# Handbook T-XVIII
## CIERMMI Women in Science
# Health Sciences

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# ECORFAN CIERMMI Women in Science

## Volume XVIII

The Handbook will offer volumes of selected contributions from researchers who contribute to the scientific dissemination activity of the Colegio de Ingenieros en Energías Renovables de Querétaro A.C. in their areas of research in Health Sciences. In addition to having a total evaluation, in the hands of the directors of the Colegio de Ingenieros en Energías Renovables de Querétaro A.C., the quality and timeliness of its chapters, each individual contribution was refereed to international standards (RESEARCH GATE, MENDELEY, GOOGLE SCHOLAR and REDIB), the Handbook thus proposes to the academic community, recent reports on new developments in the most interesting and promising areas of research in the Health Sciences.

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# CIERMMI Women in Science T-XVIII
# Health Sciences
# *Handbooks*

# Prologue

Since time immemorial, women have contributed to science with their sensitivity to visualize the world and its phenomena. It is precisely through the feminine eye that the solutions to the problems of yesteryear and today have found in this perspective an alternative solution in which, of course, integral approaches are part of it.

The recognition to the female researchers who congregate through the contribution of knowledge in the area of health, which, as always, is a reference in science since it implies the improvement of the conditions that humanity faces.

The development of new perspectives that enrich current medical practices is necessary and urgent since there are conditions such as mental health, cancer, air quality, physical care, use of medicinal plants, the context of professional training that cannot wait and require solutions that can be found in these contributions.

In my first collaboration with CIERMMI I have found a wealth of contributions led by women that demonstrate the involvement and evolution of equity practices, in this area, research.

Undoubtedly, the road to equity, equality and equality will be traveled slowly but surely for future generations. The researchers who precede us and those who come will see in these works an inspiration for the new challenges that life demands of us.

*Morado-Huerta, Guadalupe. PhD*
*Universidad Tecnológica de San Juan del Río*

# Introduction

The Colegio de Ingenieros en Energías Renovables de Querétaro A.C. (CIER-QUERÉTARO), and its chapters of Renewable Energy, Industrial Maintenance, Mechatronics and Informatics, technical sponsors of the International Interdisciplinary Congress on Renewable Energy, Maintenance, Mechatronics and Informatics, CIERMMI 2022 has as general objective to establish a space for discussion and reflection on issues related to the areas of: renewable energy, industrial maintenance, mechatronics and informatics with the participation of students, teachers, researchers and national and international speakers, promoting the formation and consolidation of research networks. Contributing to provide a space for dissemination and discussion of the presentations of students, graduates, academics and researchers, representatives of various higher education institutions, research centers in our country, as well as educational institutions beyond our borders. Promoting the formation of research networks between different institutions. Offering a space for undergraduate, master's, doctoral and postdoctoral students, in which they can present the progress of the research they carry out in their different educational centers. Providing a space in which study groups and members of academic bodies, linked to the curricular program of renewable energy, industrial maintenance, mechatronics and computer science careers, can present the research work developed within their institution and in collaboration with other national or international educational institutions. Establishing a training space for the attendees, through the development of specific lectures and conferences.

This volume, Women in Science T-XVIII-2022 contains 8 refereed chapters dealing with these issues, chosen from among the contributions, we gathered some researchers and graduate students from the 32 states of our country. We thank the reviewers for their feedback that contributed greatly in improving the book chapters for publication in these proceedings by reviewing the manuscripts that were submitted.

*CARDENAS, ALTAMIRANO, ARREOLA and RESÉNDEZ* introduce the Modelling of human polyglutamine neurological disorders in *Drosophila*, *DÍAZ, GONZÁLEZ, UVALLE and MEDEROS* analyse the association between triglycerides and insulin resistance as a predictor of cardiometabolic diseases in university students; *REYES, ROMERO, MIGUEL and FERNANDEZ* present the use of medicinal plants by dentists in the state of Guerrero, Mexico; *MERAZ, HERNÁNDEZ, GARCÍA and CÁRDENAS* are investigating the association between sleep quality and executive functions in a sample of first-semester medical students at a public university; *VALLEJO, ROJAS, VERDUGO and LIMON* welcome us to the omics era: proteomics importance in cancer research; *CERÓN, CERÓN, LARA and VICHIQUE* submit diurnal variation and health risk of atmospheric aromatic hydrocarbons concentrations in an urban site located in Nuevo Leon, Mexico; *QUITL & JIMÉNEZ* expose mental health and family dynamics in university students from Tlaxcala after confinement. Finally, *SALGADO, AGUILAR, CRUZ and CÉLIS* study the effects of a training program for fitness instructors based on STD and BPN on the attitude of users of a private university gym.

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# Chapter 1 Modelling of human polyglutamine neurological disorders in *Drosophila*

# Capítulo 1 Modelado de enfermedades neurológicas por expansión de glutaminas en *Drosophila*

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**Abstract**

Polyglutamine (PolyQ) expansion diseases are a family of autosomal dominant neurodegenerative disorders that includes Huntington's disease and spinocerebellar ataxias. These diseases are caused by an abnormal number of glutamines repeats in the affected proteins. Different in vitro and in vivo models have been developed to study these diseases; in this review, we will focus on the fruit fly, *Drosophila melanogaster*, as a model organism to study PolyQ diseases in humans, resulting in a better understanding of PolyQ pathologies and opening avenues to potential therapeutical treatments.

**Neurodegenerative disorders, *Drosophila melanogaster*, Animal models, Autosomal dominant diseases, Polyglutamine expansions**

**Resumen**

Las enfermedades causadas por expansiones de poliglutaminas (PolyQ) son una familia de enfermedades neurodegenerativas autosómicas dominantes que incluyen la enfermedad de Huntington y las ataxias espinocerebelares. Estas enfermedades están causadas por un numero anormal de repeticiones de glutamina en las proteínas afectadas. Diferentes modelos in vitro e in vivo han sido desarrollados para estudiar estas enfermedades; en esta revisión, nos enfocaremos en la mosca de la fruta, *Drosophila melanogaster*, como modelo para el estudio de las enfermedades PolyQ en humanos, obteniendo un mejor entendimiento de estas patologías y abriendo nuevas avenidas para terapias potenciales.

**Enfermedades neurodegenerativas, *Drosophila melanogaster*, Modelos animales, Enfermedades autosómicas dominantes, Enfermedades poliglutamínicas**

## 1.1 Introduction

Polyglutamine (PolyQ) expansion diseases encompass a family of forty autosomal dominant neurodegenerative disorders that affect neurons in the cerebral cortex, basal ganglia, the cerebellum, and sometimes the retina (Shao and Diamond, 2007; Srinivasan *et al*., 2023). These disorders share symptoms like chorea, ataxia, weakness, cognitive impairment, and eye degeneration among others (Takahashi *et al*., 2010). The most widely known PolyQ disease is Huntington's Disease (HD), which affects approximately 41 000 people in the United States (Huntington's Disease Society of America, 2022), and 8000 people in Mexico (Instituto Nacional de Neurología y Neurocirugía MVS, 2018). Other PolyQ diseases are spinal bulbar muscular atrophy (SBMA), dentatorubral-pallidoluysian atrophy (DRPLA), and seven different spinocerebral ataxias (SCA) classified as 1, 2, 3, 6, 7, 8 and 17, depending on the affected protein (Shao & Diamond, 2007). SCA3, SCA2, and SCA6 are the most common ataxias worldwide; SCA17 is the rarest (Salas-Vargas *et al*., 2015; McIntosh *et al*., 2021). SCA7, although rare, has a high prevalence in Veracruz, México due to a founder effect, and it accounts for 7.4% of all SCA cases in the country (García-Velázquez *et al*., 2013; Salas-Vargas *et al*., 2015).

These pathologies are caused by an increased number of CAG (glutamine) repeats in the affected proteins; healthy proteins contain between 35–50 glutamine repeats, whereas mutated proteins contain between 40 to more than 100 repeats (Table 1.1) (Cohen-Carmon and Meshorer, 2012). These expansions are unstable and, consequently, the number of repeats changes between different cells in the same individual. These diseases also show "genetic anticipation" meaning that the number of repeats increases with every generation, and with it the onset and severity of the disease (Cohen-Carmon and Meshorer, 2012; Goswami *et al*., 2022; Srinivasan *et al*., 2023). In some cases, a non-pathological allele can expand to become pathological within a generation (McIntosh *et al*., 2021, Goswami *et al*., 2022).

**Table 1.1** PolyQ expansions in human polyglutamine diseases

| Disease | Protein affected | PolyQ repeat length | |
|---|---|---|---|
| | | Normal | Pathogenic |
| Huntington´s disease (HD) | Huntingtin (HTT) | 6-35 | 36-180 |
| Spinal and bulbar muscular atrophy (SBMA) | Androgen receptor (AR) | 9-36 | 38-65 |
| Dentatorubral-pallidoluysian atrophy (DRPLA) | Atrophin 1 (ATN1) | 6-36 | 49-80 |
| Spinocerebellar ataxia type 1 (SCA1) | Ataxin 1 (ATXN1) | 6-39 | 39-83 |
| Spinocerebellar ataxia type 2 (SCA2) | Ataxin 2 (ATXN2) | 14-32 | 32-200 |
| Spinocerebellar ataxia type 3 (SCA3) | Ataxin 3 (ATXN3) | 12-41 | 55-84 |
| Spinocerebellar ataxia type 6 (SCA6) | Calcium channel α1A subunit (CACNA1A) | 4-19 | 20-33 |
| Spinocerebellar ataxia type 7 (SCA7) | Ataxin 7 (ATXN7) | 4-35 | 37-306 |
| Spinocerebellar ataxia type 8 (SCA8) | Ataxin 8 (ATXN8) | 15-50 | 54-250 |
| Spinocerebellar ataxia type 17 (SCA17) | TATA-binding protein (TBP) | 25-44 | 46-63 |

The molecular mechanism behind PolyQ diseases is not completely understood, but it is clear that the PolyQ regions are the common denominator for the diseases, given that the proteins involved differ in sequence, structure, and biological function (McIntosh *et al*., 2021). It is thought that, just like in the case of Alzheimer's Disease and Parkinson's Disease, protein aggregates might play a role in the toxicity that causes development of neurodegeneration (Takahashi *et al*., 2010). In this case, the conformational changes brought on by the PolyQ extensions would cause misfolded proteins to combine, and in turn, these aggregates would either sequester proteins, leading to a loss of function, or interfere with nuclear or neuronal function (Takahashi *et al*., 2010; McIntosh *et al*., 2021). Takahashi and colleagues (2010) claim that aggregation alone does not explain the pathological features of PolyQ diseases, but rather the interaction between mutated proteins with other proteins in the different neurons affected.

In Huntington's disease, the mutant huntingtin protein (mHTT) has an abnormally expanded polyglutamine repeat. Mutant huntingtin causes neuronal dysfunction and death due to aggregates that cause cell toxicity and interfere with neural processes like axonal transport, transcription, translation, and synaptic function. Medium spiny neurons (MSNs) of the striatum are the most affected by mHTT, resulting in the clinical marks of this disease like motor disturbances with prominent chorea in the early stages. Cognitive issues may present many years before symptoms start to show, and they are characterized by impaired visuospatial and executive function, processing speed, and emotion recognition (McColgan and Tabrizi 2017). It was recently reported that HD also affects peripheral tissues via a crosstalk with the nervous system (Gómez-Jaramillo, 2022)

Spinal and bulbar muscular atrophy (SBMA) is a rare hereditary lower motor neuron disease characterized by progressive muscular weakness. This was the first evidence of a pathogenic expanded trinucleotide repeat causing a disease. The mutation responsible occurs in the androgen receptor gene (AR) with an expanded trinucleotide repeat (CAG > 37). In SBMA, toxicity is caused by the formation of nuclear inclusions of the mutant receptor. This impairs AR function, affecting both motor neurons and muscles, and causing endocrine manifestations such as gynecomastia and infertility (Breza and Koutsis, 2018).

Dentatorubral-pallidoluysian atrophy (DRPLA) is an autosomal dominant hereditary ataxia caused by mutation in the Atrophin-1 (ATN1) gene with a CAG trinucleotide repeat expansion (≥48 tandem copies). DRPLA symptoms include epilepsy, ataxia, choreoathetosis, and dementia. Clinical presentation varies: in younger people, the disease is often characterized by seizures, and it more commonly presents with ataxia and cognitive impairment in older patients. Clinical symptoms are progressive, with life expectancy typically 8–16 years (Tsuji, 2012).

Spinocerebellar ataxias (SCAs) are a genetically heterogeneous conditions that can be inherited, and are autosomal dominant or recessive (Orr, 2012; Paulson *et al*., 2017). Patients present a cerebellar syndrome marked by loss of balance and coordination accompanied by cortical symptoms (seizures, cognitive impairment), peripheral neuropathy and slurred speech; onset most often occurs in adult life (Manto and Marmolino, 2009). SCA mutations cause cerebellar atrophy by prominent damage to Purkinje neurons. About 30 of these conditions have been described, however, only seven —SCA1, SCA2, SCA3, SCA6, SCA7, SCA8 and SCA17—are caused by an expanded polyglutamine region in the protein affected. The PolyQ proteins tend to accumulate in the nucleus or cytoplasm of cells, affecting gene expression, organization and cell function, and other functions. Some mutant PolyQ proteins responsible for SCAs are ataxin-1, -2, -3, -7, and -8 (SCA1, SCA2, SCA3, SCA7, and SCA8), the α1A subunit of the calcium channel CACNA1A (SCA6), and the TATA-binding protein (TBP) in SCA17 (Orr and Zoghbi, 2007; Chongtham and Agrawal, 2016).

## 1.2 Structure of PolyQ aggregates

The conformation of the PolyQ expanded aggregates was discovered in 1994 by Perutz and collaborators; they reported that chemically synthesized expanded PolyQs, under normal physiological conditions, form rich aggregates of properly folded a-sheet structures (Figure 1.1). These a-sheet-rich monomers are preceded by a helical conformation and followed by a random coil that forms soluble oligomers. In the expanded protein, the PolyQ stretch folds into β-sheet-rich monomers that form soluble oligomers that aggregate in an insoluble amyloid-like structure (Perutz *et al*., 1994; Minakawa and Nagai 2021; Mier & Andrade-Navarro 2021). Normally, PolyQ fibers with a single, 20-glutamine helical twist are unstable and therefore removed, but a double stretch of 40 glutamines is held together by polar zippers formed by hydrogen bonds between amides of successive turns (Perutz *et al*., 1994). It has been described that α-helical coiled-coil structures also contribute to PolyQ protein toxicity (Fiumara *et al*., 2010: Kwon *et al*., 2018). It is not yet clear whether the aggregates adopt this conformation before or after polymerization, that is, whether the transition to a β-sheet conformation causes the PolyQ oligomerization or whether PolyQ oligomerization causes the conformational change (Hoffner and DJian, 2015).

**Figure 1.1** Expanded PolyQ aggregation and toxicity. A) Schematic representation of a protein containing a normal PolyQ extension, which is properly folded into an α-helix to perform its cellular functions in the brain. B) A disease-causing protein with an expanded PolyQ region undergoes aberrant folding from an α-helix-rich structure into a β-sheet-rich conformation state, followed by the formation of insoluble aggregates with amyloid-like structures. The insoluble aggregates lead to neural cell toxicity, and eventually neurodegeneration
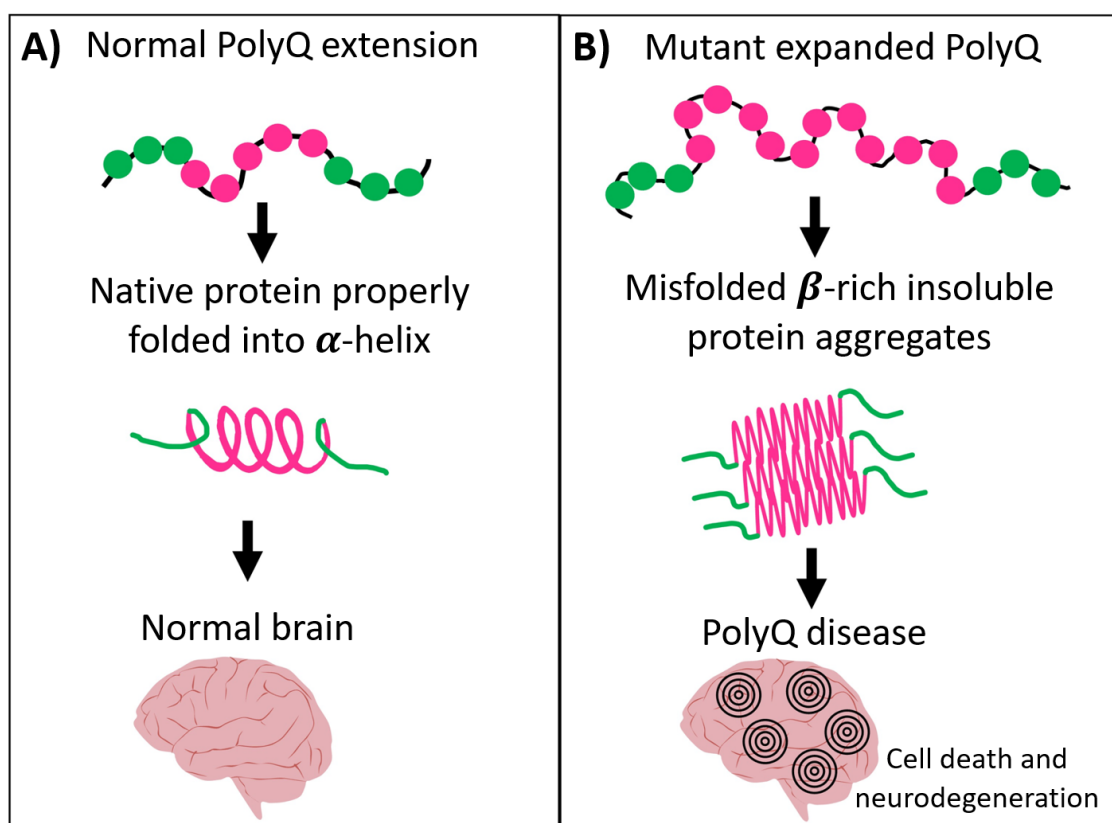
Different microscopy techniques have been used to elucidate additional structures that could be present in these protein aggregates; this may contribute to describe not only structure or toxicity features, but also how the aggregates are formed within cells (Legleiter *et al.*, 2010; Nucifora *et al.*, 2012; Olshina *et al.*, 2010).

PolyQ diseases are being studied with in vitro and in vivo models. Microarrays and fluorescence resonance energy transfer (FRET) have been used to study gene expression and the interactions of mutated proteins in living cells, respectively (Luthi-Carter *et al.*, 2002; Takahashi *et al.*, 2010). Pluripotent stem cells are also a promising model to study these diseases (Cohen-Carmon & Mesroher, 2012; Naphade *et al.*, 2019). Transgenic mice have been used to study spinocerebellar ataxias (Burright *et al.*, 1995; Bichelmeier *et al.*, 2007; Watase, 2014; Meierhofer *et al.*, 2016) and Huntington's disease (Stack *et al.*, 2005, Brooks & Dunnett, 2013, Yang *et al.*, 2017, Dunnett & Brooks, 2018, Farshim & Bates, 2018, Kosior & Leavitt, 2018, Back *et al.*, 2021). The fruit fly, *Drosophila melanogaster*, has also been used as a model to replicate the phenotypic characteristics of PolyQ diseases (Koon & Chan, 2017; Rosas-Arellano *et al.*, 2018,)

Model organisms have greatly increased our understanding of these pathologies, and in the development of potential treatments. In this review, we will focus on the fruit fly, *Drosophila melanogaster*, as an idoneous model organism for neurodegenerative diseases.

### 1.3 *Drosophila* as a model for neurodegenerative diseases

In 1900, entomologist Charles W. Woodworth proposed using *Drosophila melanogaster* as a genetic model organism. Nine years later, Prof. Morgan established his lab for genetic experiments using the fruit fly (Sturtevant, 1959). Since then, hundreds of studies have been carried out with *D. melanogaster*, consolidating it as a model organism and expanding the knowledge in crucial matters such as genes, chromosomes, and inheritance of genetic information. Its success as a model organism also relies on its small size, easy husbandry, and inexpensive maintenance and manipulation in the laboratory: *D. melanogaster* has a rapid life cycle and produces large amounts of genetically identical progeny in 10 days at 25 °C, generating large amounts of data for statistical analyses. Another valuable characteristic is its external development which facilitates visualization (Yamaguchi & Yoshida, 2018).

Genes between fruit flies and humans are highly conserved, moreover, the similarity does not stop at the genetic level: *D. melanogaster* possesses a complex nervous system and behaviors including social activity, learning and memory. Additionally, about 75% of the genes involved in human diseases have their homologue in *D. melanogaster* (Yamamoto *et al.*, 2014), making it an excellent prospect for studying neuronal dysfunction and neuronal death derived from various neurodegenerative diseases (Chan & Bonini, 2000).

Some neurodegenerative disorders studied in *Drosophila* include Alzheimer, Parkinson, tauopathies, ALS, prions (PrD), dystonia, noncoding expansions (SCA8, myotonic dystrophy), some recessive disorders including fragile X syndrome and Friedreich's ataxia, and various polyglutamine disorders (Huntington disease, SCA1, SCA3, and spinobulbar muscular atrophy) (Xu *et al.*, 2015; Tzou *et al.*, 2022).

