse and at all stimulation frequencies, taking the stimulus artifact as a reference. The ratio of the amplitude of the paired H2/H1 pulses will then be determined to establish the modulation of spinal excitability. A ratio ≥ 0.6 for any stimulation frequency will be considered as an indicator of dysfunction in somatosensory processing according to Marshall et al. (18)

8.8 STATISTICAL ANALYSIS

A descriptive analysis of all dependent variables, as well as demographic variables, will be performed, expressing them as mean ± standard deviation or, where appropriate, as median and range (min-max), according to the normality of the data; nominal variables will be expressed as frequencies. Subsequently, an intragroup inferential analysis will be carried out for weight, waist circumference, abdominal circumference and blood pressure with ANOVA for repeated samples with Tukey *post hoc*, and in case the data distribution does not meet the normality criteria, the corresponding nonparametric test will be performed (e.g.,

Friedman, followed by Dunn's *post hoc*). For biochemical analysis (glycemic and lipid profile and markers of inflammation), as well as the evaluations corresponding to diabetic peripheral and autonomic neuropathy, and quality of life, a Student's T test will be performed for dependent groups, or where appropriate, with Willcoxon.

The intergroup comparison will be made with Student's T test for independent samples, or if applicable, with Mann-Whitney U at each measurement time. A $p < 0.05$ will be considered as statistical significance. All of the above will be carried out using version 5 of the GraphPad Prism software.

9. ETHICAL CONSIDERATIONS

This study complies with the guidelines stipulated in the International Ethical Guidelines for Biomedical Research Involving Human Subjects, in the Declaration of Helsinki (69) and the Nuremberg Code (70), as well as the NOM-012-SSA3-2012. (71) In terms of national regulation, it adheres to the Regulations of the General Health Law on Research, within whose article 17, this protocol is considered research with greater than minimum risk due to the random method of assignment to the intervention scheme. (72) Considering the level of risk, and in accordance with the provisions of article 14, informed consent must be in writing, so each subject who participates in the study, as detailed in the Methodology section, will be informed and explained the procedures to be carried out in order to subsequently record in writing their decision to participate in an informed consent letter, in accordance with Chapter I of the General Health Law regarding the ethical aspects of research in human beings (Annex 1).

Likewise, the protocol will be submitted to the research and Bioethics committees of the Faculty of Health Sciences of the Universidad Anáhuac México Norte.

A nutrition plan will be given for the prevention or correction of obesity and to contribute to metabolic control, as indicated by the Mexican Official Standard 015-SSA2-2010 for the Prevention, Treatment and Control of Diabetes Mellitus. (76)

10. SCHEDULE OF ACTIVITIES

ACTIVITY	August-December 2020	January – December 2021	January – June 2022	July – December 2022	January – June 2023
Submission, evaluation, correction Health Science Faculty Committee protocol	X	X			
Patient recruitment and experimental development		X			
Information coding and emptying in the database		X	X		
Statistical analysis			X		
Preparation of final report				X	
Publication of Results and obtaining a degree					X

11. BUDGET

Institutional financing will be requested (from the CICSA of the UAMN), subject to approval of this document, according to the following breakdown:

Stationery	$4,000
Cocoa capsules	$104,000 *
Placebo capsules	$5300*
ELISA kits (TNFa, IL-6, IL-10, IL-1b)	$128,000
Kits (MDA, carbonils, TAS)	$55,000
Laboratory studies (glycemic and lipidic profile and CRP)	$64,430**
Statistical software (annuity)	$5,600
Disposable electrodes for surface recording	$15,000
Computer, measuring tape	$13,500*
Electrophysiology equipment	$850,000*
Electromyography/nerve conduction velocity studies	$5,000 – 10,000*
TOTAL	$1’254,830.00
TOTAL TO REQUEST	$207,600.00
*** They are already counted on ** They are already included as part of the institutions**	

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13. Appendices

Appendix 1: Informed Consent

Informed Consent

"EVALUATION OF THE EFFECT OF COCOA SUPPLEMENTATION ON THE BIOCHEMICAL AND CLINICAL PROFILE AND SENSORY-MOTOR PROCESSING OF PERIPHERAL AND AUTONOMIC DIABETIC NEUROPATHY: RANDOMIZED CLINICAL TRIAL"

Date: _____

The study you are being invited to participate in will evaluate the effect of cocoa supplementation on diabetic peripheral and autonomic neuropathy over a 3-month period of treatment.