Modeling of human neurodegenerative diseases in *D. melanogaster* is achieved through the "humanization" of the fly, which is modified using the UAS/GAL4 binary system (Brand & Perrimon, 1993) to express the pathogenic human protein related to the disease in question (Marsh & Thompson, 2006). Once "humanized", *D. melanogaster* offers multiple advantages to study the molecular and cellular mechanisms involved in the disease to be studied: well-known anatomy, well-characterized gene promoters, and a wide variety of mutants (Venken & Bellen, 2005). In the next section, we will describe some *Drosophila* models for PolyQ diseases

**1.4 *Drosophila* as a model for PolyQ diseases**

The first PolyQ diseases modeled in *D. melanogaster* were SCA1 and SCA3 (Warrick *et al*., 1998; Fernandez-Funez *et al*., 2000). The modeling of SCA1 in *D. melanogaster* elucidated the genetic and molecular mechanisms underlying neuronal degeneration by expressing the human SCA1 gene in *Drosophila*. According to the experiments, high levels of wild-type ATXN1 were found to cause degenerative phenotypes like those caused by expanded PolyQ proteins. In addition, the research team corroborated that flies that expressed high-levels of wild-type ATXN1 shared the same toxic effects of the ones expressing the ATXN1 protein with an expanded PolyQ region. This was patent in the negative geotaxis assay, were both cohorts of flies were unable to fly as high as the flies in the control group (Fernandez-Funez *et al*., 2000). Recently, by performing a cross-species genetic screening, a total of 22 mutant regulators of ATXN1 were described in *Drosophila melanogaster*. Among them, transglutaminase 5 (TG5) stood out; in this case, TG5 preferentially regulated mutant ATXN1 over the wild-type protein. In physiological conditions, TG enzymes catalyze the cross-linking of ATXN1 in a polyQ-length-dependent manner, modulating mutant ATXN1 stability and oligomerization. When Tg expression was silenced in a *Drosophila* SCA1 model, mutant ATXN1 toxicity was modulated (Lee *et al*., 2022).

Another *D. melanogaster* model was created for SCA3, where only a segment of the ATXN3 protein containing the expanded polyglutamine region was expressed in the flies. This time, however, the protein was expressed in the photoreceptor neurons. This experiment allowed the researchers to observe the degeneration of the eye tissues in the fruit fly, which indicated cell degeneration. The most affected fly was the one expressing the protein with 78 glutamines, which presented abnormally thin and severely depigmented eyes, due to severe loss of eye cells (Xu *et al*., 2015). Another possible mechanism involved in SCA3 pathogenesis was thought to be non-AUG translation or also known as RAN translation which has been documented in polyglutamine (polyQ) disorders. However, when studied in a SCA3/MJD *Drosophila* model, there was no unconventional translation in fly neurons or glia (Johnson *et al*., 2022).

Another model for SCA7 was attempted in *D. melanogaster* but the expanded PolyQ ATXN7 protein remained stable regardless of the context in which it was expressed. Consequently, it was posited that, during evolution, selective pressure allowed *D. melanogaster* to develop robust mechanisms to maintain PolyQs within a controlled range, which has not been observed in mammals (Jackson *et al*., 2005).

Additionally, Jun Ma's group established a model of SCA17 in *Drosophila*, in which hTBP34, 54 and 80Q was expressed in the eye. Transgenic flies expressing a mutant hTBP protein with an expanded PolyQ tract (hTBP80Q) show progressive degeneration in the ommatidia of the eye, a characteristic that was extrapolated with the neurodegeneration present in the brains of SCA17 patients. The authors focused mainly on gene expression, which they measured with PolyQ, found deregulations in transcription that suggested that the activity of the transcription factor Su(H) is involved in the pathological progression in SCA17 patients (Ren *et al*., 2011).

SMBA has also been modeled in *D. melanogaster*, where it was observed that the androgen receptor (AR) protein with the expanded PolyQ region can translocate to the nucleus and activate AR-dependent pathway transcription. *Drosophila melanogaster* loses motor neurons when the expanded PolyQ protein is expressed, hence, showing loss of climbing ability and affected gait (Nedelsky et al., 2010).

Last but not least, Huntington's disease PolyQ has also been modeled in the fruit fly. This model displayed the same phenotypes as the other PolyQ: the degeneration of the eye and loss of climbing ability. Fly lines with the expanded PolyQs HD-Q75 and HD-Q120 had normal eye morphology and intact ommatids on hatching day (day 0), but by day 10, many rhabdomeres were disrupted, with more obvious and severe degeneration in HD-Q120 flies than in HD-Q75 (Zhang et al., 2010).

## 1.5 Humanizing the fly

The "humanization" of flies—their capacity to express human proteins—is possible thanks to a system called UAS-GAL4. Andrea Brand and Norbert Perrimon standardized this method in flies in 1993 and it has been used to study the expression of genes ever since. The system consists of crossing a GAL4 producer line or driver and a UAS reporter line. The producer line encodes and expresses the GAL4 trans-activator protein under the control of a tissue-specific promoter or enhancer; the reporter line contains the gene encoding the protein of interest under the control of UAS (Upstream Activator Sequence) sequences where GAL4 binds to activate transcription (Fig. 1.2). When crossing both flies' lines, the progeny that contains both transgenes, in a tissue-specific manner, will express the GAL4 protein which in turn will bind to the UAS sequence and promote the transcription of the gene of interest only in the regions of the promoter's specificity.

**Figure 1.2** Expression of human TBP gene through the UAS/GAL4 binary system. The GAL4 gene encodes a trans-activator protein introduced into *D. melanogaster* genome and its expression is regulated by a tissue-specific promoter in the producer line that will express GAL4 in specific tissues and cells. In this case, because GMR is the promoter, GAL4 will be expressed in the eye neurons of *Drosophila melanogaster,* where it will bind to the UAS sequences and allow the expression of hTBP 34Q or hTBP 80Q
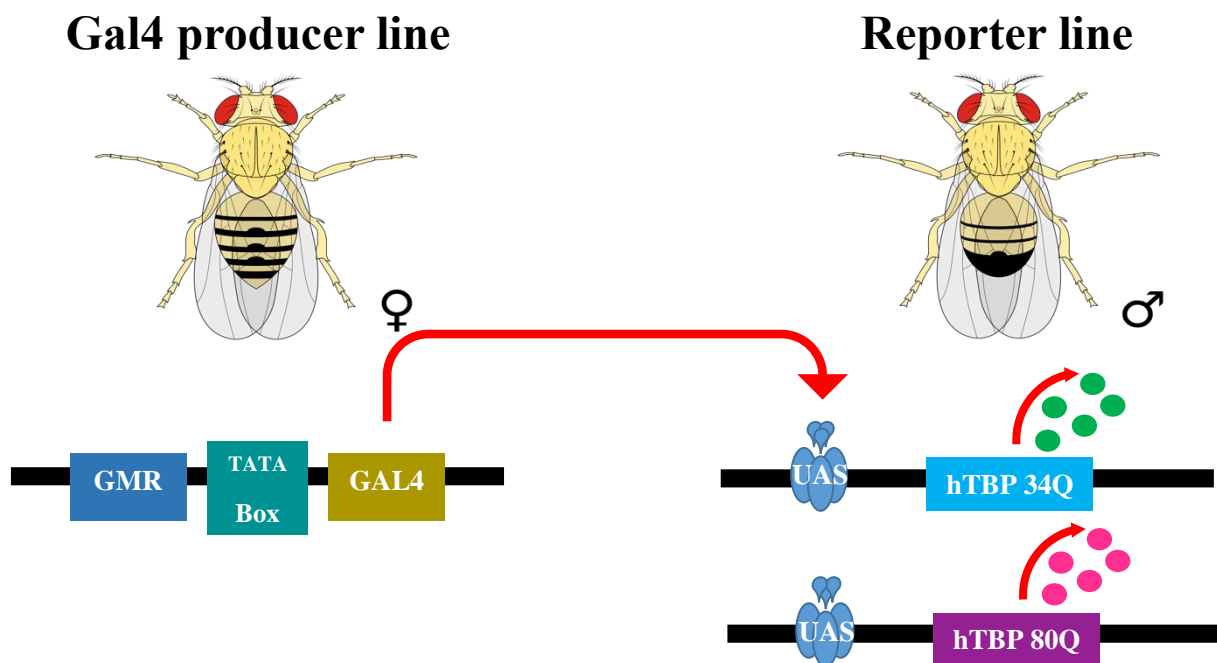


In our laboratory, we modeled SCA17 in *D. melanogaster* to determine the cytotoxic effect of elongated glutamines in the TATA binding-protein (TBP). Using the UAS-Gal4 system (Fig. 1.2), we generated flies that, in their eyes, expressed human TBP with 34 (hTBP 34Q) and 80 (hTBP 80Q) glutamines and evaluated them for 50 days. We found that, in the flies with hTBP 80Q, the eyes degenerated more as the fly aged, as shown by eye color decrease and sometimes tissue disorganization (Fig. 1.3) (Cárdenas-Tueme, 2017).

**Figure 1.3** Expression of hTBP34Q and hTBP80Q targeting the eyes of *Drosophila melanogaster*. Brightfield micrographs of the *Drosophila* eye show that GMR-directed expression of hTBP 34Q and hTBP 80Q causes impairments in the fruit fly eye. (A-B) Expression of hTBP 34Q at different times: Day 1 and 50 days after hatching, the phenotype caused by hTBP 34Q is not aggressive, a slight persistent discoloration is observed throughout the days in the perimeter of the eye. (C-D) Expression of hTBP 80Q at 1 and 50 days after hatching. (C) On day 1, depigmentation is seen around the eye's perimeter. (D) The discoloration has spread towards the center of the eye at day 50, so the hTBP 80Q protein appears to be more toxic
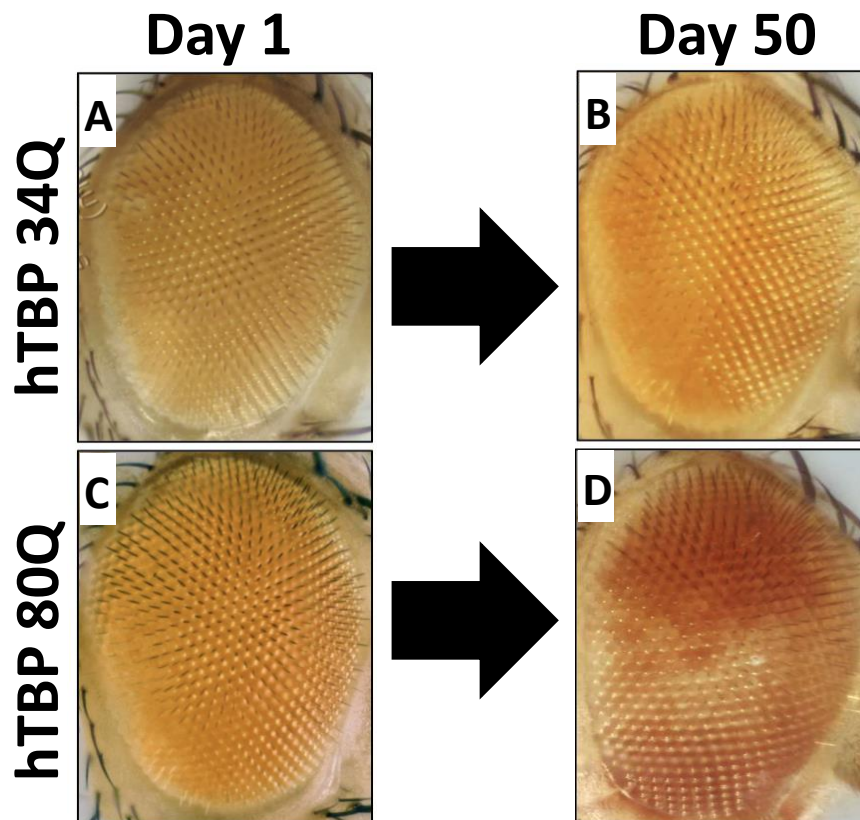


## 1.6 Drug screening in *Drosophila melanogaster*

Drug development has high failure rates, is extremely expensive (Wouters *et al*., 2018), and usually encompasses a long design and testing process. Alternative approaches for drug discovery, such as the use of *D. melanogaster* as a model to test new drugs, would greatly benefit the pharmaceutical industry.

For instance, the efficacy of diverse drugs or chemical compounds against neurological diseases can be studied in *Drosophila* (Lawal *et al*., 2014). Humanized *Drosophila* may help to determine the adequacy of a drug at an early stage before it is tested in more expensive rodent assays and in clinical trials.

The easiest and most common way to do this is to keep 10-15 flies in a vial and add the chemical or drug directly to the food where the flies are kept. The flies will be monitored for several days, and it will be documented whether the flies exposed to the chemical/drug have a shorter life expectancy than those that were not exposed. Behavioral changes, such as flying, can also be assessed. Furthermore, thanks to the genome structure of the fly and its reduced genetic redundancy, the molecular mechanism of drug action will be easier to elucidate.

Even though *Drosophila* holds promise for drug discovery, there are limitations, particularly related to toxicity and pharmacodynamics that deserve attention, however this can be resolved sooner rather than later to benefit from this model organism.

## 1.7 Acknowledgements

## 1.8 Funding

## 1.9 Conclusions

Neurodegenerative diseases, including the polyQ disorders such as Huntington disease and spinocerebellar ataxias share the feature of abnormal protein accumulation. Here we contend that *Drosophila* is a promising model of polyQ diseases including HD, SCA1, SCA3 and SCA17 in the study of the pathological effects of polyQ expansions. In our lab, we created a SCA17 model in *Drosophila* using an expansion of a polyQ repeat in the TBP transcriptional factor. The transgenic flies expressing an expanded polyQ hTBP protein exhibited progressive neurodegeneration very similar to SCA17 patients. The use of animal models, such as *Drosophila* may also open new therapeutic avenues for the diagnosis and treatment of PolyQ diseases.

Since polyQ diseases remain uncurable, modeling of these diseases in organisms such as *Drosophila* presents great potential and advantages to examine numerous therapeutic strategies effective against polyQ diseases. Humanizing the fly allows to test pharmacological and biological molecules to block the early events of the aggregation process and inclusions, as well as degradation of toxic proteins and regulation of cellular function.

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# Chapter 2 Association between triglycerides and insulin resistance as a predictor of cardiometabolic diseases in university students

# Capítulo 2 Asociación de triglicéridos con resistencia a la insulina como predictor de enfermedades cardiometabólicas en universitarios

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**Abstract**

Cardiometabolic diseases represents the main cause of death in the world and several risk factors influence in different way. On the other hand, those risk factors appear at younger ages each time. One common factor is the insulin resistance and dyslipidemia. Some studies have suggested that higher levels of triglycerides are an independent risk factor for insulin resistance and in the future the possible development of diabetes and cardiovascular diseases. We performed a cross sectional study. Obtaining data of 189 university students from the faculty of pharmacy. Which 30% presented insulin resistance and 17% hypertriglyceridemia. We obtained an OR of 3.890 (IC 1.711-8.45; $p<0.0004$). Identify at early stage the possible risk factors for cardiometabolic disease comes important for the prevention of the same disease.

**Triglycerides, Cardiovascular, Resistance**

**Resumen**

Las enfermedades cardiometabólicas representan la principal causa de muerte en el mundo y los diversos factores de riesgo influyen en diferente magnitud. Por otro lado, estos factores se presentan cada vez a más temprana edad. Un común denominador de estos padecimientos es la resistencia a la insulina y las dislipidemias. Diversos estudios han sugerido que niveles elevados de triglicéridos son un factor de riesgo independiente de resistencia a la insulina y en un futuro el posible desarrollo de diabetes y enfermedades cardiovasculares. Se realizó un estudio de tipo transversal analítico. Se obtuvieron datos de 189 estudiantes universitarios de la Facultad de Farmacia. De estos el 30 % presentó resistencia a la insulina y el 17% hipertrigliceridemia. Se obtuvo un OR de 3.890 (IC 1.771-8.545; $p <0.0004$). Identificar de manera temprana posibles factores de riesgo modificables de enfermedades cardiometabólicas resulta de vital importancia para la prevención temprana de las mismas.

**Triglicéridos, Cardiovascular, Resistencia**

**2.1 Introduction**

The prevalence of cardio-metabolic diseases and their associated consequences is currently increasing (Weschenfelder *et al.*, 2020). Of these, cardiovascular diseases (CVD) constitute a major public health problem. In the last decade, the number of deaths from CVD has increased worldwide by 12.5% over the previous decade (Joseph P. *et al.,* 2020). Diseases of the cardiovascular system are now the leading cause of death in the world's adult population (World Health Organization, 2017).

In some regions of the world, including Latin America, life expectancy at birth continues to increase; however, the quality of life in many cases does not increase at the same rate, due to the large number of health complications that go from chronic diseases, caused by the consumption of diets high in saturated fats to smoking and sedentary lifestyles; these facts become more common and result in high mortality from CVD (Pan American Health Organization, 2017).

CVD is multifactorial in origin and one risk factor must be considered to interfere in the context of others. Cardiovascular risk factors (CVRFs) are a set of biological characteristics or behaviors that, in those individuals who exhibit them, increase the likelihood of developing or dying from CVD (Baeradeh *et al.*, 2022). The WHO considers smoking, a sedentary lifestyle, a diet low in fiber and high in cholesterol and saturated fat, DM2, dyslipidemia and high blood pressure as the main modifiable cardiometabolic risk factors (Wu Y. *et al.*, 2014). Of these factors, dyslipidemia, and insulin resistance as a precursor to DM2, are two factors that are related and could themselves have an impact on CVD.

**2.1.1 Dyslipidemia**

Dyslipidemia is one of the major cardiovascular risk factors (Kopin *et al.*, 2017). It is characterized by increased levels of triglycerides (TG) and/or low-density lipoproteins (LDL), in addition to decreased levels of high-density lipoproteins (HDL). (Lui & Li, 2014). In Mexico, the most common dyslipidemia is increased triglycerides known as hypertriglyceridemia. In the last National Health and Nutrition Survey (ENSANUT) it was reported that 57.5% of the population had triglyceride levels above 150 mg/dL. (National Institute of Public Health, 2019).

## 2.1.2 Hypertriglyceridemia

Hypertriglyceridemia (HTG) is a common abnormality of lipid metabolism (Pedragosa, et al 2013). It is often clinically silent and is most frequently found during screening of patients without any symptoms. According to the most recent clinical guidelines, triglyceride levels are considered normal if they are less than 150 mg/dL, borderline high if 150 to 199 mg/dL, high if 200 to 499 mg/dL, and very high if 500 mg/dL or higher. (Elkins & Friedich, 2018).

It is a disorder usually accompanied by other lipid profile alterations and is currently a cardiovascular risk factor that should be investigated in at-risk individuals. Early detection of high plasma triglyceride concentrations in children, adolescents and young adults is of paramount importance to prevent future cardiovascular events (González-Sandoval *et al.*, 2014). Recent evidence suggests that this pathology is strongly associated with an insulin resistance (IR) profile, since it favors lipogenesis and there tends to be an elevation of plasma triglyceride levels (Fiuza *et al.*, 2018). The mechanisms by which this situation occurs have been empirically demonstrated and encourage further research into both pathologies.

## 2.1.3 Insulin resistance

Insulin resistance (IR) is a metabolic disorder characterized by an attenuated biological response to the action of insulin. It is defined as a state in which an increased amount of insulin is required to produce a normal biological response to a glucose load, i.e., compensatory hyperinsulinism develops. (Centers for Disease Control and Prevention, 2017).

The development of IR is influenced by genetic and environmental factors, such as a high-trans-fat, high-calorie diet, obesity, ageing and sedentary lifestyle. It results in decreased glucose uptake by skeletal muscle and adipose tissue cells, decreased hepatic glycogen production (glycogenolysis) and increased hepatic glucose production (glycogenolysis) (Martínez Basila *et al.* 2011).

The standard method to identify IR is the hyperglycemic clamp method, however, due to its high cost and difficulty to perform, the HOMA index has been proposed as a suitable model to assess and determine IR at an affordable cost.

## 2.1.4 HOMA Index

The Homeostasis Model Assessment (HOMA) index was proposed by Mathews et al in 1985. It is the most widely used method for diagnosing IR in humans and was derived from a mathematical equation relating β-pancreatic cell function and fasting glucose and insulin concentrations. The equation is calibrated with a β-cell function assumed to be 100% and with a normal RI of 1 (Martinez-Basila *et al.*, 2011) according to the following formula:

**Figure 1.1** Insulin resistance HOMA formula

$$HOMA - IR = \frac{\text{insulina } plasmática \text{ en ayuno (mU/L)} \times \text{glucosa plasmática en ayuno (mmol/L)}}{22.5}$$

*Source: Martínez, 2011*

The HOMA index can also be used to assess β-pancreatic cell function. Thus, the HOMA model is used to estimate insulin sensitivity and β-cell function. This is because plasma insulin and glucose concentrations have a relationship in the basal state and reflect the balance between hepatic glucose and insulin secretion that is maintained by feedback between the liver and β-cells (Wallace *et al.*, 2004).

HOMA index values in a population of Mexican descent are considered normal when they are less than 2.60. However, values between 2.60 and 3.80 refer to moderate IR, while values above 3.80 represent severe IR (Qu et al. 2011). The growing increase in metabolic diseases such as those mentioned above, especially in the young population, has increased interest in studying this population and the possible factors that could influence the development of these diseases.

University students have been specifically singled out as a population of interest for reasons such as age, educational level, lifestyles associated with long hours of study and class attendance, among others. Students from the health field, have the potential, soon, to apply their knowledge in society (Maldonado et al, 2013).

Students are subjected to conditions when entering the university system. They are at a critical stage in the development of their eating habits, characterized by little time to eat, frequent skipping of meals, eating between meals, high consumption of fast food, among others. In addition to this, there is a decrease in physical activity due to the rise of passive entertainment. Furthermore, there has been an increase in the prevalence of tobacco and alcohol consumption. These unhealthy lifestyles are contributing to the development of cardio-metabolic risk factors in university students (Kim & Park, 2016)

There is a high prevalence of risk factors for chronic non-communicable diseases in university students, including overweight and obesity, insulin resistance especially in women and hypertriglyceridemia in men (Morales, 2013). Early identification of possible modifiable risk factors for cardio-metabolic diseases is of vital importance for their early prevention. Therefore, the aim of this study was to look for the possible association between two risk factors such as hypertriglyceridemia and insulin resistance as a possible predictor of cardio-metabolic risk.

## 2.2 Materials and Methods

Students from the Centro Universitario de Ciencias Exactas e Ingenierías of the University of Guadalajara, Mexico, with the following characteristics was recruit: aged between 18 and 25, with 10 to 12 hours of fasting, were recruited for this study. They attended to the Biochemistry Laboratory, where personal data was registered, a clinical history was filled out and somatometric measurements were obtained. At the end, a blood sample was taken.

Transversal analytic study type. The next parameters were determined.

- Body Mass Index. Weight and height were measured using a TANITA30A scale that automatically calculates the BMI, which was used to characterize the population as normal weight, overweight and obese, considering: 19 - 24.9 kg/m$^2$, 25 - 29.9 kg/m$^2$ and $\geq$ 30 kg/m$^2$ BMI, respectively.

- Serum analysis of total cholesterol (TC), triglycerides and glucose were measurement using a dry chemist technique in the FujiFilm DriChem NX500i equipment. LDL was calculate using Friedwald formula.

- The determination of insulin was using a ELISA sandwich type technique in the Magpi Luminex equipment. A cut off point of >150 mg/dL was considered for hypertriglyceridemia and for insulin resistance HOMA index > 2.5 was used.

## 2.3 Results

We recruit a total of 189 students, 61% of whom were female and the rest male. Table 2.1 shows the general characteristics obtained, separated by gender, and the percentage of students with alterations in triglycerides and insulin resistance.

**Table 2.1** Descriptive anthropometric and biochemical results

| Variable | Women n= 116 | Men n= 73 | Impaired (%) |
|---|---|---|---|
| Age (years) | 20.52 ± 1.44 | 21.0 ± 1.91 | |
| BMI (Kg/m$^2$) | 24.8 ± 5.26 | 28.11 ± 6.48 | %67 (127) |
| Total Cholesterol (mg/dL) | 168.5 ± 34.57 | 162.34 ± 34.30 | |
| **Triglycerides (mg/dL)** | **97.99 ± 51.03** | **112.90 ± 55.36** | **%17 (33)** |
| HDL (mg/dL) | 50.13 ± 13.03 | 40.3 ± 9.18 | |
| LDL (mg/dL) | 99.61 ± 26.06 | 95.14 ± 38.47 | |
| Glucose (mg/dL) | 83.61 ± 7.99 | 85.55 ± 8.82 | |
| Insulin (mU/L) | 10.28 ± 0.44 | 12.51 ± 9.62 | |
| **HOMA (mU*mmol/L$^2$)** | **2.06 ±1.54** | **2.55 ± 1.86** | **%30 (57)** |

Table 2.1 shows that the results are within normal parameters, except for the BMI in men, which is on average in the overweight range. It can also be seen that men have the highest HOMA levels, slightly above the normal range. A prevalence of hypertriglyceridemia of 17% was found in our population. 34% of which were women, the rest men. Insulin resistance was diagnosed in 57 volunteers, which corresponds to a prevalence of 30%. Of these, 49% were female. In addition, 9.5% had both disorders.

The association between IR and hypertriglyceridemia was performed. An Odds Ratio (OR) of 3.890 p<0.0004 (CI 1.771-8.545) was obtained. This result shows that the presence of elevated triglyceride levels can be considered per se a cardio-metabolic risk factor, since it increases the likelihood of insulin resistance to a great extent, that along with these two factors, represent an increased risk of these diseases.

## 2.4 Discussion

The relevance of this study lies in the population being studied, although these are apparently healthy young university students, the frequency of dyslipidemias was observed to be high. In addition, a correlation was found between plasma triglyceride values and the HOMA index of insulin resistance in non-diabetic males. Fasting hypertriglyceridemia is an abnormality commonly found in insulin-resistant subjects and is frequently associated with other elements of insulin-resistant dyslipidemia.

There are explanations for this close association, as mechanisms have been demonstrated by which insulin resistance leads to hypertriglyceridemia and there are also mechanisms by which the degree of insulin resistance increases. Visceral adipocytes are generally more insulin resistant, so they show marked lipolysis and an increased flow of fatty acids to the liver. The increase in fatty acids has two consequences in the liver: firstly, it increases the synthesis of triglycerides and secondly, it favors the synthesis of very low-density lipoproteins (VLDL) that pass into the blood in large quantities and cause hypertriglyceridemia (Miguel Soca et al., 2011).

The increase in triglycerides in the blood is possibly due to a decrease in the clearance of VLDL, triglyceride-rich lipoproteins that are removed from the circulation by an endothelial enzyme, whose activity depends on insulin and is reduced in insulin resistance. Hypertriglyceridemia affects the lipoprotein pattern by promoting lipid exchange between VLDL and high-density lipoproteins (HDL), through the action of cholesteryl ester transfer protein. In this exchange, triglycerides are transferred to HDL and cholesteryl esters to VLDL, enriching the triglyceride content of HDL and increasing the cholesterol content of VLDL. Triglyceride-rich HDL are more susceptible to degradation by hepatic lipase, which reduces their blood concentrations and affects the role of these lipoproteins in reverse cholesterol transport, the main cardio-protective mechanism of HDL (Tilg et al., 2008).

Resistance to insulin action (decreased response to the hormone) in skeletal muscle is one of the first detectable defects in humans with type 2 diabetes. Obesity is the most important risk factor for the development of this pathology and specifically central fat deposits (visceral obesity). Initially, insulin resistance generates compensatory mechanisms so that, for a certain period, insulin hypersecretion keeps blood glucose levels under control. This period, which could be called pre-diabetic, is difficult to detect because blood glucose levels remain within normal limits. However, this situation deteriorates until pancreatic failure occurs, which is when the beta cells are no longer able to maintain insulin hypersecretion, begin to deteriorate and this deterioration leads to reduced insulin secretion. This is the point at which most cases of type 2 diabetes mellitus and metabolic syndrome start to be diagnosed (Genoni et al., 2017).

The progression of insulin resistance not only leads to type 2 diabetes, but if appropriate measures are not taken, patients will become insulin dependent. Although the etiology of resistance is not yet clearly established, it is thought that there is a polygenic genetic component that is mediated by the environment. In this sense, the lifestyle with little physical exercise and constant availability of food, which is the case in our society, seems to be responsible for the escalation in recent years in the incidence of insulin resistance related diseases. More than 90% of diabetics are classified as type 2. According to the World Health Organization, the number of people worldwide affected by this disease has tripled in the last 20 years. Forecasts for the not-too-distant future are that almost 30% of the population will develop insulin resistance and its complications during their lifetime (Benito M., 2014).

Insulin resistance is associated with hyperinsulinemia, hypertension, dyslipidemia, and is a risk factor for cardiovascular disease, including in children. It is for this reason that the diagnosis of young people with insulin resistance has been proposed as a strategy to identify those at high risk of becoming diabetic (Haymond, 2003), since in the pathophysiology of type 2 diabetes mellitus, the first step is the presence of insulin resistance.

Insulin resistance and the so-called metabolic syndrome where hypertriglyceridemia is implicated as a primary factor, are constituted as two complex pathological entities, which represent a real challenge for clinicians and specialists in terms of their proper definition and diagnosis. Even today, with advanced technological resources and well-established clinical principles, it is necessary to continue the research into the pathophysiological mechanisms and the most effective and safe therapeutic approaches to these entities.

The basis of these primary prevention measures, so far, continues to be lifestyle modifications (weight reduction, low-calorie diet, and exercise); in addition, an individual assessment should be performed in populations at higher risk (family history of diabetes, macrocosmic product, central obesity, arterial hypertension, glucose intolerance and hypertriglyceridemia and some other early markers) that may lead to the presence of hyperinsulinemia and metabolic syndrome. The presence of both hypertriglyceridemia and insulin resistance, and their probable complications, in all population groups compromises the present and future health of people, which is why it is considered a public health problem and therefore requires the attention not only of health professionals but also of the economic and political authorities of our entity.

## 2.5 Conclusions

–   Higher BMI, triglyceride, glucose, insulin and HOMA values were observed in males.

–   Similarly, females have a lower prevalence of hypertriglyceridemia. However, they are almost equally prevalent as the male gender in terms of insulin resistance.

–   With these data, we can conclude that hypertriglyceridemia can indeed be associated with insulin resistance. The volunteers were young students, reason for which it is necessary to insist on a lifestyle improvement, because despite their young age, some severe disorders have been observed, which will have repercussions in their adult life.

## 2.6 Funding

## 2.7 Conflicts of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this document.

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# Chapter 3 Use of medicinal plants by dentists in the state of Guerrero, Mexico

# Capítulo 3 Uso de plantas medicinales por odontólogos en el estado de Guerrero, México

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**Abstract**

Objective: To identify some medicinal plants used by dentists in their clinical practice for the treatment of oral diseases Methods: A survey was applied to identify the use of plants by dentists in the state Guerrero state, the oral pathologies in which they are applied, and frequency of use Results: 21% (22) of the dentists use medicinal plants in their clinical practice, 77% of the dentists who use plants, report having obtained knowledge by local tradition Contribution: 22 plants were identified for use in pathologies oral, the most used was the clove with 27% (6) and the paulillo with 18% (4). Conclusion: 21% of the dentists in the state of Guerrero who participated in the study use medicinal plants in their professional practice based mostly on the knowledge of traditional medicine. The sociodemographic characteristics of the dentists did not show differences between the use or not of medicinal plants.

**Medicinal plants, Dentistry, Oral pathologies**

**Resumen**

Objetivo: Identificar algunas plantas medicinales utilizadas por los odontólogos en su práctica clínica para el tratamiento de enfermedades bucales Métodos: Se aplicó una encuesta para identificar el uso de plantas por parte de los odontólogos del estado de Guerrero, las patologías orales en las que se aplican y la frecuencia de uso Resultados: El 21% (22) de los odontólogos utilizan plantas medicinales en su práctica clínica, el 77% de los odontólogos que utilizan plantas, reportan haber obtenido el conocimiento por tradición local Contribución: Se identificaron 22 plantas para su uso en patologías orales, la más utilizada fue el clavo de olor con 27% (6) y el paulillo con 18% (4). Conclusión: El 21% de los odontólogos del estado de Guerrero que participaron en el estudio utilizan plantas medicinales en su práctica profesional basándose principalmente en el conocimiento de la medicina tradicional. Las características sociodemográficas de los odontólogos no mostraron diferencias entre el uso o no de plantas medicinales.

**Plantas medicinales, Odontólogos, Patologías orales**

**3.1 Introduction**

We will explain in general the importance of the use of medicinal plants, as it seems to be in apogee nowadays, however, we must remember that their use has been proposed since ancient times, the use of plants has been documented by ancient civilisations such as the Chinese and Indian cultures, for the treatment of different diseases, interestingly both countries have integrated traditional medicine in their national health programmes. Mexico has an ancient tradition in the use of medicinal plants, in fact it is considered that after China, our country has the largest number of inventoried medicinal plants" it is also noteworthy that 80% of the Mexican population makes frequent use of herbal medicine, it is estimated a record of 4, 500 plants of which 3,000 are registered in the IMSS Herbarium, Mexico also has the Herbal Pharmacopoeia of the United Mexican States (FHEUM), a document issued by the Ministry of Health that sets out the general methods of analysis and technical specifications that plants and their derivatives used in the production of herbal medicines and remedies must comply with (Biodiversidad, 2022; Pharmacopoeia, 2022). Plants have been used since prehistoric times as medicines; health among cultures such as the Nahuatl and Mayan was obtained with treatments that required natural alternatives to heal and treat illnesses. With the arrival of the Spanish in Mexico in the 16th century, Fray Bernardino de Sahagún, who was interested in the myths, customs and, of course, the diseases and plant resources that were used to treat the health of the population, compiled in the book Historia general de las cosas de la Nueva España, written in 1548, the names in Nahuatl of the plants that were used for certain ailments. Many of the uses survive today thanks to the application of herbal medicine, which is a basic resource in the cure of the most common illnesses. Thanks to the fact that Mexico has a very diverse flora, its herbal medicine is one of the most varied, as it includes hundreds of home remedies that are used in infusions, poultices or in salads, to cure different ailments. Herbal medicine is based on the presence of chemical compounds with pharmacological actions in plant species, which constitute the primary ingredients that pharmaceutical laboratories use in their patented commercial medicines (Government of Mexico, 2020).

It is important to know about this topic because the familiarity that the population has with the use of medicinal plants has led to the increasing use of herbal medicine as an adjuvant in the treatment of different pathologies. This makes us think that health professionals may be using traditional medicine for the treatment or adjuvant to treat various pathologies, even sometimes on a daily basis, such as the use of cloves for dental pain.

The added value of this work is that by identifying the medicinal plants used by dentists, it will allow us to link herbal medicine with clinical practice. It can also guide basic science researchers in investigating the effects of plants that may not have been reviewed.

Ethnobotanical studies have an interdisciplinary impact ranging from natural, medical-clinical and social sciences, reviewing not only the spiritual effects of plants but how to apply them to our benefit for health recovery.

Applying ethnobotanical surveys allows us to know how the clinician adopts traditional medicine for the benefit of patients either as a complement to allopathic medicine or even as the sole or main treatment.

The use of medicinal plants has a direct impact on one of the most important problems of our time, which is bacterial resistance to antimicrobials used today, and it should be noted that there are bacteria that are resistant to practically all antimicrobials, and this is not a problem that will disappear soon. Identifying the components that have antimicrobial activity against microorganisms associated with oral pathologies can help to provide alternatives or targets for the development of new pharmaceuticals.

Furthermore, knowing the anaesthetic or anti-inflammatory properties of these plants would allow us to use them locally in pathologies such as reversible pulpitis, improving the prognosis and treatment of various inflammatory pathologies of dental origin and even having a systemic impact, improving the patient's quality of life.

The problem to be solved in this research is to answer whether dental surgeons in the state of Guerrero use medicinal plants in their clinical practice for the treatment of oral diseases, and whether some of the socio-demographic characteristics of the dentists influence whether or not they prescribe medicinal plants, to know their doses and frequency of application; starting from the central hypothesis that clinical dentistry is supported by traditional medicine for the treatment of some oral diseases, which is why some dentists still use herbal medicine.

This chapter consists of the following sections:

3.2 Description of the method, where we will discuss the type of study and the design of the data collection instruments.

3.3 Results, where we present the results expressed in simple frequencies, a logistic regression analysis to see whether or not the socio-demographic characteristics of dental surgeons influence the prescription of medicinal plants, and tables showing in detail the use of plants, dosage, frequency and form of prescription.

3.4 Discussion, in which we contrast our results with those obtained by other authors in similar studies.

3.5 Conclusions, where we set out the main findings of the present work.

3.6 References.

**3.2 Description of the method**

A descriptive cross-sectional study was carried out between July 2021 and May 2022, using convenience sampling. 104 dentists from different localities in the state of Guerrero were interviewed with prior informed consent. A survey was designed to identify the use of medicinal plants, the part that is used and the pathology that is treated with these plants, as well as the demographic characteristics of the population. The main conditions of the oral cavity treated with plants, the form of application and frequency of treatments were also considered.

**3.3 Results**

A total of 104 dentists residing in the state of Guerrero, Mexico were interviewed, ranging in age from 24 to 65 years with a mean of 37 and SD + 8. Of these, 52% (54) were women and 48% (50) were men. The surveyed population resides in 20 different localities in the state, 51% (53) are from Acapulco, and 20% (21) from Chilpancingo. Graphic 3.1 shows the distribution of 80 localities, the participating dentists were grouped according to the region to which the locality of residence belongs, for this study Chilpancingo was considered independent because it is the capital of the state and an urban area, however individuals from Tixtla and Zumpango were interviewed, also belonging to the Central Zone; From the Costa Grande, places of residence included Papanoa, Zihuatanejo, Atoyac and San Luis la Loma; from the Costa Chica, San Marcos, Tlacochistlahuaca, Ometepec, Cuajinicuilapa, Cruz Grande and Copala; from the Zona Norte, Iguala and Taxco; and from the Montaña Alta, Chilapa and Metlatónoc.

**Graphic 3.1** Place of residence
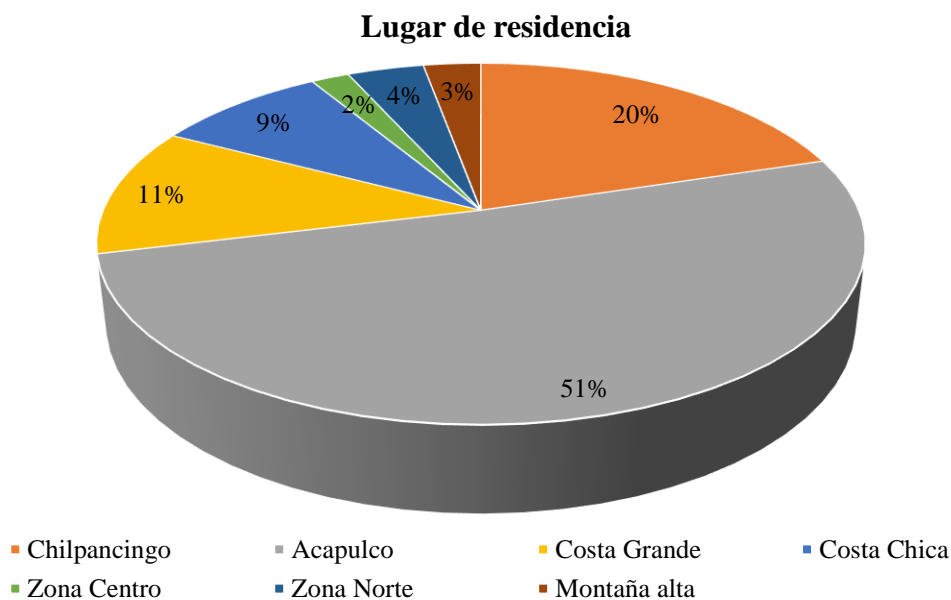


*Source: Own elaboration*

Table 3.1 shows the socio-demographic characteristics of the population studied, and an analysis of differences to determine whether the use of medicinal plants is influenced by age, gender, place of residence, the university where they studied, whether they work in the public or private sector or both; we can see that there are no differences between these variables and those who use medicinal plants or do not use them.

**Table 3.1** Socio-demographic characteristics of the dentists interviewed classified by the use or non-use of medicinal plants in their dental practice. For age the median is shown (p25-p75), as there is no normal distribution the p-value was calculated by the Mann Whitney test, for the rest of the variables the number (n) and percentages (%) the p-value was calculated by the $X^2$ test

| Characteristic | | Total n=104 (100%) | Use plants n= 22 (21%) | Do not use plants n= 82 (79 %) | p-value |
|---|---|---|---|---|---|
| **Age** | | 36 (32-40) | 37 (33-41) | 35.5 (32-40) | 0.5 |
| **Gender** | Female | 54 (52%) | 12 (22.2%) | 42 (77.8%) | 0.7 |
| | Male | 50 (48%) | 10 (20%) | 40 (80%) | |
| **Place of residence** | Urban | 74 (71%) | 16 (21.6%) | 58 (78.4%) | 0.8 |
| | Rural | 30 (28%) | 6 (20%) | 24 (80%) | |
| **University where you studied** | Universidad Autónoma de Guerrero | 82 (79%) | 18 (22%) | 64 (78%) | 0.7 |
| | Universidad Nacional Autónoma de México Benemérita | 4 (4%) | 1 (25%) | 3 (75%) | |
| | Universidad Autónoma de Puebla | 3 (3%) | 1 (33.3%) | 2 (66.7%) | |
| | Universidad Autónoma Metropolitana | 3 (3%) | 1 (33.3%) | 2 (66.7%) | |
| | Others | 12 (11%) | 1 (8.3%) | 11 (91.7%) | |
| **Degree of studies** | General Dentist | 33 (31.7%) | 7 (21.2%) | 26 (78.8%) | 0.9 |
| | Diploma | 10 (9.6%) | 1 (10%) | 9 (90%) | |
| | Speciality | 43 (41.4%) | 10 (23.3%) | 33 (76.7%) | |
| | Master's degree | 14 (13.5%) | 3 (21.4%) | 11 (78.6%) | |
| | Doctorate | 4 (3.8%) | 1 (25%) | 3 (75%) | |
| | Periodontology | 5 (8.2%) | 0 (0) | 5 (100%) | 0.4 |
| **Area of specialisation** | Endodontics | 11 (18%) | 2 (18.2%) | 9 (81.8%) | |
| | Orthodontics | 15 (24.6%) | 6 (40%) | 9 (60%) | |
| | Pathology | 2 (3.3%) | 0 (0%) | 2 (100%) | |
| | Surgery | 5 (8.2%) | 1 (20%) | 4 (80%) | |
| | Implantology | 10 (16.4%) | 3 (30%) | 7 (70%) | |
| | Rehabilitation | 3 (4.9%) | 0 (0%) | 3 (100%) | |
| | Paediatric dentistry | 3 (4.9%) | 0 (0%) | 3 (100%) | |
| | Other | 7 (11.4%) | 1 (14.3%) | 6 (85.7%) | |
| **Sector of work** | Public | 14 (13.5%) | 0 (0) | 14 (00%) | 0.1 |
| | Private | 65 (62.5%) | 16 (24.6%) | 49 (75.4%) | |
| | Both | 25 (24%) | 6 (24%) | 19 (76%) | |

*Source: Own elaboration*

Of the dentists interviewed, 21% (22) use medicinal plants in their clinical practice; a total of 22 species were identified, with cloves being the most used with 27% (6), followed by paulillo with 18% (4). Table 3.2 shows the distribution of plants used by the dentists, their scientific name, common name, the pathology they treat, the part of the plant they use and how they are used.