The study will consist of the following: once a month—and until completing 3 months of treatment—you will be asked to come in for a consultation, where the following assessments will be performed: a) routine anthropometric measurements (weight, height, and abdominal circumference), b) blood pressure, c) a noninvasive study—called the paired H-reflex test—to assess changes in your nerves, d) a questionnaire about your quality of life, and f) a scale for the shape and consistency of your bowel movements—called the Bristol scale. None of the above assessments will cause you any side effects or pain.

In addition, laboratory tests will be performed at the start of treatment and at the end of the three months, during which glycated hemoglobin, glucose, insulin, blood lipids, as well as markers of inflammation and oxidative stress will be assessed.

Once these evaluations are completed, you will be provided with an individualized dietary plan and given the capsules to take daily for the duration of the three months. At this point, you should be aware that you may be randomly assigned to receive either the active ingredient capsules or placebo capsules (you won't be able to tell because they both look the same); this information will be disclosed only at the end of the study.

It should be noted that your participation in the study and the tests and treatments provided as part of it will not represent any cost to you; however, you will not receive any payment. Your participation in this study is of great importance to us, but it is not mandatory. You have the right to withdraw this consent at any time, without affecting the care and attention provided at the Institution.

All information you provide for the study will be strictly confidential in accordance with the General Health Law on health research. It will be used only by the project's research team and will not be made available for any other purpose. You will be identified by a number, not your name. The results of this study will be published for scientific purposes, but will be presented in a manner that prevents you from being identified.

We are committed to answering any questions you may have regarding the study procedure at any time (such as evaluation procedures, risks, benefits, etc.), as well as providing you with any information obtained during the study. Finally, all information related to this project will be kept accessible only to the participating team as researchers. The personal information of each participating patient will be available at all times, but will be provided only to you. Therefore, please be assured that your data will be handled confidentially, respecting your anonymity.

We thank you in advance for your cooperation and remain at your disposal for any questions or comments you may have.

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Senior researcher

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PhD candidate in Nutritional Sciences

Phone number: 55 85502972

I,_____I have read and understood the above information, and my questions have been answered satisfactorily. I have been informed and understand the procedures that will be performed, as well as that the results obtained may be published and disseminated for scientific purposes.

Appendix 2: Cocoa capsules

Appendix 3 "*The Michigan Neuropathy Screening Instrument*" (MNSI) Instrument

1.	Are your legs and/or feet numb?	☐ No	☐ Yes
2.	Do you ever have any burning pain in your legs and/or feet?	☐ No	☐ Yes
3.	Are your feet too sensitive to touch?	☐ No	☐ Yes
4.	Do you get muscle cramps in your legs and/or feet?	☐ No	☐ Yes
5.	Do you ever have any prickling feelings in your legs or feet?	☐ No	☐ Yes
6.	Does it hurt when the bed covers touch your skin?	☐ No	☐ Yes
7.	When you get into the tub or shower, are you able to tell the hot water from the cold water?	☐ No	☐ Yes
8.	Have you ever had an open sore on your foot?	☐ No	☐ Yes
9.	Has your doctor ever told you that you have diabetic neuropathy?	☐ No	☐ Yes
10.	Do you feel weak all over most of the time?	☐ No	☐ Yes
11.	Are your symptoms worse at night?	☐ No	☐ Yes
12.	Do your legs hurt when you walk?	☐ No	☐ Yes
13.	Are you able to sense your feet when you walk?	☐ No	☐ Yes
14.	Is the skin on your feet so dry that it cracks open?	☐ No	☐ Yes
15.	Have you ever had an amputation?	☐ No	☐ Yes

Total: _____

Appendix 4: Toronto Clinical Scoring System (TCSS)

Symptom scores	Reflex scores	Sensory test scores
Foot	Knee reflexes	Pinprick
Pain	Ankle reflexes	Temperature
Numbness		Light touch
Tingling		Vibration
Weakness		Position
Ataxia		
Upper-limb symptoms		

Sensory testing was performed on the first toe. Symptom scores: present = 1; absent = 0. Reflex scores: absent = 2; reduced = 1, normal = 0. Sensory test score: abnormal = 1. normal = 0. Total scores range from normal = 0 to maximum of 19.