**Table 3.2** Use of plants by dental surgeons in the state of Guerrero. It shows: scientific name of the plant, common name of the plant, pathology treated with the plant, part of the plant used, frequency of use of the plant, quantity of the plant used, state where the plant is used, number and percentage of dentists who use the plant

| Scientific name | Common name | Use | Part used | Form of use | Frequency of use | Quantity | Status |
|---|---|---|---|---|---|---|---|
| *Syzygium aromaticum* | Clove | Pain, inflammation | Fruit, Seed | Infusion, powder, poultice, rinse, ointment | 1 time, 2 times | Little, Slightly, Slightly, Medium | Dry |
| *Rauvolfia tetraphylla* | Daffodil | Periodontitis, Periodontal abscesses, Periodontal abscesses, Inflammation, Scarring | Leaves, Flowers, Stem, Peel or bark, Fruit | Infusion, rinse, decoction | 2 times, 3 times | A little, Quite a lot, A lot | Fresh |
| *Arnica montana* | Arnica | Healing, Inflammation, Inflammation, Aphthous ulcers, pain | Leaves, Flowers | Infusion, rinse, decoction | 2 times, 3 times | Quite a lot, Medium | Fresh, Dry |
| *Chamaemelum nobile* | Chamomile | Inflammation, Aphthous ulcers, Erosions on oral mucosa, Pain | Leaves, Flowers, Stem | Infusion, Rinse | 3 times, 1 time | A little, Quite a lot | Fresh, Dry |
| *Lavandula angustifolia* | Lavender | Healing | Root | Infusion | 1 time | A little | Fresh |
| *Salvia rosmarinus* | Rosemary | Periodontal abscesses | Leaves | Infusion, Rinse | 1 time | Medium | Dry |
| *Laurus nobilis* | Laurel | Inflammation | Leaves | Rinse | 3 times | Medium | Dry |
| *Allium sativum* | Garlic | Pain, Candidiasis, Periodontal disease | Fruit, Sap | Infusion, Juice, Powder, Ointment, Rinsing | 1 time | A little | Fresh |
| *Aloe vera* | Aloe | Wounds, gingival erosions, Inflammation, Scarring | Sap, Stem | Poultice, Ointment, Ointment, Ointment, Juice | 2 times | Fairly, Medium | Fresh |
| *Amphipterygium adstringens* | Quachalalate | Mobility, Periodontal Disease, Inflammation | Leaves, Bark | Infusion, Rinse | 1 time, 3 times | A little | Fresh |
| *Quercus xalapensis* | Holm oak | Pain | Husk or Bark | Rinse, Infusion | 2 times | Slight | Dry |
| Cannabis | Marijuana | Inflammation, Pain | Leaves | Infusion | 1 time | A little | Dry |
| *Cinnamomum verum* | Cinnamon | Ulcers | Bark | Infusion, Rinse | 1 time | Medium | Dry |
| *Eysenhardtia polystachya* | Palo azul | Healing | Leaves | Rinse | 3 times | Medium | Fresh |
| *Dracaena draco* | Drago | Inflammation, Healing, Periodontal disease | Stem | Infusion, Rinse | 2 times | Little, Medium | Dry, Fresh |
| *Sesamum indicum* | Sesame | Inflammation, Healing, Canker sores, Periodontal disease | Seed | Powder (Local) | 4 times | Fairly | Dry |
| *Citrus limon* | Lemon | Inflammation, Canker sores | Fruit | Juice | 1 time | Medium | Fresh |
| *Rubus ulmifolius* | Blackberry | Periodontal disease | Fruit | Juice | 1 time | Medium | Freso |
| *Dysphania ambrosioides* | Epazote | Pain, Inflammation, Healing, Periodontal disease | Leaves | Infusion, Rinse | 3 times | Slightly | Fresh |
| *Mentha spicata* | Peppermint | Halitosis, Pain | Leaves | Rinse | 2 times | A little | Fresh |
| *Thymus vulgaris* | Thyme | Periodontal disease | Sap | Powder, ointment | 2 times | Slightly | Dry |

*Source: Own elaboration*

It was analyzed which oral pathologies are treated with medicinal plants, among which inflammation, healing and pain stand out. Graphic 3.2.

**Graphic 3.2** Oral pathologies treated with medicinal plants. 14 of the medicinal plants were used to treat inflammation, 10 to treat scarring, 9 for pain, 4 for periodontal disease, 3 for canker sores and 2 for candidiasis

**Oral diseases treated with medicinal plants**
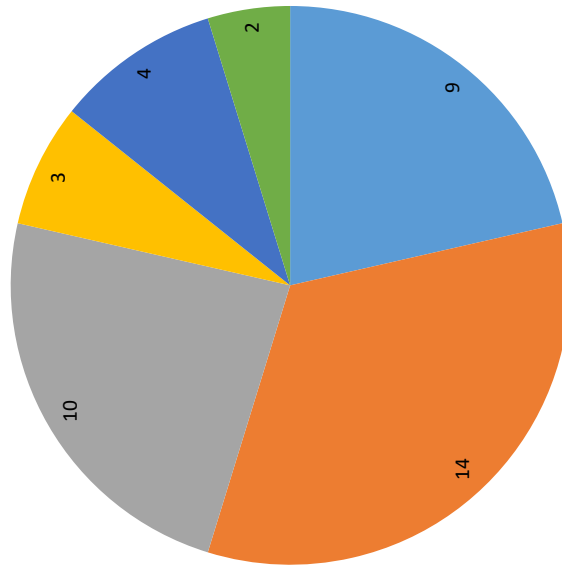


*Source: Own elaboration*

With regard to knowledge of the use of these plants, 77% of the dentists who used plants reported having obtained knowledge of the use of plants through local tradition, family members or the recommendation of a colleague; only 23% reported having obtained knowledge of the use of plants from the university, scientific articles or a course on herbal medicine in dentistry. They were also asked whether they had used the plant alone or in combination, of which 68% (15) used it alone and 32% (7) combined it with different substances, table 3.3.

**Table 3.3** Substances with which plants are mixed for the treatment of oral pathologies

| Substance | Frequency |
|---|---|
| Analgesic | 1 |
| Antibiotic | 1 |
| Anti-inflammatory | 1 |
| Alcohol | 1 |
| ZOE | 1 |
| Metronidazole | 1 |
| With another plant | 2 |

*Source: Own elaboration*

Of the dentists who use plants in their clinical practice, 91% (20) use plants as an adjuvant, but 9% (2) use them as a treatment. Interestingly, although 21% of the dentists use plants, 54% (52) consider the use of medicinal plants to be justified.

**3.4 Discussion**

The use of herbal remedies has assumed a global dimension, culminating in their use in the treatment of various ailments in both developed and developing countries. However, only a few of these herbs have been approved for their medicinal properties, a vast majority of them in natural form. Kumar, G. (2013). It should not be overlooked that plants have been shown to have various metabolites with therapeutic activity, so much so that many of these are the main source (active ingredient) of various drugs.

In this study, 22 medicinal plants applied for dental use were identified, which are used alone or in combination with another plant or patent medicine, in which case the effect that the drug could provide should be considered. The use of plants in this study population is not only based on scientific evidence (articles), but mainly on traditions or advice from patients and relatives, so, although the use of plants seems to have a beneficial effect, it is recommended to review the scientific evidence to know both the beneficial and toxic effects of each of the recommended plants, as it is erroneously considered as a general belief that herbal medicines are benign and do not cause severe toxicity, however, the use of medicinal herbs can cause severe toxicity and even death. Kumar, G. (2013)

In this research it was found that the most used plants are cloves, this is different from what is reported by some authors such as Montoya (2011), which can be explained due to the region to which the interviewed professionals belong, However, several plants used coincide with those reported by Waizel-Bucay (2007) and Waizel-Bucay (2011), such as garlic and aloe vera; however, not all of them were reported for the same condition. D., (2020) in Mérida, Venezuela, obtaining similarities in plants such as garlic, aloe vera, chamomile, chamomile, mint, cloves, among others.

It is striking that almost a quarter of the dentists interviewed (21%) use medicinal plants, mostly based on empirical practices of traditional medicine, although there is scientific evidence of the benefits they provide. The diseases for which the dentists we interviewed used medicinal plants were: inflammation, healing, pain, periodontal disease, aphthous ulcers and candidiasis. (2006) who points out that medicinal plants are used in dentistry in two ways: one, through information from traditional medicine and the other, in the form of preparations such as toothpaste, topical paste, mouthwashes, mouthwashes for the treatment of gingivitis, aphthae, odontalgia, inflammatory processes, as fungicides and antibacterials.

## 3.5 Conclusions

21% of the dentists in the state of Guerrero who participated in the study use medicinal plants in their professional practice. The socio-demographic characteristics of the dentists showed no differences between the use or non-use of medicinal plants. In future similar research, we could increase the sample size to obtain more significant and conclusive results, as well as directing our efforts towards finding out the medicinal properties of the most commonly used plants.

## 3.6 Acknowledgements

## 3.7 Funding

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# Chapter 4 Association between sleep quality and executive functions in a sample of first-semester medical students at a public university

# Capítulo 4 Asociación entre la calidad del sueño y funciones ejecutivas de una muestra de alumnos del primer semestre de medicina de una universidad pública

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**Abstract**

Poor sleep quality is common among medical students, it has been attributed to high demand of medicine careers; meanwhile sleep insufficiency has been associated with lower academic performance. Nevertheless, still not fully understood the processes that underlie such effect, like the alteration of executive functions that could occur due to sleep insufficiency. In this study, were assessed the sleep quality using the Pittsburgh Sleep Quality Index (PSQI) and executive functions using the WisconPC program, in 38 first-semester medical students, the instruments were applied at the beginning and at the end of school cycle and correlations were estimated. The results of PSQI evidenced a sleep quality worsened towards the end of the semester (start: 8.31±3.41, end: 10.92±1.81), a reduction in the average of sleep hours from 6 to 2.4 at the beginning and end of the semester respectively; however, overall score revealed low sleep quality since the initial evaluation in most of participants. On the other hand, we did not find significant changes in the components of the executive functions; except the correlation between the average reaction time per response and the average reaction time of hits, which indicates the development of cognitive flexibility at the end of semester. No correlation was found between sleep quality and executive functions, further studies are needed to understand the functional relations between sleep and cognitive processes, also to explain the mechanisms underlying the academic performance impairment, attributed to poor sleep. Besides, it is needed to research the etiology of the poor sleep quality that students inform from the beginning as university students, to implement interventions to improve the overall quality of life of medical students, that is reflected in their academic performance.

**Quality sleep, Executive functions, Medical students**

**Resumen**

La mala calidad del sueño es común entre los estudiantes de medicina, lo que se ha atribuido a la alta demanda académica de la carrera; a su vez, dormir inadecuadamente se ha asociado a un menor desempeño académico. Sin embargo, aún no se comprenden del todo los procesos que subyacen a tal efecto, como la alteración de funciones ejecutivas que podrían ocurrir debido al sueño insuficiente. En este estudio se evaluaron la calidad del sueño mediante el Índice de calidad de sueño de Pittsburg (ICSP) y las funciones ejecutivas mediante el programa WisconPC; en 38 alumnos de primer semestre de medicina, los instrumentos se aplicaron al inicio y final del ciclo escolar y se buscaron correlaciones entre variables. Los resultados del ICSP evidenciaron un deterioro en la calidad del sueño hacia el final del semestre (inicio: 8.31±3.41, final: 10.92±1.81), al igual que una reducción del promedio de horas de sueño de 6 a 2.4 al inicio y final del semestre respectivamente; sin embargo, el puntaje general reflejó baja calidad de sueño desde la evaluación inicial en la mayoría de participantes. Por otro lado, no encontramos cambios significativos entre los dos momentos del semestre en los componentes de las funciones ejecutivas; con excepción de la correlación entre el tiempo de reacción promedio por respuesta y el tiempo de reacción promedio de aciertos, lo que indica el desarrollo de flexibilidad cognitiva hacia el final del semestre. No se encontró correlación entre la calidad de sueño y las funciones ejecutivas, es necesario realizar otros estudios que profundicen en las relaciones funcionales del sueño y los procesos cognitivos, y que expliquen los mecanismos de afectación del desempeño académico adjudicados a dormir inadecuadamente. Además, se requiere investigar la etiología de la mala calidad del sueño que los estudiantes reportan desde el inicio de su primer semestre universitario, de manera que se puedan implementar intervenciones para mejorar la calidad de vida general de los estudiantes de medicina, que se refleje en su rendimiento académico.

**Calidad del sueño, Funciones ejecutivas, Estudiantes de medicina.**

## 4.1 Introduction

Sleep is a physiological state that consists of sequential and cyclic phases, during each stage vital processes occur; as metabolic and endocrine regulation, and others mechanism to maintain homeostasis. In recent decades, the prevalence of sleep disorders has increased worldwide, as well as sleep insufficiency associated with work or school schedules and demands (D'Ambrosio *et al.*, 2019). The sleep quantity and quality are physical and mental health determinants, with effects on different systems such as the nervous, associated primarily with cognitive functions; currently, the lack of sleep or poor quality has become a major health public problem (Carrillo, Barajas, Sánchez, & Rangel, 2018; Lira & Custodio, 2018). Sleep quality involves quantitative and qualitative aspects such its duration, onset latency, efficiency to achieve rest and restorative effect, daytime dysfunction and sleep disturbances (Krystal & Edinger, 2008).

The sleep insufficiency is usual among university students, they tend to reduce the time spent sleeping in order to achieve their academic goals; particularly in medicine career (García-López & Navarro-Bravo, 2017; Palatty, Fernandes, Suresh & Baliga, 2011; Saad *et al.*, 2016). Nevertheless, it is suggested that poor sleep quality impairs cognitive functions, then academic performance could be affected (Curcio, Ferrara & De Gennaro, 2006; Gomes & Azevedo, 2011; Quevedo-Blasco, R. & Quevedo-Blasco, V. J., 2011; Vecsey *et al.*, 2009). Some authors suggest that first-year students are more vulnerable, due to the changes involved in the transition to university level, which entails irregular sleep patterns, others claim to find differences between advanced school grades (Cheng *et al.*, 2012; Lemma *et al.*, 2012).

The mechanisms underlying the impact on academic performance attributed to poor sleep quality, are still not clear; some researchers suggest it is due to daytime sleepiness and dysfunction (de la Portilla-Maya et al., 2019). Others have shown that sleeping interrupts the damage induced by reactive oxygen species (ROS), produced by the activity inherent to wakefulness. Likewise, sleep facilitates the synthesis and function of molecules that protect nerve cells and glia from oxidative stress and gives time to repair or replace essential cellular components of nerve tissue that were damaged (Siegel, 2005). These protective functions of sleep help maintain cognitive abilities essential for learning, whose efficiency could be reflected in student academic performance, reaffirming the importance of well sleeping (Benavides-Endaras & Ramos, 2019).

On the other hand, sleep quality could influence the cognitive brain processes that underlie academic performance, as the ability to assimilate new knowledge through attention, comprehension, memory, and executive functions, which can be altered (Jiménez-Puig, Broche-Pérez, Hernández-Caro & Díaz-Falcón, 2019). These functions are cognitive abilities that allow transforming thoughts and emotions into actions, it include four domains: attentional control, information processing, goal setting, and cognitive flexibility (Anderson, 2002; Lezak, Howieson, & Loring, 2004); in wich, memory is implicit. Memory is the ability to encode, store and retrieve information and it is essential in all cognitive processes. The role of sleep-in memory consolidation has generated controversy and research continues to elucidate it (Siegel, 2001; Wamsley, Tucker, Payne & Stickgold, 2010), in that regard, has been shown sleep to facilitate neurogenesis in the dentate gyrus of hippocampus (Siegel, 2005 Op. cit), being already known the hippocampus role in memory processes. Executive functions are essential to carry out learning processes as well to plan and achieve objectives set by the students. Nevertheless the sleep rol in academic performance is still unclear, so more specific research about underlying cognitive processes is needed.

Nevertheless there are no regional or local epidemiological studies, to allow us to know the situation of sleep problems of student population, even less about the causal relations of poor sleep quality or sleep insufficiency on executive functions. More research is needed to implement specific strategies to improve the students sleep, health and academic performance. This study was carried out to evaluate the sleep quality by use the Pittsburgh Sleep Quality Index (PSQI), of first semester students of medicine career of the University Center of the South, and its associations with their executive functions through a computerized version of WCST instrument (WisconPC), this program was developed by the Institute of Neurosciences, of the Guadalajara University in México (Guevara, Aguilar & Hernández, 2017). WisconPC allows us to standardize the test application, faster qualification and adjusting to the needs imposed by COVID-19.

## 4.2 Methods

### 4.2.1 Study Design and Participants

The study was quantitative, because the level of analysis was considered descriptive, correlational, cross-sectional, non-experimental. The study was conducted during the period from August to December 2021, the population was taken as first semester students of the midwife surgeon medical career (MCP) of the public University in the South of Jalisco, Mexico.

The sample was calculated with a 95% confidence interval (CI), expected error of 5%, n=38, the sampling was probabilistic by clusters, the inclusion criteria considered were first semester MCP students who agreed to participate, the sociodemographic variables measured were: sex, age, whether they live with their parents or not, with whom they share a room, whether they work or not. The study variables considered were sleep quality as an independent variable and executive functions as a dependent variable.

### 4.2.2 Instruments

Two instruments were used: the first called the Pittsburgh Sleep Quality Index (PSQI), which consists of 19 items self-assessed by the patient. They are grouped into 7 sleep components: quality, latency, duration, efficiency, disturbances, use of sleep medication and daytime dysfunction. Each component is scored from 0 to 3. The sum of the 7 components gives the total score of the PSQI, which ranges from 0 to 21 points (the higher score means the worse quality of sleep). The Pittsburg index: Buysse et al. 1989 found an internal consistency for the 19 items of 0.83 Cronbach's alpha, consisting of two factors: sleep quality per se and sleep duration.

The second instrument used was the WisConPC test, which evaluates executive functions in 4 domains: attentional control, information processing, cognitive flexibility, and goal setting. The test consists of the presentation of four sample cards (pattern cards), together with a block of 128 stimulus (response) cards. The application time is 15 to 20 minutes. The test allows us to know: total number of stimuli (response cards) presented, total and percentage of correct and incorrect responses, total perseverations, completed sequences, total average reaction time (RT), average RT of correct responses and average RT of incorrect responses. The Wisconsin test, has been used extensively in research and clinical settings to measure executive functions, proving to be highly reliable (Drewe, 1974).

The procedure was developed in two stages, one >pre<, two weeks after the beginning of the semester, with the objective that the students had a period of adaptation to the entrance to the university in its virtual modality; and an application in a >post< stage, 15 days before the end of the semester, to avoid acquiescence due to the workload and final exams.

An online survey with 19 items was applied to collect information on the sociodemographic status of the students. The link to access the Google Forms platform where the survey was located was shared with the students by email, and the survey began with informed consent, in case the student did not wish to participate, the form thanked him/her and did not require more information. In case the student decided to participate, the form displayed the sociodemographic survey and the Pittsburgh test for them to answer. The results were added to an individual folder with the student's name on the Google Drive platform, which only the participant and the researchers could access (data protection). At the end of the study, participants were able to review the results of the tests that were applied to them in the research, which allowed them to know their quality of sleep and their performance in executive functions.

### 4.2.3 Ethical Considerations

The present research work is based on the Regulations of the General Health Law on research, taking as a basis the following articles: article 16 to protect the privacy of the individual, article 17 was considered a research with risk minimum because it answered psychological tests, article 57 was also considered where the informed consent was influenced by some authority, in this case the present study was authorized by the Ethics Committee of the University Center, with folio CEI/045/2021. The application of the principles of the Declaration of Helsinki (beneficence, respect for human dignity and justice) of the World Medical Association for Medical Research Involving Human Subjects was considered for the development of the study.

## 4.2.4 Statistical analysis

The statistical analysis was correlational between quantitative variables, descriptive and dispersion data are reported, and a normality test was performed to verify the distribution of the data. It was determined that both the Pittsburgh sleep quality index and the Wisconsin test do not have normal distributions, the Kolmogorov-Smirnov test yields a significance level (95%) less than 0.05, so there are no normal data, so the Wilcoxon test was performed, this was applied for both tests. To determine the strength of correlation between variables, Spearman's correlation coefficient test was used. The statistical tool Statistical Package for the Social Sciences v. 25.0 (SPSS) in Spanish and freely available was used for data processing.

## 4.3 Results

### 4.3.1 Sociodemographic information

In the present study, 100% of the participants were medical students, most of them were female (63%). Of the total number of students, a high percentage still lived with their parents (73%) and did not work (71%), according to the sociodemographic data shown in Table 4.1.

**Table 4.1** Sociodemographic data

| Variable | | Frequency |
|---|---|---|
| Age | | 18.21 (DE=.41) |
| Sex | Women | n = 24 (63%) |
| | Men | n = 14 (37%) |
| Studying | Medical student | n = 38 (100%) |
| With whom do you currently live? | Nuclear family (parents and siblings) | n = 28 (73%) |
| | Others | n = 10 (27%) |
| With whom do you currently share a room? | Friend/Partner | n = 3 (8%) |
| | Family | n = 10 (27%) |
| | I do not share | n = 25 (65%) |
| Are you currently working? | Weekends | n = 9 (24%) |
| | Half time | n = 2 (5%) |
| | I do not work | n = 27 (71%) |
| Notes: n (%) = Frequency (percentage). | | |

*Source: Own elaboration*

Of all participants, 60% or more rated their sleep quality, sleep latency and sleep efficiency at the beginning of the semester as good and fairly good; they reported sleeping between 6 and 7 hours. On the other hand, at the end of the semester, 50% of students reported poor and fairly poor sleep quality, while the time spent sleeping was reported to be less than 6 hours, and the percentage with very poor sleep efficiency increased. Sleep disturbance and use of medication for sleep showed similar percentages at both times of the study; however, an increase was perceived at the end of the semester, 11% have problems with daytime dysfunction more than 3 times per week and 20% once or twice per week. At the beginning of the semester 78.94% of the students resulted as poor sleepers, towards the end of the semester 100% were categorized as that, it can be seen in Table 4.2.

**Table 4.2** Sleep quality of medical students at the beginning and end of the semester

| Component | Scale | Beginning of semester | | End of semester | |
|---|---|---|---|---|---|
| | | N | % | N | % |
| Sleep quality | Very good | 7 | 18% | 0 | 0% |
| | Fairly good | 16 | 42% | 18 | 47% |
| | Fairly bad | 0 | 0% | 12 | 32% |
| | Very bad | 14 | 37% | 7 | 18% |
| Latency | Very good | 14 | 37% | 16 | 42% |
| | Fairly good | 11 | 29% | 12 | 32% |
| | Fairly bad | 11 | 29% | 3 | 8% |
| | Very bad | 1 | 3% | 0 | 0% |
| Sleep duration | > 7 hours | 6 | 16% | 0 | 0% |
| | 6-7 hours | 14 | 37% | 6 | 16% |
| | 5-6 hours | 18 | 47% | 10 | 26% |
| | < 5 hours | 0 | 0% | 22 | 58% |
| Sleep efficiency | >85% | 14 | 37% | 0 | 0% |
| | 75-84% | 12 | 32% | 13 | 34% |
| | 65-74% | 6 | 16% | 0 | 0% |
| | < 65% | 6 | 16% | 25 | 66% |
| Sleep disturbance | Not during past month | 5 | 13% | 14 | 37% |
| | Less than once a week | 23 | 61% | 13 | 34% |
| | Once or twice a week | 7 | 18% | 9 | 24% |
| | Three or more times a week | 2 | 5% | 2 | 5% |
| Use of sleep medication | Not during past month | 25 | 66% | 21 | 55% |
| | Less than once a week | 13 | 34% | 12 | 32% |
| | Once or twice a week | 0 | 0% | 5 | 13% |
| | Three or more times a week | 0 | 0% | 0 | 0% |
| Daytime dysfunction | Not during past month | 3 | 8% | 1 | 3% |
| | Less than once a week | 12 | 32% | 6 | 16% |
| | Once or twice a week | 11 | 29% | 20 | 53% |
| | Three or more times a week | 12 | 32% | 11 | 29% |
| Global PSQI score | Good sleepers | 8 | 21.05% | 0 | 0% |
| | Bad sleepers | 30 | 78.94% | 38 | 100% |
| Average sleeping hours per day at the beginning of semester | 6.05 | | | | |
| Average sleeping hours per day at the end of semester | 2.42 | | | | |

*Source: Own elaboration*

## 4.3.2 Comparisons of sleep quality between the beginning and end of semester period in medical students

There are statistically significant changes between pre-assessment and post-assessment. At the beginning of the semester the duration component scores were $1.32\pm0.73$, while at the end of the semester they were $2.42\pm0.75$. The efficiency component scores were $1.11\pm1.08$ and $2.32\pm0.96$ respectively. The increased scores indicate that sleep duration and efficiency was poor at the end of the semester. The total PSQI score showed statistically significant changes in the score at the beginning of the semester $8.31\pm3.41$ compared to that at the end of the semester $10.92\pm1.81$, comparisons are shown in the total score, the score increased between the pre evaluation and post evaluation, which translates into sleep problems; however, it is necessary to take into account that from the beginning of the semester the students presented an overall score higher than 5, which places them as poor sleepers or with sleep problems.(Table 4.3)

**Table 4.3** Wilcoxon t-test to compare the score of the 7 components of the Pittsburg sleep quality index

| Component | Beginning of semester | End of semester | P |
|---|---|---|---|
| Sleep quality | 1.55 (1.17) | 1.71 (.76) | .56 |
| Sleep Latency | 1 (.90) | .84 (.82) | .36 |
| Sleep duration | 1.32 (.73) | 2.42 (.75) | .001 |
| Sleep efficiency | 1.11 (1.08) | 2.32 (.96) | .001 |
| Sleep disturbance | 1.16 (.71) | .97 (.94) | .301 |
| Use of sleep medication | .34 (.48) | .58 (.72) | .060 |
| Daytime dysfunction | 1.84 (.97) | 2.08 (.74) | .231 |
| Global PSQI score | 8.31 (3.41) | 10.92 (1.81) | .001 |
| Notes: X: media; SD: Standard deviation | | | |

*Source: Own elaboration*

### 4.3.3 WisconPC test: initial and final semester results

The results of the WisconPC test showed no significant changes between the initial and final application, only the variable of average time of correct answers showed a significant difference, indicating that students took less time to answer correctly in the test applied at the end of the semester. The results are shown in Table 4.4.

**Table 4.4** Wilcoxon test to compare the score of the WisconsinPC test

| Component | Beginning of semester | End of semester | p |
|---|---|---|---|
| Total number of correct responses | 48.89 (7.10) | 50 (6.53) | .631 |
| Average hit time | 2.41 (1.26) | 1.83 (.44) | .042 |
| Total numbers of errors | 2.40 (.86) | 2.21 (.81) | .261 |
| Average per response (seconds) | 2.30 (.95) | 1.97 (.60) | .115 |
| Total of perseverations | 7.26 (4.64) | 5.94 (3.02) | .216 |
| Notes: X: media; SD: Standard deviation | | | |

*Source: Own elaboration*

### 4.3.4 Correlation between Sleep Quality Index scores and Wisconsin test results

Correlation was performed between the total score of the PSQI, executive functions and hours of sleep, another one between all the components of the PSQI and the components of the Wisconsin test. Correlations were performed using Spearman's test, from which it was determined that there is no correlation between the variables of the total score of the PSQI and executive functions, in addition to hours of sleep and executive functions.

Graphic 4.1 shows the correlation between the PSQI components and the Wisconsin test components (lower left gray box), correlation between the PSQI components (upper green triangle) and correlation between the Wisconsin test components (lower orange triangle).

**Graphic 4.1** Spearman correlation between the components of the Pittsburg sleep quality index and the Wisconsin components

| | C | L | D | E | A | UDM | DD | NDA | TPA | TPE | PPR | TP |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | 1 | | | | | | | | | | | |
| L | .05 | 1 | | | | | | | | | | |
| D | .40* | -.02 | 1 | | | | | | | | | |
| E | -.20 | -.07 | - 33* | 1 | | | | | | | | |
| A | -.01 | .42* | .01 | -.39 | 1 | | | | | | | |
| UDM | -.27 | -.06 | .08 | .11 | -.18 | 1 | | | | | | |
| DD | .08 | .02 | .08 | -.37 | -.03 | -.18 | 1 | | | | | |
| NDA | .17 | -.14 | -.10 | -.13 | -.09 | .21 | .02 | 1 | | | | |
| TPA | .02 | -.07 | .26 | .06 | .140 | -.03 | -.06 | -.30 | 1 | | | |
| TPE | .02 | .01 | .31 | .09 | .13 | .22 | -.20 | -.20 | .68* | 1 | | |
| TPR | -.07 | .14 | .19 | .14 | .01 | -.05 | -.09 | -.35* | .86** | .56** | 1 | |
| TP | .07 | .13 | -.07 | -.16 | .25 | -.17 | -.12 | -.27 | -.09 | -.27 | .80 | 1 |

*Source: Own elaboration*

Note: Quality (C), Latency (L), Duration (D), Efficiency (E), Alterations (A), Use of sleep medication (UDM), Daytime dysfunction (DD), Number of hits (NDA), Average reaction time of hits (TPA), Average reaction time of errors (TPE), Average reaction time per response. (TPR), Total perseverations (TP). ***p<.001, ** p<.01, *p<.05.

The correlation between the components of the Wisconsin test showed that the greater the average reaction time per response, the greater the average reaction time for successes (r=.86, p<.01), and the greater the average reaction time for errors, the greater the average reaction time for successes (r=.68, p<05.). Finally, we observed that the greater the total average reaction time, the greater the average reaction time for errors (r=.56, p<.01).

## 4.4 Discussion

Sleep is an essential part of human health and life; this action has been related to different cognitive functions. It is well documented, that lack of sleep or poor sleep quality affects individual learning ability, academic performance and performance of neurocognitive functions (Gutiérrez-Ruíz *et al*., 2020; Jalali *et al*., 2020; Granados-Carrasco *et al*., 2013). This study aimed to determine the relationship between sleep quality and executive functions in first semester medical students at a public university in Jalisco.

Multiple studies have been conducted on sleep quality and various cognitive functions in medical students, especially in those who are in their last year of study and in those who are in a medical residency specialty, however, little attention has been paid to first semester or first year students according to the university program. This population is especially susceptible to sleep disturbances due to the change that represents the transition from high school student to university demands, even more so in a career that historically, has been characterized by its high academic demand (Baars *et al*., 2015; Knouse, Feldman, & Blevins, 2014).

It is important to mention that the study was planned to be carried out in a normal university environment; however, the arrival of the COVID-19 pandemic forced the universities to migrate to virtuality, making it necessary to adapt and apply the survey and tests virtually. The fact that the subjects were taught virtually for the first time resulted in a decrease in the academic demands of the professors, since most had to adapt their syllabus to virtuality in a matter of days, so that many of the practices, exams and assignments were excluded, thus reducing the academic load, even so distance education represented a challenge for the students.

At the beginning of the semester students slept on average more than 6 hours a day, however according to the PSQI score the highest percentage was classified as poor sleepers, while at the end of the semester 100% of the students were classified as poor sleepers with an average of 2.4 hours of sleep per day, in addition significant changes were observed in the duration and efficiency of the hours of sleep. These results are not surprising since, as mentioned above, despite the changes that occurred in the academic load due to the change to virtuality, the academic demand continues to be high (Rao *et al.*, 2020; Jalali *et al.*, 2020 Op. cit).

Meta-analyses and systematic reviews have concluded that sleep quality and duration have an association on academic performance (Dewald *et al.*, 2010; Shochat, Cohen-Zion & Tzischinsky, 2014), however, there are no conclusive results about the impact of sleep duration and efficiency on learning and academic performance (Hershner & Chervin, 2014; Taras & Potts-Datema, 2005; Gruber *et al.*, 2010). In this sense, there is a possibility of sleep disturbance in students, and this possibility is expressed as lack of academic performance, however, this relationship has not been clearly explained (Gaultney *et al.*, 2010).

Cognitive processes are determinants of academic performance; executive functions are cognitive variables that play an important role in the development and maintenance of academic skills. The WisconPC test allowed us to know the predominant domains such as working memory and cognitive flexibility.

Through Wilcoxon test it was possible to identify changes in the executive functions between the two times of the study, the most outstanding was the improvement in cognitive flexibility, since the students had more correct answers, the average reaction time per answer was directly proportional to the average reaction time for correct answers and for errors, which makes us suppose that the decrease in the rest time caused the students to be slower when performing the test, however the number of correct answers was higher. The increase in the number of correct answers is logical since, as the subject has more correct answers, it is easier to identify the different categories proposed by the task (Ramírez-Flores & Ostrosky, 2012), reinforcing the assumption of improvement in cognitive flexibility.

In the present study we found no significant correlations between the components of the Pittsburgh Sleep Quality Index and the components of the Wisconsin test, leading us to conclude that good sleep has no impact on executive functions and probably on academic performance.

## 4.5 Acknowledgments

## 4.6 Founding

## 4.7 Conclusions

The academic load in first semester medical students reduces the time allocated to sleep and reduces the efficiency and duration of sleep resulting in students with sleep problems or "bad sleepers"; however, sleep problems have not been associated with executive functions processes that may have implications on academic performance of first semester medical students.

The results presented highlight the need to explore other cognitive, behavioral and psychological variables that could influence sleep quality and cognitive functions related to overall academic performance, with the aim of developing a multivariate model that allows designing intervention programs that promote the well-being of the student community.

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# Chapter 5 The omics era: proteomics importance in cancer research

# Capítulo 5 La era ómica: importancia de la proteómica en la investigación del cáncer

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## Abstract

Cancer is a pathology that leads the causes of death in the population worldwide also is reported its increase with enhancing of life expectancy. In addition, this pathology is multifactorial, including genetic mutations and environmental effectors such as germs or environmental compositional changes, considered as contaminating elements to the organism. For example, other direct factors are associated with chronic diseases that induced continue inflammation.

Therefore, understanding cancer biology and its mechanism of action is a fundamental part of mitigating its effect on public health. As a heterogeneous disease, his study is a constant challenge, identifying metastasis in the early stages and the resistance to drugs are problems with an unmet need that could be solved through the study of the disease at the molecular level.

Omics sciences have proven to be a promising option for the study of heterogeneous pathologies, due to their ability to analyze a biological system at the molecular level, quantify its composition, and group it according to its function. The study on which each science is based is by which it takes its name, genomics studies the genome, metabolomics the metabolome, proteomics the proteome, among others.

In this chapter, we will limit ourselves to proteomics, the study of the set of proteins of a biological system, which from our point of view is the omics science with the widest understanding and from which satisfactory results have been used in clinical application. Especially, because it has been possible to identify biomarkers that may be useful during the diagnosis or prognosis of the disease or therapeutic targets for personalized medicine in patients and thus minimize the adverse effects caused by drugs on healthy cells. We expose different proteomics studies applied in different biological systems such as cell lines, xenografts, and patient tissues or fluids, to reveal the versatility of the technique and the functionality of the data that have been obtained with it.

**Proteomics, Pathology, Inflammation, Mechanisms, Biomarkers**

## Resumen

El cáncer es una patología que lidera las causas de muerte en la población a nivel mundial, y que ha aumentado con el incremento de la esperanza de vida. Además, se trata de una patología multifactorial, que incluye mutaciones genéticas y efectores ambientales como gérmenes o cambios en la composición ambiental que son considerados como contaminantes externos del organismo. Por ejemplo, otros factores directos están asociados con enfermedades crónicas que producen inflamación continua.

Por lo tanto, el entendimiento de la biología del cáncer y sus mecanismos de acción es una parte fundamental para mitigar su impacto en la salud pública. Al ser una enfermedad heterogénea, su estudio representa un constante reto, identificar la metástasis en etapas tempranas y la resistencia a medicamentos son problemas que no se han resuelto y podrían encontrar solución en el estudio de la enfermedad a nivel molecular. Las ciencias ómicas han demostrado ser una opción prometedora para el estudio de patologías heterogéneas, debido a su capacidad para analizar un sistema biológico a nivel molecular, cuantificar su composición y agruparlo según su función. El estudio al que se basa la ciencia le da su nombre, así, la genómica estudia el genoma, metabolómica se encarga del metaboloma, proteómica de las proteínas, entre otras.

En este capítulo nos limitaremos a la proteómica, el estudio del conjunto de proteínas de un sistema biológico, que desde nuestro punto de vista es la ciencia ómica mayormente estudiada y de la que se han obtenido resultados satisfactorios en su aplicación clínica, al permitir identificar biomarcadores que pueden ser utilizados durante el diagnóstico o pronóstico de la enfermedad al permitir encontrar dianas terapéuticas para la medicina personalizada en los pacientes y así minimizar los efectos adversos que provocan los fármacos sobre las células sanas. Exponemos diferentes estudios de proteómica aplicados en diferentes sistemas biológicos como líneas celulares, xenoinjertos y tejidos o fluidos de pacientes, para revelar la versatilidad de la técnica y la funcionalidad de los datos que se han obtenido con ella.

**Proteómica, Patología, Inflamación, Mecanismos, Biomarcadores**

## 5.1 Introduction

When we talk about cancer, we refer to conditions characterized by abnormal cellular behaviors outside of biological controls. All cancer (except for brain cancer and skin carcinoma that do not cause metastasis) has three characteristics: uncontrolled cell division, invasion, and destruction of normal tissues and colonization of distant sites being able to survive in an external environment of its origins and continuing its invasion (Compton, 2020).

A key step in the onset of cancer is the failure of cell death signaling pathways, which are activated by multiple causes of cell damage, such as DNA damage or mutation, activating caspase -9, -2, and -8 pathways or RIP kinases, the lack of control of these pathways will lead to the initiation of cancer (Gregory, 2016).

Knowing the origin of cancer is not the only option for the development of biomarkers and therapeutic targets is also important to study the tumor microenvironment as well as the mechanism involved in one of the most critical parts of cancer, the metastasis.

The tumor microenvironment includes Cancer-associated fibroblast (CAF), blood and lymphatic vessels, inflammatory cells, adipocytes, and neuroendocrine cells, all of them are present in the extracellular matrix (ECM) and made the structure of tissues and organs. Signaling proteins are involved too, such as cytokines and growth factors. All this interaction created signaling pathways that contribute to the progression and metastasis of cancer (Coban *et al.*, 2021).

To cancer get to metastasis, it must follow a few steps, like a local invasion of the ECM and stromal cell layer, blood vessels, survival to the transportation around the vasculature, deposition in distant organs, extravasation, survival in a microenvironment different to his original one and finally the proliferation (Feng *et al.*, 2020).

We can notice that cancer is a multifaceted disease. Over that 11 million people are diagnosed every year. The lack of early diagnosis is more related to cancer mortality than the availability of treatment. The advantages of early detection are detected cancer when is still localized and curable, which prevents mortality and reduces the comorbidities, this represents a lower cost to the health systems (Parisa Karimi *et al.*, 2014).

One of the aims of actual medicine is the development of personalized medicine. In cancer, we know signaling pathways that are used as a therapeutic target. An example is the mutation of *EGFR* present in some cases of non-small cell lung cancer (NCSLC), this gen codified the epidermal growth factor receptor. If this mutation is identified, the patient is a candidate for treatment with erlotinib, a drug that blocks the action of the receptor and prevents the promotion of cell cancer proliferation (Mathé *et al.*, 2018).

Even with the recent advance in treatments and diagnosis methods, most of them are invasives and inefficient in a lot of patients. It is still a challenge to expand and get better options for the patients, therefore, it is fundamental to continue with the search for new biomolecules or molecular changes that can work in the diagnosis, prognosis, or treatment of cancer.

New techniques and tools have revolutionized clinical medicine with the application of sciences such as genomics, proteomics, transcriptomics, metabolomics, and epigenomics that are integrated to form a new science field known as "omics". The development of these 'omics' technologies had a significant impact and created techniques of diagnosis faster, more sensitive, and more specific, as well as in the prognosis, treatment, and prevention of diseases, allowing the advent of new therapeutic strategies, with the identification of molecules that may be useful as new therapeutic targets and thus be able to offer personalized medicine to the patient (Figure 1.1) (Farfán & Torres, 2018; Ocana & Pandiella, 2010; Velásquez-Fernández D, 2011).