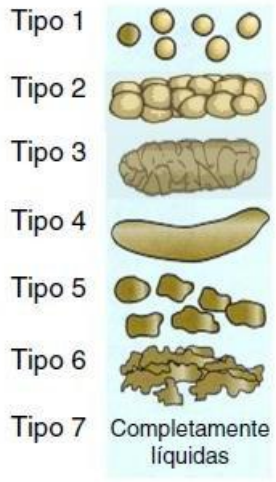
Appendix 5: BEST questionnaire

TABLA 2. Cuestionario BEST para evaluar el impacto de los síntomas de un intestino irritable sobre la CVRS

1. **Durante las últimas 4 semanas, ¿Cómo se ha sentido en relación a su hábito de defecar?**
   - Muy bien: mis deposiciones han sido normales.
   - Bien: apenas ha habido alteraciones en mi ritmo intestinal y, de hecho, las he ignorado.
   - Regular: no he podido ignorar las alteraciones en mi ritmo intestinal.
   - Mal: las alteraciones de mi ritmo intestinal han llegado a afectar a mi estilo de vida.
   - Muy mal: las alteraciones del ritmo intestinal han afectado gravemente a mi estilo de vida.
2. **Lea Vd. la siguiente declaración y diga lo que opina sobre ella: "Los síntomas intestinales que estoy sufriendo significan que algo muy serio está ocurriendo en mi cuerpo".**
   - No, de ninguna manera.
   - Sí, quizás en algún momento, pero muy pocas veces.
   - Sí, algo sí.
   - Sí, bastante.
   - Sí, mucho.
3. **Durante las últimas 4 semanas, ¿con qué frecuencia sus síntomas intestinales han hecho que se sienta tenso o profundamente afectado?**
   - La mayoría del tiempo.
   - En muchas ocasiones.
   - Solo muy de vez en cuando.
   - En ningún momento.
4. **Díganos lo que piensa sobre la siguiente frase: "Debido a los síntomas intestinales que sufro, durante las últimas 4 semanas no he podido disfrutar de las cosas que antes me divertían".**
   - De ninguna manera me ha ocurrido esto.
   - Sí, quizás en algún momento, pero muy pocas veces.
   - Sí, algo sí.
   - Sí, bastante.
   - Sí, mucho.

Las respuestas a estas preguntas se miden en una escala de 0 (el mejor estado de salud) a 100 (el peor estado de salud). La calificación final asignada de acuerdo a un algoritmo estándar se entrega al médico, antes de que éste entre en la consulta. [Spiegel BMR, Naliboff, Mayer E, *et al*. Development and initial validation of a concise point-of-care IBS severity index: the 4-item BEST questionnaire. *Gastroenterology* 2006;130:S1040].

Appendix 6: Bristol Scale



- Tipo 1: Trozos duros separados, como nueces, que pasan con dificultad.
- Tipo 2: Como una salchicha compuesta de fragmentos.
- Tipo 3: Con forma de morcilla con grietas en la superficie.
- Tipo 4: Como una salchicha; o serpiente, lisa y blanda.
- Tipo 5: Trozos de masa pastosa con bordes definidos, que son defecados fácilmente.
- Tipo 6: Fragmentos blandos y esponjosos con bordes irregulares y consistencia pastosa.
- Tipo 7: Acuosa, sin pedazos sólidos, totalmente líquida.

Traducido de: Heaton, KW, Lewis, SJ. Stool form scale as a useful guide to intestinal transit time. *Scandinavian Journal of Gastroenterology* 1997 (32): 9, 920-4

Appendix 7: Quality of Life Questionnaire “Diabetes 39”

La calidad de vida de las personas está afectada por muchas causas. Estas causas pueden incluir el estado de salud, la oportunidad para vacacionar o divertirse, los amigos, la familia o el trabajo. El siguiente cuestionario se diseñó para ayudar a conocer lo que afecta la calidad de vida en las personas con diabetes.