**Figure 5.1** Use of multi-omic analysis for biomarker detection
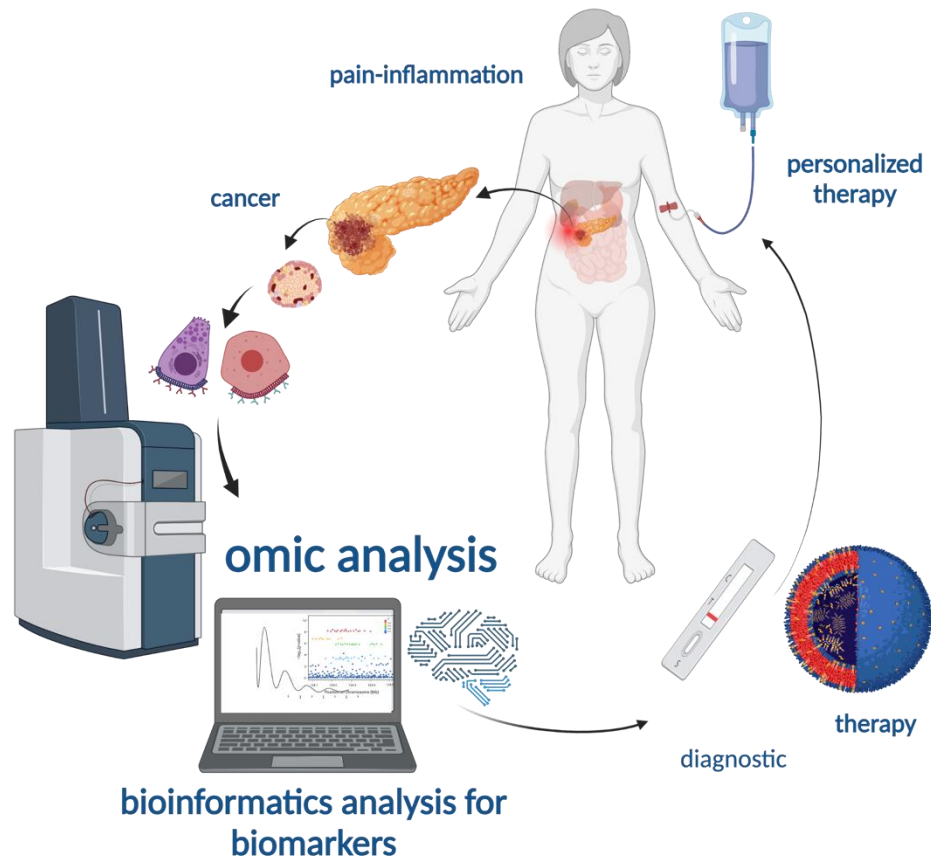


## 5.2 Omics: understanding the biological systems

The investigation of omics science is referred to the use of technologies to analyze biological systems at a molecular level. These techniques are capable of reproducing data that are correlated with specific states of the biological systems. The molecules that the omics studied give it the name: metabolomics, proteomics, metabolomics, genomics, etc. (Rajesh *et al.*, 2021).

The beginning of the omics era has significant big advances in biological sciences. Omics is the evolution from molecular biology to systems biology, changing the way to try to understand biological entities. The phenomenon of the interaction of biomolecules like DNA and proteins cannot be explained such as the determinate dogma central, it is imperative to study different levels of biomolecules and their interactions. If we get to integrate this information in the best way possible, we were able to reach the objective of omics in the clinic: recognize, evaluate, and applied biomarkers of diagnosis and prognosis (Colnaghi Simionato, 2021; Nguyen & Wangid, 2020).

The omics tools have permitted big progress in the identification of cancer markers like in Acute lymphoblastic leukemia (ALL). With genomics, it was possible to reveal acquired somatic disorders that can be used as markers such as translocation and aneuploidies, also it has been studied the role that plays the heritable genetic variations in the risk to have ALL. It has been important too, to understand the response to the drugs and the effects of the antineoplastic therapy. It is still necessary to continue with the identification of marks that can work to give information about the malignancy of pathologies such as ALL (Jiménez-Morales *et al.*, 2017).

An important fact in the omics era is haven been the capacity to recollect useful information about biological systems, this issue is well explained in the review made by Kwon and collaborates (2021) where the recompilation of data is called multi-omics, which consists of the approach of the data generation of omics, creating a big-scale database given the possibility to make a big analysis of this information and use it to understand the biological systems. This can be focused on looking for data about a specific disease and its physiopathology and proteins, genes, metabolites, etc., and their interaction related to specific events.

We can mention a lot of sites of databases that integrated omics science such as The Cancer Genome Atlas (TCGA), The Human Protein Atlas, BAMS, BioGpS, GenBank, Catalogue of Somatic Mutations in Cancer (COSMIC), ENCODE project, UniProtKB, NIH cancer, UNIPROT, Proteomics DB, PRIDE, NCBI Structure Group, just to name a few ones.

The omics sciences have given us a lot of knowledge about cancer. Each one of them has provided valuable information to find out mechanisms of metastasis, resistance to drugs, metabolic pathways, etc. In all these omics science it is important to mention one that had given different biomarkers and therapeutics targets that are widely used in clinical practice, and it is necessary to personalized medicine, the proteomics.

The genome has all the hereditable information in the DNA, this biomolecule can transcript to RNA fragments, these fragments can or cannot codify proteins, and all the set of RNA is called transcriptome. The messenger RNA (mRNA) translates to proteins. Proteins are going to determine the functions of the cell and are necessary to cell life. But the quantity of proteins is not just related to the translation and its variation, also is involve post-translational modifications this generated a very large catalog of proteins. The set of proteins is called proteome. All these characteristics and variability are very important in the investigation of cell modification and tissue alteration. Proteomics not only revealed the complexity of life it is important to identify and study the alteration of the cell and its environment, during disease, treatment, or any specific situation. The proteome of a specific disease can give us clues about disease physiopathology (Ciocan-Cartita *et al.*, 2019).

Knowing information about the genome is very important, as the exposed case of ALL and lung cancer, all these discoveries have made us question interesting things about cancer, but the conventional way of studying only one gene or one protein it is not enough to completely understand the disease, it is necessary to make a big-scale study. Genome study is very useful but another way to study the pathologies is using the genes products: the proteins (Lin *et al.*, 2016).

If we think of cancer biomarkers that are used currently, we can mention CA 19-9, CA125, and CEA (carcinoembryonic antigen) all of them have common that are proteins (Mocan *et al.*, 2022). Treatments like erlotinib or imatinib that can block the action of kinases to treat different types of cancer are a perfect example that the proteins are very important also like therapeutic targets (Asami, 2014; Buclin *et al.*, 2020). It is easy to identify that studying these biomolecules is essential for clinical advances.

## 5.3 Proteomics and its usefulness in the study of cancer

Proteomics is the omic science in charge of studying the proteome of any biological system, this includes the structure of proteins, their functions, interactions, and modifications in any situation, using quantitative measurements to characterize any biological process and decipher its mechanisms.

The proteome of a living system provides fundamental details in the understanding of its biology, and how its biology is altered in the face of a certain specific physiological or pathological condition, the proteome tends to change in response to modifications of its normal conditions, providing fundamental information for the study of any pathology. Within diseases, the use of proteomics allows early diagnosis of diseases, prognosis, and monitoring of the disease, it is also necessary for the development of drugs as it allows the identification of therapeutic targets (Aslam *et al.*, 2017).

Biochemical changes in the proteome of biological systems have provided important knowledge for the study of complex diseases, such as cancer, infectious diseases, neurodegenerative diseases, and diabetes, among others. Proteomic approaches locate and identify differential proteins at each stage of the disease for their correct treatment or eradication (Monti *et al.*, 2019).

All cancers share a series of crucial capacities for the development of the tumor phenotype, such as proliferation even in the absence of growth factors, insensitivity to growth suppression mechanisms, angiogenesis, avoidance of apoptosis, tissue invasion, and metastasis. All the proteins involved in these characteristics will differ between the diseased and healthy individuals (Kruse *et al.*, 2008).

Proteins are thought to drive tumor growth, tumor invasion, and response to targeted therapies, so identifying changes in protein signaling helps understand the mechanism of cancer progression. Cancer proteomics bases its studies on the quantification and analysis of proteins concerning their healthy counterparts, for the identification of information that helps the treatment of the disease (Shruthi *et al*., 2016; Srinivas *et al*., 2001)

In cancer, proteomics has become a fundamental omics science for the study of tumor growth and metastasis, contributing knowledge to the molecular biology of cancer. It allows us to explain the molecular mechanisms of malignant cells, as well as being able to analyze the tumor microenvironment (Hoi et al., 2017). As cancer progresses, the protein profiles present changes, which can be examined through quantitative proteomics, based on this it has been possible to identify biomarkers and locate protein expressions that could be used for tumor staging, prediction, or possible therapies (Kwon *et al*., 2021). Quantitative proteomics has been a good option for cell classification according to a pathological state, and in turn understanding its biological mechanism, also supporting the understanding of the mechanism of action and interaction of some drugs (Zhu *et al*., 2018). Since cancer could be considered a disease caused by abnormal transductions, aberrant signaling pathways are a promising therapeutic target (Pan *et al*., 2013). Proteomics is a very powerful tool to study signaling pathways and their possible alterations.

Proteins, being key molecules in biological processes, can be used as characteristic molecules of the different stages of cancer. The study of its structure, function, and quantification could improve patient outcomes. Some of the techniques for protein detection are ELISA, immunohistochemistry, fluorescence-activated cell sorting, and mass spectrometry, among others.

Pancreatic cancer is a type of cancer that is distinguished by its poor prognosis, which is why the identification of a biomarker in the early stage of cancer indicates an unmet need. Proteomic studies of pancreatic cancer have been carried out in patients (tissue, plasma, pancreatic juice, fluids), in cell cultures, or employing xenografts, where information has been sought on the mechanism of the disease. The proteomic approach appears promising in trying to identify a biomarker for early-stage disease however, it is an ongoing effort as this identification is challenging. Proteins that have been identified in the cell membrane have shown promise for biomarker development (Chen *et al*., 2005; Law *et al*., 2020; Le Large *et al*., 2019; Sanh N. *et al*., 2018).

## 5.4 The study of proteins

## 5.4.1 Cell line proteomics

Cancer cell lines are useful for the proteomic study of cancer in vivo, allowing a better understanding of cancer mechanisms and basic biology since for the most part, they retain the same characteristics as the tumor of origin. The use of cell lines presents easy manipulation and characterization, and high reproducibility, in addition to the fact that it is not necessary to obtain ethical permits for its use, however, any inadequate condition during its culture or the extraction of proteins will result in a loss in its morphology and turn to impair the proteomic characterization (D. Ferreira *et al*., 2013; van Staveren *et al*., 2009)

In a study of lung cancer, two cell lines characteristic of the disease, A549, and SW900, were analyzed by mass spectrometry, through which 68 proteins that were overexpressed in both cell lines could be identified. These proteins were classified into different functional processes. The comparison between the two proteomes allowed a more specific characterization of each cell line, A549 was related to cellular respiration, homeostasis, response to drugs, oxidative stress, and intracellular transport, while SW900 was related to the negative regulation of apoptosis, protein translation, and response to organic and inorganic substances (Korrodi-Gregório *et al*., 2016). Similarly, Katsogiannou *et al*. carried out a proteomic study using mass spectrometry of four cell lines belonging to prostate cancer, identified the over-expressed proteins in each cell line, and according to the identified proteins, functionally classified each cell line related to metabolism. transport and localization and identified some proteins related to resistance (Katsogiannou *et al*., 2019)

Cell lines are useful tools to investigate the molecular basis of cancer invasion. In a study by Pei et al., using a combination of electrophoresis and mass spectrometry, they analyzed the proteomes of cell lines U87MG and U343MG-A, which are lines cell characteristics of gliomas, the most lethal tumors of the brain, where it was possible to identify that annexin A2 was related to the invasion of the glioma, in the same way, Cathepsin D was seen as an important part in tumor progression, expressed more significantly in the U87MG cell line (Pei *et al.*, 2014).

Chemoresistance is an obstacle during the treatment of diseases, so elucidation of the molecular mechanisms of resistance could lead to a response to therapy. In vitro analyzes of cell lines are a good option to elucidate these mechanisms. In an important study by Qinghong *et al.*, they identified possible protein targets related to resistance to doxorubicin. The proteomes of cell lines belonging to leukemia were compared with control cells and the possible protective strategies to resist the drug were listed, concluding that it is a series of mechanisms that provides this resistance (Qinghong *et al.*, 2015).

In another similar study by Albrethsen and colleagues, they compared the proteomics of six cell lines to test for resistance to agents that interact with microtubules and induce cell death. Galectin-1 was identified, as directly related to the resistance of the applied drugs, as well as stathmin, among other proteins (Albrethsen *et al.*, 2014). Therefore, the proteomic analyzes applied to cell lines are not only functional for the characterization of the lines, but also for the application of various in vitro studies that support a better therapeutic response of the patient, as well as to explain the mechanisms and functions of the basic biology of cancer.

## 5.4.2 Proteomics in murine models

Cancer cells from cell line cultures or cells from cancer patients can be grafted into mouse models generating xenografts. D. Ferreira *et al.* (2013) mentioned that represents a greater similarity to the mechanisms of cancer in humans, however, the environment in which the tumor develops varies significantly, in addition to this it is necessary to have ethical permits for their manipulation.

Cancer-derived cachexia and muscle loss are associated with increased morbidity and mortality, so Hulmi and his team took on the task of developing an animal model of this condition, analyzing its proteome. With this analysis, they were able to study the alterations of the oxidative phosphorylation system and NAD+ homeostasis, with this information they proposed and tested an activin blocker that can restore depleted muscle NAD+ and Nrk2, being an example of the opportunities, it offers the study of the proteome and the applications of this knowledge (Hulmi *et al.*, 2020).

R. Ferreira *et al.* (2019) used murine models of drug-induced urothelial carcinoma, a group that underwent exercise to study the change in the cardiac mitochondrial proteome that this activity caused, to study the cardiac effects observed in patients with cancer and the cardioprotective effect that exercise could have, and its mechanism. It was observed that from the outset, tumor induction by itself induces cardiac remodeling characterized by positive regulation of cardiac tissue morphogenesis and decreased cellular respiration, in mice that exercised it was shown that this was counteracted through the overexpression of cardiac muscle contraction proteins and ATP metabolism.

## 5.4.3 Proteomics in patients

The proteomic analysis of patients can be analyzed with a sample of the tissue of the tumor that has developed, but it is an invasive analysis for the patient and on some occasions, it is difficult to obtain, so biofluids such as blood, urine, saliva, tears, cerebrospinal fluid, prostatic secretions, among others, have also made it possible to identify clinically relevant proteins of the disease (Macklin *et al.*, 2020).

Versatility in the use of samples is a crucial point. Currently, the characteristics and advantages of using these fluids to replace tissue biopsies have been analyzed and liquid biopsies have been determined. This option allows cover all aspects of cancer, unlike tissue biopsy, liquid biopsies could be useful for early detection, diagnosis, prognosis, and monitoring (Ding *et al.*, 2021). The procedure consists of isolating entities derived from tumors from these samples, such as circulating tumor cells, circulating tumor DNA, or extracellular tumor vesicles (Lone *et al.*, 2021).

Proteomic strategies have become necessary in the discovery of biomarkers that could be applied to these samples, for example, in a study that analyzed the proteome of nipple secretion to find a method for early detection of cancer in women. youths. The results in this study identified an average of 1,265 proteins, of which 46 are on the list of 195 proteins under investigation by the Department of Early Detection of the National Cancer Institute of the United States, the interesting part is that 22 of these 46 were found in all samples with cancer and 7 of these 22 were not found in plasma (Shaheed *et al*., 2017). In a study by Chu and colleagues (2019), using body fluids to identify key proteins that might be useful in detecting oral cancer, they collected saliva samples from healthy and sick patients over two years who were subjected to proteomic analysis. Chu et al. identified 24 proteins with the potential to be biomarkers, in addition to some that could be useful for early detection of the disease (Chu *et al*., 2019).

As already mentioned, proteomics is versatile in terms of the samples to be used. An example of the use of a biopsy sample is the study by Madoz and his team, who used biopsies from 15 patients with colorectal carcinoma and used antibody microarrays which allow observing protein expression patterns of tumor cells and compared them with mucosal cells that were not yet affected. The microarray was made up of 224 antibodies specific for proteins of signaling pathways of interest in cancer such as apoptosis, cell cycle, signal transduction, nuclear proteins, and neurobiology, in addition, 8 antibodies were specific for phosphorylations of associated protein kinases (FAK, histone H3, MAPK, etc.). This study identified proteins of interest such as MDM2, CHK1, and ERK, which showed overexpression in patient samples. It is also important to mention the interesting use of microarrays for proteins and how these panels can be made specifically for other types of cancer (Madoz-Gúrpide *et al*., 2007).

## 5.5 Conclusion

Proteomics is an omic science that has revolutionized cancer studies by expanding existing knowledge, elucidating growth mechanisms and basic biology, and based on this, applying it to the clinical care of patients.

Personalized medicine has been based largely on the study of the proteome, since it allows the identification of biomarkers and therapeutic targets to direct a treatment according to the type of pathology and level of the patient, and in this way minimizing side effects of current chemotherapies. Proteomics allows the study of both patient samples, xenografts or, through the cultivation of immortalized cell lines, the comparative and complementary study in each of these techniques has provided a greater understanding of the mechanisms with which said pathology is addressed, in addition to being able to identify the main function that certain cells have in the behavior of metastasis or resistance to treatment drugs, which has allowed important advances in the adequate treatment of patients, although in some cases the disease is not eradicated, the survival time has presented a significant increase.

The examples of studies shown here have shown how this omics science has highlighted the advantage of using this omics science for the analysis of different pathologies, that although the experimental study is of the utmost importance for the collection of optimal data, each time they have revealed techniques with easier implementation and analysis. In the same way, the use of this omics science was evidenced to translate the knowledge of basic science into the clinic and how its use shows promising and competent data to generate a great impact on public health.

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## 5.8 References

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# Chapter 6 Diurnal variation and health risk of atmospheric aromatic hydrocarbons concentrations in an urban site located in Nuevo Leon, Mexico

# Capítulo 6 Variación diurna y riesgo a la salud de las concentraciones atmosféricas de hidrocarburos aromáticos en un sitio urbano localizado en Nuevo León, México

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**Abstract**

Diurnal variation of aromatic hydrocarbons (BTEX: benzene, toluene, ethylbenzene and p-xylene) in ambient air was determined in an urban site located in Monterrey, during North's season 2020. Samples were collected using active sampling by a vacuum pump at a controlled flow of 200 ml/min during 1.5 h, considering three sampling periods: morning (07:00 - 08:30 h), midday (14:00 - 15:30 h) and afternoon (17:30- 19:00 h). Samples were desorbed with carbon disulfide and the extracts were analyzed by gas chromatography with ionization flame detection. Ethylbenzene and p-xylene were the dominant hydrocarbons (mean concentration: 18.581 $\mu g/m^3$ and 18.039 $\mu g/m^3$, respectively). Mean values for benzene and toluene were 15.137 $\mu g/m^3$ and 15.503 $\mu g/m^3$, respectively. All BTEX showed a diurnal pattern with higher values during the afternoon. From a meteorological study (wind roses) and chimiometric analysis (principal component analysis) were identified relations among the measured variables and their possible emission sources (industrial and vehicular sources). It was carried out a health risk assessment, considering both, carcinogenic and non-carcinogenic (respiratory and cardiovascular diseases) related to BTEX inhalation founding that population in the study site could develop cancer in the lifetime by benzene inhalation.

**Hydrocarbons, Chromatography, Carcinogenic, Aromatic, Benzene**

**Resumen**

Se determinó la variación diurna de hidrocarburos aromáticos (BTEX: benceno, tolueno, etilbenceno y p-xileno) en aire ambiente de un sitio urbano ubicado en Monterrey, Nuevo León durante Nortes 2020. Las muestras fueron colectadas usando muestreo activo en tubos absorbentes empacados con carbón activado usando una bomba de vacío a flujo controlado de 200 ml/min durante 1.5 horas, considerando 3 periodos de muestreo: mañana (07:00 a 08:30 h), medido día (14:00 a 15:30 h) y tarde (17:30 a 19:00 h). Las muestras fueron desorbidas con disulfuro de carbono y los extractos fueron analizados por cromatografía de gases con detección de ionización de flama. Etilbenceno y p-xileno fueron los BTEX dominantes (concentraciones promedio: 18.581 $\mu g/m^3$ y 18.039 $\mu g/m^3$, respectivamente). Los valores promedio para benceno y tolueno fueron 15.137 $\mu g/m^3$ y 15.503 $\mu g/m^3$, respectivamente. Todos los BTEX mostraron un patrón diurno con mayores valores durante el mediodía y por la tarde. A partir de un análisis meteorológico (rosas de viento) y un análisis quimiométrico (análisis de componentes principales) se identificaron las relaciones entre las variables medidas y las posibles fuentes de emisión (fuentes industriales y vehiculares). Se llevó a cabo un análisis de riesgo a la salud por inhalación de BTEX considerando tanto riesgo carcinogénico como no carcinogénico (enfermedades cardiovasculares y respiratorias), encontrándose que la población en el sitio de estudio podría desarrollar cáncer en el tiempo de vida por inhalación de benceno a los niveles observados.

**Hidrocarburos, Cromatografía, Cancerígenos, Aromáticos, Benceno**

**6.1 Introduction**

The deterioration of air quality either by anthropogenic or natural causes, can generate negative effects on human health and ecosystems (Torres *et al*., apud Valls, 2017). Atmospheric pollution has become an issue of interest for the scientific community and population in general since that in different studies has been demonstrated that a degraded air quality is related to diverse health conditions and diseases of the cardiovascular and respiratory system, even with different kinds of cancer (Instituto Nacional de Ecología y Cambio Climático, 2020). On the other hand, several studies affirm that atmospheric pollution around the world is associated to the global warming process and its respective consequences (Zhang et al., 2012). Some factors as use of fossil fuels, operation of coal-fired power plants, the increased dependency of motor vehicles for private transport, inefficient use of energy in buildings and the use of biomass burning to cook food and for space heating, produce the called Volatile Organic Compounds (VOC's) (World Health Organization-WHO, 2014). Within this group of compounds exists a sub-group named as BTEX, that includes to benzene and its alkyl-derivatives: benzene, toluene, ethylbenzene and xylenes. These compounds can cause both carcinogenic and non-carcinogenic (cardiovascular and respiratory diseases) and they are tropospheric ozone precursors (Marć *et al*., 2014).

Lan and Binh (2012 apud IARC, 2017) indicate that benzene is highly toxic, inducer of leukemia in humans, and it has been identified as carcinogenic in humans for the International Agency for Research on Cancer. For this reason, WHO and EPA do not recommend a safe level of exposure for this compound. The rest of BTEX have adverse effects on human health and are considered as toxics of the air. For this reason, in order to evaluate air quality in given location it is important to determine the levels of concentration of these compounds in ambient air. Many cities around the world commonly monitor the levels of BTEX in ambient air, but in Mexico, even when the risks and consequences of the inhalation of these compounds are well known, neither there is an air quality standard that regulates the maximum levels in ambient air nor a continuous monitoring is carried out in the air quality stations belonging to the Mexican air quality network, excepting in Mexico City.

The general objective of this study was to evaluate the atmospheric levels of aromatic hidrocarbons (BTEX: benzene, toluene, ethylbenzene and p-xylene), their origin and their relationship with criteria pollutants, as well as their impact on health in an urban site located within the Metropolitan Area of Monterrey during the Norths season 2020. The atmospheric concentrations of BTEX were determined by gas-chromatography with ionization flame detection. The influence of the winds at the local level on the concentrations of the pollutants measured was analyzed by means of an analysis of wind roses. The probable sources of the measured pollutants were inferred from the results of the meteorological and statistical analysis (Pearson's correlation matrix and Principal Component Analysis). Finally, the health risk of BTEX was evaluated considering carcinogenic and non-carcinogenic risk (potential of development of cardiovascular and respiratory diseases).

This chapter is structured as follows: The first section provides the Introduction to the research work, Background Section (Section 6.2) gives information on the study of BTEX atmospheric registered by other researchers around the world. In Section 6.3, a background of BTEX measurements is provided, including the results of BTEX measurements in Mexico and other sites around the world, as well as the results of emissions inventory and the main sources of BTEX in the study area. Section 6.4 provides the study methodology, considering both the collection and analysis methods for the determination of BTEX by Gas Chromatography – Ionization flame Detection. A description of the method used for the meteorological analysis is also provided, which made it possible to determine the influence of local winds on the levels of the measured BTEX, as well as the identification of possible sources contributing to the levels of these pollutants. This section also provides descriptive information on the statistical tools and analysis applied to the data to determine the relationships between the measured air pollutants and the meteorological variables recorded (bi-varied and multivariate analysis: principal component analysis).

This section describes the method used for the evaluation of health risk considering both the cancer risk coefficient in the life time and the non-cancer risk coefficient (risk of contracting diseases other than cancer: cardiovascular and respiratory diseases) due to inhalation of BTEX. Section 4 shows the results of the study, the descriptive statistics of the BTEX concentrations and their diurnal variation, and the possible sources contributing to the levels of the pollutants measured and its location, based on statistical analysis and meteorological analysis. An analysis of relations between the measured BTEX is provided in order to know the relative contribution of sources to the BTEX levels and the grade of processing of air masses (photochemical age of the air masses), then benzene/toluene and ethylbenzene/p-xylene were determined. This section also shows the results of the health risk assessment for inhalation of these pollutants. Finally, section 6.6 provides the conclusions of the study, as well as the recommendations for future work.

## 6.2 Background

In recent decades, we are facing an ever-increasing energy consumption in the world due to the increase in population and the consumption of fossil fuels, nevertheless, the promotion of industries and the improvement of social welfare. This has resulted in a degradation of air quality that can be a risk to the health of the population due to the concentrations of toxic substances in the air, such as BTEX.

Some studies have been carried out around the world and in Mexico in order to know the behavior, distribution and characteristics of these compounds in ambient air. Rad and collaborators (2014) measured atmospheric BTEX by active sampling at 12 sampling points in Ahvaz, the capital city of Khuzestan province, from July to September 2012 and January to March 2013. BTEX were analyzed by chemical desorption followed by chromatography gas/flame ionization detector (GC-FID). The mean concentrations of benzene, toluene, ethylbenzene, xylenes, and BTEX were 1.78, 5.19, 0.51, 1.13, and 8.61 $\mu g \cdot m^{-3}$, respectively, and it was found that they were highly correlated. They found a clear seasonal and spatial variability in the atmospheric concentrations of BTEX at the sampling sites, showing great differences between high-traffic and residential areas and also pointed to traffic as the main source of emission of these compounds. The lowest levels of BTEX occurred during the summer due to increased solar radiation and photochemical reactions. However, the average concentrations of benzene did not exceed the European limits in any of the monitored points. Cai and collaborators (2022) studied BTEX levels in a peri-urban area in France during COVID pandemic, collecting and analyzing air samples using a fully automated online gas chromatography system (GC-FID, AirmoVOC C6–C16 Chromatotec ®, Val-deVirvée, France) at an air quality monitoring station located in the suburbs of Orleans, France. The mean concentrations of BTEX (benzene, toluene, ethylbenzene, and xylenes) before, during, and after confinement were 402 ± 143, 800 ± 378, and 851 ± 445 pptv, respectively. Their results suggested that although anomalous local anthropogenic activity can cause significant changes in BTEX concentrations, contamination levels in Orleans are primarily dependent on meteorological conditions, specifically whether the winds are coming from the Paris region. The variation in mean BTEX concentration suggested that, in addition to strength source, seasonal and diurnal variations in atmospheric BTEX in peri-urban areas are also highly dependent on meteorological conditions and photochemical activity.

In Mexico, some studies have also been carried out in order to know the levels of BTEX in the low atmosphere. Rodríguez et al. (2018) studied diurnal variation of BTEX in Tijuana, Baja California, Mexico collecting samples at intervals of 1.5 hours in different sampling periods: 7:30-9:00 a.m., 12:00-1:30 p.m., 3:00-4:30 p.m., and 6:00-7:30 p.m. and later, analyzing them by means of gas chromatography with a flame ionization detector. The highest concentrations of BTEX occurred during the morning (7:30-9:00 a.m.) and night (6:00-7:30 p.m.) periods. The average concentrations (in $\mu g\ m^{-3}$) of BTEX were 32.40, 13.28, 17.16, and 7.02, for benzene, p-xylene, toluene, and ethylbenzene, respectively. These results indicate that there should be changes in environmental policies to improve air quality in this region. The relative abundance of BTEX had the following order: benzene (32.398 $\mu g\ m^{-3}$) > p-xylene (17,155 $\mu g\ m^{-3}$) > toluene (13,277 $\mu g\ m^{-3}$) > ethylbenzene (7.023 $\mu g\ m^{-3}$). Pearson's correlation analysis and Principal Component Analysis confirmed that vehicular traffic was the main source of benzene, toluene, and p-xylene emissions, and that toluene and p-xylene may have contributed to the formation of tropospheric ozone through photochemical reactions. Ethylbenzene did not correlate with the rest of the pollutants measured, indicating that its origin probably came from a different source than the rest. Estévez et al. (2015) found a relative abundance of the BTEX in an industrial site in Orizaba Veracruz, Mexico that exhibited the following order: Benzene > Toluene> Xylenes > Ethylbenzene. The average concentrations were: 74.51 $\mu g\ m^{-3}$ for Benzene, 5.33 $\mu g\ m^{-3}$ for Toluene, 2.26 $\mu g\ m^{-3}$ for Ethylbenzene, and 3.35 $\mu g\ m^{-3}$ for p-Xylene. BTEX levels were strongly influenced by winds coming from the S and SSE where an important road, the Veracruz-Mexico highway, is located, indicating that these compounds were mainly originating from vehicular sources. The ratios T/B, X/Ebz and PCA analysis showed that B was strongly influenced by fresh vehicle emissions. The results found for B are greater than those previously observed in Europe and the United States, and the lifetime risk of cancer due to exposure to B in ambient air exceeded the acceptable levels reported by the USEPA.

Although some studies have been carried out in Mexico (Cerón *et al.*, 2021; Cerón *et al.*, 2015; Cerón *et al.*, 2013), they are not enough, and the distribution of these compounds is currently unknown in big cities of Mexico. Thus, the Metropolitan Area of Monterrey in Nuevo Leon State, is one of the biggest population centers in the country. This Metropoli is constituted by 12 municipalities (Guadalupe, Apodaca, San Nicolás de los Garza, General Escobedo, Santa Catarina, Benito Juárez, García, San Pedro Garza García, Cadereyta Jiménez, Santiago, Salinas Victoria, El Carmen) and Monterrey city, according to the National Institute of Statistics and Geography (INEGI) is the second most populous metropolitan area in Mexico with 5,341,171 inhabitants and the second with the largest territorial extension (7657 km$^2$), being the 84th largest metropolitan area in the world.

According to the INEM (Criteria Pollutant Emissions Inventory) for the Metropolitan Area of Monterrey 2016, VOC emissions for these municipalities are largely due to the use and consumption of products containing volatile organic compounds. These emissions are mainly due to work in mining, the construction industry, based on electricity consumption, transportation, fossil fuel industries, industrial processes, waste management, agriculture, treatment of hazardous waste, and other anthropogenic factors. Table 6.1 shows the results for the emissions inventory 2016 for VOC's in Nuevo Leon State.

**Table 6.1** Results for the emissions inventory 2016 for VOC's in Nuevo Leon State

| Source distribution | Municipalities | COV's (tons/year) |
|---|---|---|
| Area Sources | Sabinas Hidalgo, Salinas Victoria, General Zuazua, General Zuazua, Apodaca, García, Santa Catarina, Monterrey, San Nicolás de los Garza, Pesquería, Cadereyta Jiménez, Santiago, Montemorelos, Allende, Linares, Galeana, Doctor Arroyo. | > 6,750.00 - 12,197.72 |
| Fixed sources | Salinas Victoria, Ciénega de Flores, El Carmen, Abasolo, Apodaca, García, General Escobedo, Monterrey, Cadereyta Jiménez, Santa Catarina, Juárez, Montemorelos, Linares, Agualeguas. | > 1,155.30 - 2,060.77 |
| Mobile Sources | Sabinas Hidalgo, Apodaca, García, San Nicolás de los Garza, Monterrey, Cadereyta Jiménez, Santa Catarina, Montemorelos, Allende, Linares, Santiago | > 3,098.94 - 7,047.72 |
| Natural Sources | Vallecillo, Sabinas Hidalgo, Villaldama, Bustamante, General Treviño, Cerralvo, Melchor Ocampo, los Herreras, General Bravo, Doctor Coss, Los Ramones, Cadereyta Jiménez, Santa Catarina, Juárez, Montemorelos, Linares, Galeana, Aramberri, Doctor Arroyo, Mier y Noriega, Hualahuises, General Terán, El Carmen, Juárez. | > 2,088.05 - 4,881.98 |

*Source: Inventario Nacional de Emisiones de Contaminantes Criterio 2016, INEM- SIINEM*

In the 2016 emissions inventory, the municipality of Monterrey, where Obispado is located, stands out with the main contribution to VOC (Volatile Organic Compound) emissions, being the municipality with higher emissions, this is due to the contribution of area sources such as commercial and light industrial sources, poor waste management and the use of solvents. But above all, the main contaminant for the distribution of VOCs in the metropolitan area is the use of petroleum derivatives for transportation. This study is focused on determine the levels of BTEX in ambient air in Obispado, an urban site located in Monterrey municipality, during the Norths season in 2020; and evaluate the potential of health risk by inhalation of these compounds in the population of this study area.

## 6.3 Methodology

### 6.3.1 Study Area Description

Figure 6.1 shows the location of the Metropolitan Area of Monterrey. Samples were collected in Obispado site, within an air quality monitoring station of the government of Nuevo Leon State. It is located within the coordinates 25°40′24″N 100°20′32″W / 25.67347222, -100.34217778. It is located at the tip of the Loma de la Chepe Vera, so called because the lands of José Vera were in its surroundings. This hill is currently known as the Cerro del Obispado and the palace is also known as the Palacio del Obispado (Figure 6.2).

**Figure 6.1** Location of the metropolitan area of Monterrey



*Source: INEGI (2020)*

**Figure 6.2** Location of Obispado sampling site
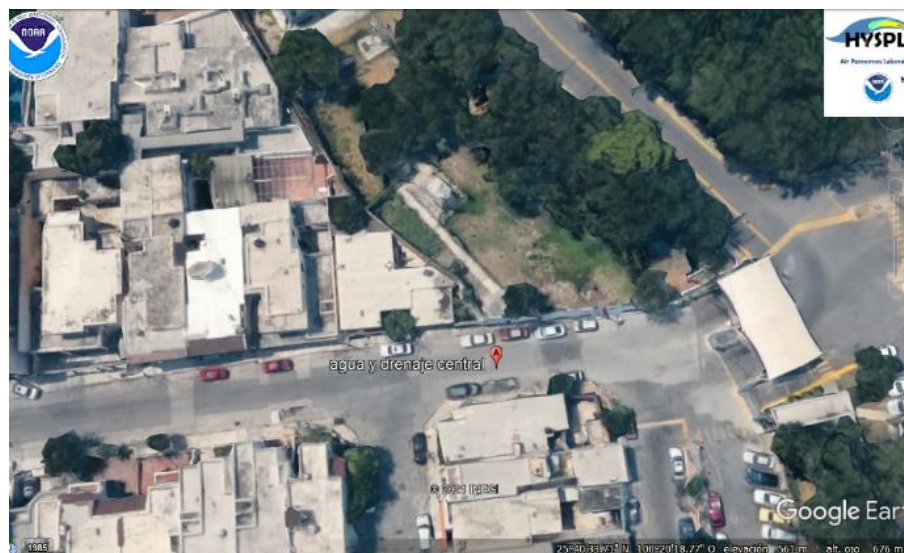


*Source: Google Earth*

## 6.3.2 Sampling Method

Ambient air samples were collected using Anasorb CSC 226-01 brand glass tubes, 70 mm long by 4.0 mm internal diameter and 6 mm external diameter, divided into two sections: 50 mg and 100 mg of activated carbon in each (Figure 6.3a), connected to a GAST-type vacuum pump operating at a controlled flow of 200 ml/min according to the Method MTA/MA-030/A92 (INSHT, 1992) (Figure 6.3.b).

**Figure 6.3** Devices and equipment used for the samples collection

a) Anasorb CSC 226-01 brand activated carbon glasses          b) GAST type vacuum pump



*Source: Own elaboration from pictures taken in the laboratory*

Three periods of sampling were considered:  morning (07:00 - 8:30 h), noon (14:00 - 15:30 h) and afternoon (17:30 - 19:00 h), with 1.5 hours of duration, from October 20 to November 18, 2020. A total of 24 samples were collected in Obispado site. At the end of each sampling, each tube was covered with plastic caps and stored in special bags to protect against of sunlight to avoid possible photochemical reactions and will later be kept refrigerated to preserve them until further analysis.

### 6.3.3 Analytical Method

For the determination of the BTEX, the desorption of each of the samples was carried out with 1 ml of carbon disulfide ($CS_2$) in amber vials of 2 ml provided with a screw cap with a rubber septa. One end of the glass tube was broken and each section of activated carbon was poured into a previously labeled vial (two vials per tube), then 1 ml of carbon disulfide ($CS_2$) was added. Then the vial was shaken for a period of approximately 5 minutes to ensure that the desorption was completed, and it was refrigerated for 24 hours until its analysis. Once the desorption was done, the chromatographic analysis was carried out using a TRACE GC Ultra Gas Chromatographs Thermoscientific brand equipment in splitless mode with flame ionization detector using extra dry air and ultra-high purity nitrogen as carrier gas (Figure 6.4). The capillary column used was 30 m x 0.32 mm ID, methyl fused silica type with a film thickness of 0.5 µm, the operator software used was Trace ChemStation. 1 µL of the sample was taken from the vial using a chromatographic syringe (Hamilton 10 µL, and then it was injected into the chromatography equipment previously conditioned for analysis. The chromatographic analysis was based on the MTA/MA-030/A92 method (INSHT, 1992).

**Figure 6.4** TRACE GC Ultra gas chromatograph



*Source: Own elaboration from pictures taken in the laboratory*

For this procedure, the equipment software (Trace ChemStation) was used, which contains files of the chromatograms generated from each injection made of the calibration curves made before the analysis of the samples for each compound to be quantified, in addition to providing the times of exact retention where each compound is present.bTo perform the calculation of concentrations in ambient air using the following equations taken from MTA/MA-030/A92 Method:

$$ms = \frac{Cf+Cp-Cb}{ED-Vd} \tag{1}$$

Where:

ms = is the total amount of analyte present in the sample, in mg.

Cf = is the analyte concentration in mg/mL in the front section of the sampling tube.

Cp = is the analyte concentration in mg/mL in the back section of the sampling tube.

Cb = is the analyte concentration in mg/mL in the two blank sections.

ED = is the desorption efficiency (depends on each compound: Table 6.2).

Vd = is the volume of desorbent solution in mL.

**Table 6.2** Desorption Efficiency Values (ED)

| Benzene | Toluene | Ethylbenzene | pXylene |
|---|---|---|---|
| 1.008 | 0.989 | 1.00 | 0.985 |

*Source: Own elaboration from lab essays*

To calculate $C_{air}$, which is the concentration of the sampled air analyte in mg/m$^3$, ms was calculated previously, being V, the volume of sampled air equal to 0.018m$^3$:

$$C_{air} = \frac{m_S}{V} \tag{2}$$

$C_{ppm}$ is the concentration in parts per million of the contaminants in ambient air, where M is the molecular weight of each compound in g/mol, P is the atmospheric pressure of the sampling site in KPa, and T is the temperature of the site at the sampling time.

$$C_{ppm} = C_{air} \times \frac{24}{M} \times \frac{101.30}{P} \times \frac{T+273.15}{293.15} \tag{3}$$

**6.3.4 BTEX ratios**

The ratio of the concentrations of toluene between benzene (T/B) and Xylene and Ethylbenzene (X/E) were calculated. The purpose of calculating the T/B and X/E ratios is based on identifying the probable origin of BTEX during a given period, being able to estimate whether the emissions were originated from mobile or area sources (T/B ratio) and to determine the grade of processing of air masses (fresh or aged) (X/E ratio).

The T/B ratio has been commonly used as an indicator of emissions from vehicular traffic. Benzene and toluene are constituents of gasoline and are emitted into the atmosphere from motor vehicle exhaust. The toluene content in gasoline is 3 to 4 times higher than the benzene content (Pekey and Yilma, 2011).

Values of this ratio less than 2-3 are characteristic of vehicle emissions and have been reported in this range for many urban areas around the world (Elbir *et al.*, 2007; Mugica *et al.*, 2003); nevertheless, values greater than 3 may indicate that BTEX levels could be associated with sources other than vehicular sources, such as industrial facilities and area sources (evaporative emissions, automotive paint shops, food cooking processes, screen printing shops), dry cleaners, among others.The X/E ratio is commonly used as an indicator of the photochemical age of air masses at a given site (Keymeulen *et al.*, 2001; Lee *et al.*, 2002). This ratio is related to the atmospheric lifetime of these pollutants in the air. High values of this ratio indicate old air masses, while low values of this ratio indicate that the air masses are fresh (recent emissions). Kuntasal *et al.*, (2005) used a value of 3.8 for this ratio. Fresh gasoline emissions provide values between 3.8 and 4.4 for this ratio.

## 6.3.5 Statistical Analysis

To identify the diurnal variation and descriptive statistics of the compounds measured in the ambient air of the city of Monterey, box plots were made which showed the mean, maximum and average of concentration for each studied compound. A bi-variate analysis was carried out in order to know the Pearson correlation coefficient and the bi-variate relation between each pair of BTEX and its grade of significance. A Principal component analysis (multivariate analysis: PCA) was applied in order to determine the relation among the studied variables using the software XLSTAT for Excel (2016 version, https://www.xlstat.com/es/). From the cosines of the variables and the factor loadings, groups of related compounds are identified, and their probable origin is inferred based on the tracer compounds present in each group of compounds. The biplot of the components that have a greater contribution to the total variability of the data is plotted.

## 6.3.6 Meteorological Analysis

### 6.3.6.1 Wind Roses Analysis

Wind speed and wind direction were examined simultaneously using a wind rose analysis. These graphics show the distribution of the direction and speed of the wind in a specific place. The wind rose model for each sampling site was made with the WRPLOT (Wind Rose Plots for Meteorological Data) software, which simulates the direction and speed of the prevailing winds in the sampling period (Software developed by Lakes Environmental, available in: https://www.weblakes.com/products/wrplot/index.html).

## 6.3.7 Health Risk Assessment

The carcinogenic potential of benzene is widely known (Zhang et al. 2015). The European Union recommends an annual limit of 5 $\mu g/m^3$ for benzene in ambient air and the level of minimum risk of cancer by inhalation (Minimal Risk Level: MRL) of 1 in 10,000, while the USEPA establishes a value of 4.0 ppbv for this pollutant (USEPA, 2013). In this study, we used the methodology proposed by Zhang and collaborators (2015), to determine the daily exposure (E) (USEPA, 2008), the lifetime risk of cancer (ILTCR) and the potential non-cancer risk (HQ), that is, the risk of developing diseases other than cancer (respiratory and cardiovascular diseases). The daily exposure (mg/kg per day) of an individual by inhalation can be calculated as:

$$E = \frac{C \times IRa \times Da}{BW}$$ (4)

Where C ($mg/m^3$) is the average concentration of benzene, IRa is the inhalation rate of an adult (0.83 $m^3$/h)[1], Da is the duration of exposure of an adult (24 h/day), and Bw is the body weight of an average adult (65 kg)[1]. The lifetime cancer risk (The Lifetime Cancer Risk: LTCR) is then calculated as:

$$LTCR = E \times SF$$ (5)

Where SF is the risk slope factor for toxic inhalation when the carcinogenic effect of exposure is considered linear. The proposed value by the United States Environmental Protection Agency (US EPA, 2009) of SF for benzene (0.029 mg/kg per day) was considered. The non-cancer risk of BTEX will be measured as a hazard quotient (HQ):

$$HQ = \frac{C}{RfC} \tag{6}$$

Where C is the average concentration received daily and RfC is the inhalation reference concentration proposed by the United States Environmental Protection Agency (USEPA, 2005) for each pollutant (Table 6.3).