Las siguientes preguntas se relacionan con el grado de afectación que la diabetes le ocasionó en su calidad de vida *durante el último mes*. Se le agradecerá que lea cuidadosamente las siguientes preguntas y conteste colocando una cruz (X) en el cuadro del número que refleje mejor el grado de afectación en su vida respecto a cada una de las preguntas señaladas, tomando en cuenta que el número 1 indica falta de afectación y, al avanzar la numeración, aumenta el grado de afectación en forma progresiva hasta llegar al máximo, que es el número 7, que indica afectación extrema. Si tiene alguna duda, con gusto se le prestará ayuda.

Se le suplica *responder todas las preguntas.*

Durante el último mes, ¿en qué medida se vio afectada la calidad de su vida por las siguientes causas?

1. El horario de los medicamentos para su diabetes

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

2. Preocupaciones por problemas económicos

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

3. Limitación en su nivel de energía

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

4. Seguir el plan indicado por su médico para el tratamiento de la diabetes

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

5. No comer ciertos alimentos para poder controlar su diabetes

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

6. Estar preocupado(a) por su futuro

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

7. Otros problemas de salud aparte de la diabetes

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

8. Tensiones o presiones en su vida

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

9. Sensación de debilidad

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

10. Restricciones sobre la distancia que puede caminar

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

11. Los ejercicios diarios que ha de hacer por su diabetes

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

12. Visión borrosa o pérdida de la visión

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

13. No poder hacer lo que quisiera

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

Durante el último mes, ¿en qué medida se vio afectada la calidad de su vida por las siguientes causas?

14. Tener diabetes

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

15. El descontrol de su azúcar en sangre

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

16. Otras enfermedades aparte de la diabetes

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

17. Hacerse análisis para comprobar sus niveles de azúcar en sangre

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

18. El tiempo requerido para controlar su diabetes

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

19. Las restricciones que su diabetes impone a su familia y amigos

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

20. La vergüenza producida por tener diabetes

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

21. La interferencia de su diabetes en su vida sexual

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

22. Sentirse triste o deprimido

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

23. Problemas con respecto a su capacidad sexual

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

24. Tener bien controlada su diabetes

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

25. Complicaciones debidas a su diabetes

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

26. Hacer cosas que su familia y amigos no hacen

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

27. Tener que anotar sus niveles de azúcar en sangre

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

28. La necesidad de tener que comer a intervalos regulares

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

29. No poder realizar labores domésticas u otros trabajos relacionados con la casa

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

30. Menor interés en su vida sexual

Nada afectada en absoluto	1	2	3	4	5	6	7	Sumamente afectada

Durante el último mes, ¿en qué medida se vio afectada la calidad de su vida por las siguientes causas?

31. Tener que organizar su vida cotidiana alrededor de la diabetes

Nada afectada en absoluto							Sumamente afectada
1	2	3	4	5	6	7	

32. Tener que descansar a menudo

Nada afectada en absoluto							Sumamente afectada
1	2	3	4	5	6	7	

33. Problemas al subir escaleras

Nada afectada en absoluto							Sumamente afectada
1	2	3	4	5	6	7	

34. Dificultades para sus cuidados personales (bañarse, vestirse o usar el sanitario)

Nada afectada en absoluto							Sumamente afectada
1	2	3	4	5	6	7	

35. Tener el sueño intranquilo

Nada afectada en absoluto							Sumamente afectada
1	2	3	4	5	6	7	

36. Andar más despacio que otras personas

Nada afectada en absoluto							Sumamente afectada
1	2	3	4	5	6	7	

37. Ser identificado como diabético

Nada afectada en absoluto							Sumamente afectada
1	2	3	4	5	6	7	

38. Interferencia de la diabetes con su vida familiar

Nada afectada en absoluto							Sumamente afectada
1	2	3	4	5	6	7	

39. La diabetes en general

Nada afectada en absoluto							Sumamente afectada
1	2	3	4	5	6	7	

Calificación global

1. Por favor, marque con una cruz (X) el cuadro que indique la calificación de su calidad devida

Mínima calidad Máxima calidad

1	2	3	4	5	6	7

2. Por favor, marque con una cruz (X) el cuadro que indique lo que usted piensa de la gravedad de sudiabetes

Ninguna gravedad Extremadamente grave

1	2	3	4	5	6	7

Gracias por sus respuestas