**Table 6.3** Reference concentration (RfC) used for BTEX

| Reference Concentration | | | |
|---|---|---|---|
| Benzene | Toluene | Ethylbenzene | p-xylene |
| (mg/m$^3$) | (mg/m$^3$) | (mg/m$^3$) | (mg/m$^3$) |
| 0.03 | 5 | 1 | 0.1 |

*Source: ATSDR (2007) for benzene, US EPA (2005) for toluene, ATSDR (2010) for ethylbenzene, and US EPA (2003) for p-xylene*

The accepted values proposed by EPA and WHO for LTCR (cancer risk for benzene inhalation) and for HQ (risk of non-cancer) for inhalation of BTEX are 1x 10$^{-6}$ and 1.0, respectively. If LTCR and HQ values are higher than these reference values, indicate a probable risk in the population health.

## 6.4 Results

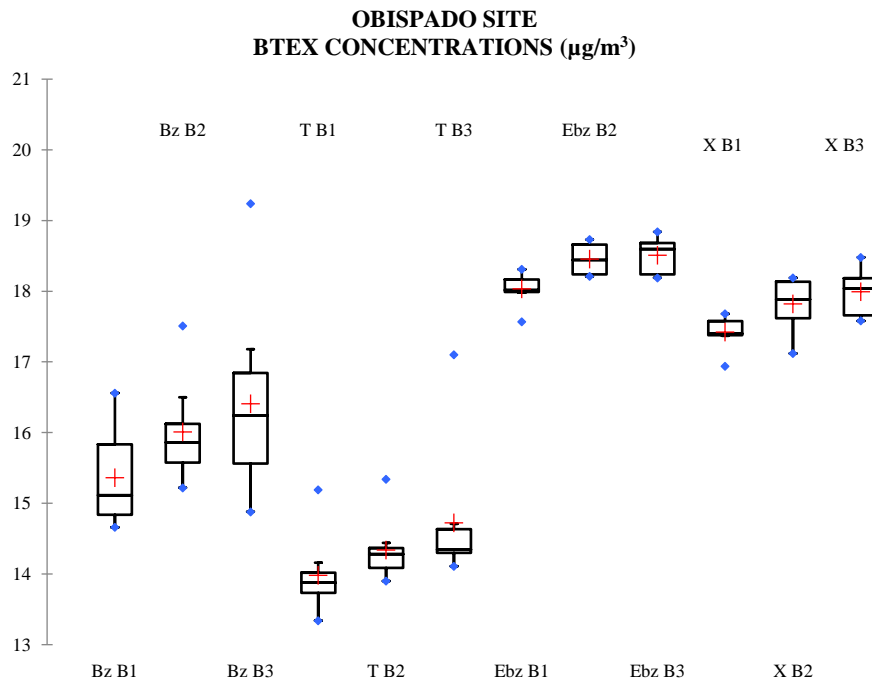### 6.4.1 BTEX Concentrations

The concentrations of benzene, toluene, ethylbenzene and p-xylene (BTEX) were measured at one sampling site (Obispado) during the Norths season. Table 6.4 shows the concentrations obtained during the Norths season. Ethylbenzene and p-xylene are the compounds with the highest levels with mean concentrations of 18.333 µg/m$^3$ and 17.747 µg/m$^3$, respectively. Mean values for benzene and toluene were the following: 15.927 µg/m$^3$ and 14.347 µg/m$^3$, respectively.

**Table 6.4** Descriptive Statistics for BTEX concentrations measured in Obispado site during the Norths season. Bz: benzene; T: toluene; Ebz: ethylbenzene; X: p-xylene. B1: morning sampling period (08:00– 09:30 h); B2: midday sampling period (13:00 – 14:30 h); and B3: afternoon sampling period (17:30 – 19:00 h)

| DESCRIPTIVE STATISTIC FOR BTEX CONCENTRATIONS IN OBISPADO SITE (µg/m$^3$) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Bz B1 | Bz B2 | Bz B3 | T B1 | T B2 | T B3 | Ebz B1 | Ebz B2 | Ebz B3 | X B1 | X B2 | X B3 |
| Minimum | 14.660 | 15.220 | 14.880 | 13.340 | 13.900 | 14.110 | 17.570 | 18.210 | 18.190 | 16.940 | 17.120 | 17.580 |
| Maximum | 16.560 | 17.510 | 19.240 | 15.190 | 15.340 | 17.100 | 18.310 | 18.730 | 18.840 | 17.680 | 18.190 | 18.480 |
| 1° Quartil | 14.840 | 15.578 | 15.563 | 13.735 | 14.085 | 14.300 | 17.988 | 18.238 | 18.240 | 17.378 | 17.620 | 17.660 |
| Median | 15.115 | 15.860 | 16.240 | 13.880 | 14.280 | 14.345 | 18.015 | 18.440 | 18.595 | 17.400 | 17.885 | 18.040 |
| 3° Quartil | 15.833 | 16.125 | 16.843 | 14.018 | 14.365 | 14.635 | 18.168 | 18.660 | 18.683 | 17.578 | 18.133 | 18.185 |
| Mean | 15.363 | 16.010 | 16.410 | 13.980 | 14.338 | 14.723 | 18.034 | 18.456 | 18.509 | 17.423 | 17.824 | 17.995 |
| Typical Deviation (n-1) | 0.672 | 0.714 | 1.397 | 0.545 | 0.443 | 0.980 | 0.225 | 0.234 | 0.249 | 0.232 | 0.380 | 0.346 |

*Source: Own elaboration from the obtained results.*

Figure 6.5 shows the box plot for BTEX concentrations in Obispado site. As it can be observed, all measured BTEX showed the same diurnal pattern, showing higher mean concentration levels during the afternoon (B3 sampling period). This behavior can be explained due to the fact that during the afternoon occurs a peak on vehicular traffic originated by a higher population mobility that commuting from their workplaces to their homes, producing higher emissions of air pollutants from automobile exhaust. Ethylbenzene and p-xylene were the dominant BTEX in Obispado site.

**Figure 6.5** Box plot for BTEX concentrations measured in Obispado site during the sampling period



**OBISPADO SITE**
**BTEX CONCENTRATIONS (µg/m³)**

+ are the mean values of BTEX concentrations, ♦ are the maximum and minimum values of BTEX concentrations and the edges of the box are the 1° and 3° quartiles, the box does not have a statistic mean only is represented for better visualization. Bz: benzene; T: toluene; Ebz: ethylbenzene; X: p-xylene. B1: morning sampling period (08:00– 09:30 h); B2: midday sampling period (13:00 – 14:30 h); and B3: afternoon sampling period (17:30 – 19:00 h)

*Source: Own elaboration from the obtained results*

A Friedman test at a significance level of α = 0.05 was applied to the data set in order to investigate if there were significant differences among the different sampling periods along the day. The results showed that there were significant differences in BTEX concentrations among the different sampling periods, which means that the measured BTEX had a clear diurnal variation. We found that that BTEX were homogeneously distributed in the study area.

## 6.4.2 Meteorological parameters

Table 6.5 shows the average values for meteorological parameters, considering wind speed, wind direction, temperature, relative humidity, solar radiation and barometric pressure. It can be observed that wind blowed predominantly from ESE with an average wind speed of 11.8 m/s, showing higher values of wind speed during the midday and afternoon sampling periods.

**Table 6.5** Meteorological parameters for both sampling sites. B1: morning sampling period (08:00– 09:30 h); B2: midday sampling period (13:00 – 14:30 h); and B3: afternoon sampling period (17:30 – 19:00 h)

| | | | | Obispado site | | | |
| Meteorological parameters | | Wind speed m/s | Wind direction | Temperature °C | Relative Humidity % | Solar radiation W/m² | Barometric Pressure mm Hg |
|---|---|---|---|---|---|---|---|
| Sampling periods | B1 | 6.9 | SSE | 18.88 | 62.75 | 167.63 | 715.74 |
| | B2 | 14.6 | E | 24.18 | 46.13 | 169.13 | 712.73 |
| | B3 | 13.9 | ESE | 21.65 | 55.5 | 148.22 | 713.56 |
| | Mean Value | 11.8 | ESE | 21.57 | 54.79 | 161.66 | 714.01 |

*Source: Own elaboration from the obtained results*

### 6.4.3 Health Risk Assessment

Table 6.6 shows the average of the non-cancer risk coefficients that represent the BTEX measured in the study site for both, adult and children population. It can be seen that the non-cancer risk coefficient (HQ: Hazard Quotient) was higher for benzene, having the following behavior: benzene>p-xylene>ethylbenzene>toluene. The values found for the non-cancer risk coefficient (HQ) were below the maximum permissible limit established by the EPA and the WHO (HQ ≤ 1.0), indicating that there is no risk of developing diseases different than cancer (respiratory and cardiovascular diseases) by inhalation of BTEX at the study site. In this study, the potential risk of non-cancer (HQ) was determined according to the methodology of Zhang (2015) and Estévez (2015).

**Table 6.6** Non-cancer risk coefficients (HQ) in Obispado site during the sampling period for a) Adult population, and b) Children population

| a) Non cancer risk coefficients (HQ) for Adult population | |
| --- | --- |
| Air Pollutant | Obispado Site |
| Benzene | 0.536 |
| Toluene | 0.003 |
| Ethylbenzene | 0.018 |
| p-Xylene | 0.178 |
| THQ = ΣHQ = 0.735 | |
| **b) Non cancer risk coefficients (HQ) for Children population** | |
| Non-cancer Risk | |
| Air Pollutant | Obispado site |
| Benzene | 0.5309 |
| Toluene | 0.0029 |
| Ethylbenzene | 0.0018 |
| p-Xylene | 0.1777 |
| THQ = ΣHQ = 0.7126 | |

*Source: Own elaboration from the obtained results*

Table 6.7 shows the life-time cancer risk coefficients in children and adult population for both sampling sites due to benzene inhalation. According to WHO and EPA, the established permissible limit is ($1 \times 10^{-6}$). The values obtained exceeded the maximum permissible levels almost by two orders of magnitude, therefore is a possible risk of developing cancer in the life-time due to the inhalation of benzene at the concentrations found at the study sampling site.

**Table 6.7** Life-time cancer risk coefficients (LTCR) in Obispado site during the sampling period for a) Adult population, and b) Children population

| a) Adult population | |
| --- | --- |
| Life-time Cancer Risk (LTCR) | |
| Air Pollutant | Obispado |
| Benzene | $1.445 \times 10^{-4}$ |
| **b) Children population** | |
| Life-time Cancer Risk (LTCR) | |
| Air Pollutant | Obispado |
| Benzene | $2.575 \times 10^{-4}$ |

*Source: Own elaboration from the obtained results*

### 6.4.4 BTEX Ratios

Analysis of BTEX ratios has been used by various authors to estimate the relative contribution of pollution sources and the photochemical processing of air masses (photochemical age of air masses) containing BTEX, and it is based on the assumption that BTEX have different rates of degradation in air (Guo, *et al.*, 2007). Figure 1.6 shows the box plots and descriptive statistic for the toluene-benzene (T/B) and the xylene-ethylbenzene (X/E) ratios for Obispado site, during each sampling period (B1, B2 and B3).

The T/B concentration ratio has been commonly used as an indicator of emissions from vehicular traffic. Benzene and toluene are constituents of gasoline and are emitted into the atmosphere from motor vehicle exhaust. The toluene content in gasoline is 3-4 times higher than the benzene content (Pekey and Yilma, 2011). Values of this T/B ratio less than 2-3 are characteristic of vehicle emissions and have been reported in this range for many urban areas around the world (Elbir *et al*., 2007; Mugica *et al*., 2002); nevertheless, while values greater than 3 may indicate that BTEX levels could be associated with sources other beyond vehicular sources such as industrial facilities and area sources (evaporative emissions, automotive paint shops, food cooking processes, screen printing shops, dry cleaners, among many others). The T/B ratio values found in this study are less than 2 and are within the typical range reported for urban sites with a strong influence of vehicle emissions. T/B ratios found in Obispado were higher during the morning sampling period (B1), when a peak of vehicular traffic occurs due to the mobility of population to their work centres and school.

The concentration ratio of p-Xylene to Ethylbenzene (X/Ebz ratio) is commonly used as an indicator of the photochemical age of air masses at a given site. A ratio of 3.6:1 (X/Ebz) has been established as a typical value of this ratio for these species (Keymeulen *et al*. 2001; Lee *et al*. 2002). This ratio is related to the atmospheric lifetime of these pollutants in the air: high values of this ratio indicate aged air masses, and low values of this ratio indicate fresh air masses (recent local type emissions). Kuntasal *et al*. (2005) used a value of 3.8 for this ratio. Fresh gasoline emissions provide values between 3.8 and 4.4 for this ratio. In the present study, the entire period registered low values of this ratio, indicating that most of the air masses in the study sites corresponded to "fresh or local emissions". The values found for this ratio (X/E) are within the typical range reported for fresh emissions with little or almost no photochemical processing, that is, emissions from primary and local sources. It can be observed in Figure 1.6 that the ratios of X/E in Obispado remained constant throughout the day, which indicates that the influence of vehicular traffic on this site is also a constant. There were no significant differences in the BTEX ratios in both study sites, which may indicate that the sources of these compounds (mainly vehicle-type emissions) are homogeneously distributed in the study area.

### 6.4.5 Influence of meteorology on BTEX concentrations

The wind flow pattern (wind direction and wind speed) influences the removal, accumulation, transport, dispersion and transformation of pollutants in the atmosphere. The winds are related to the horizontal dynamics of the atmosphere that describes both the direction from which the air masses come and the speed and turbulence with which these air masses move containing the pollutants. Based on prevailing winds, concentration roses were constructed to study the effect of wind direction on BTEX levels measured at both study sites, considering both diurnal variation (B1: morning sampling period; B2: midday sampling period; and B3: afternoon sampling period). Figures 6.7 shows concentration-wind roses for measured BTEX in the study site.
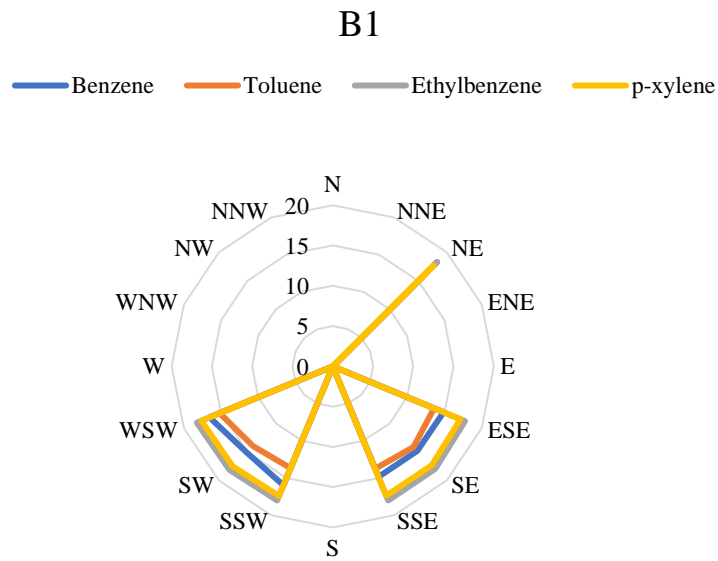
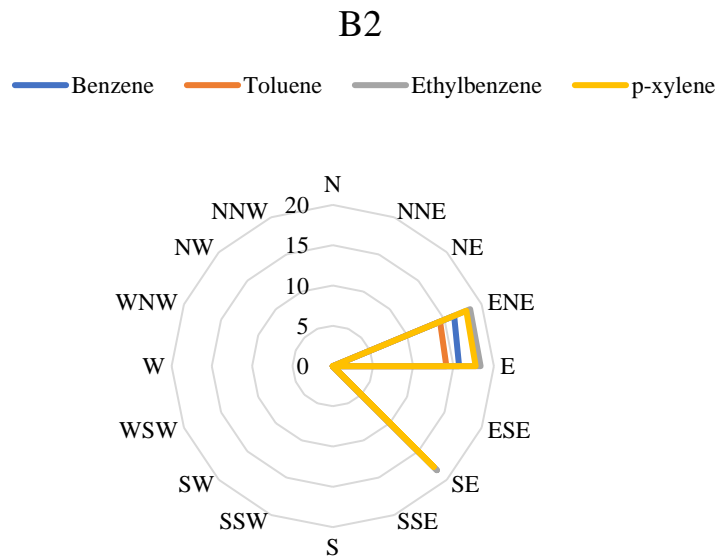**Figure 6.6** Box plot for BTEX ratios measured in Obispado site during the sampling period
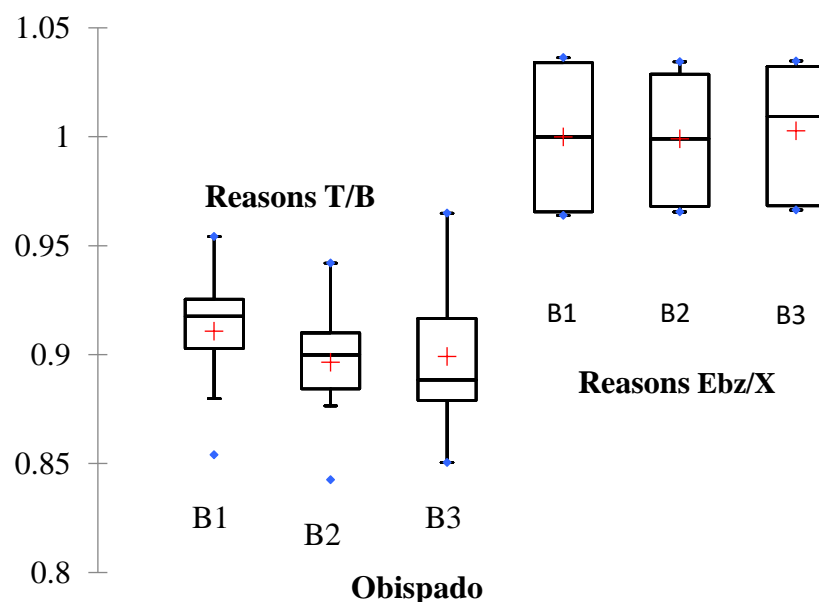


*Source: Own elaboration from the obtained results*

**Figure 6.7** Concentration-wind roses for measured BTEX in Obispado during: a) morning sampling period (B1), b) midday sampling period (B2) and c) afternoon sampling period (B3)
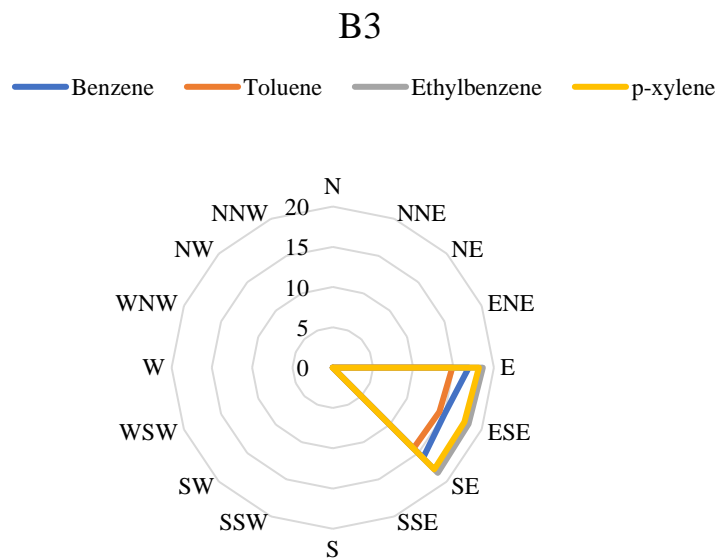
A)



B)



c)



*Source: Own elaboration from the obtained results*

As it can be observed in Figure 6.7, BTEX concentrations in Obispado site were higher when winds blowed from WSW, SSW, SW, SSE, ESE and NE during the B1 sampling period. During midday (B2 sampling period), BTEX concentrations were higher when winds blowed from ENE, E and SE. During the afternoon (B3 sampling period) BTEX concentrations were higher with winds blowing from E, ESE and SE. The municipalities of Juárez and Apodaca are located in these directions, as well as numerous avenues with high vehicular traffic with emissions that could have contributed to the BTEX levels measured at the study site.

### 6.4.6 Bi-variate and multivariate analysis

Bivariate (Pearson) and multivariate (Principal Component Analysis: PCA) statistical analysis are an useful tool to reveal more detailed information about the behavior of the pollutants studied (Polanco, 2016). Tables 6.8-6.10 show the results of the bi-variate analysis for BTEX and air criteria pollutants concentrations (CO, $SO_2$, $O_3$, $NO_2$, $PM_{10}$ and $PM_{2.5}$) in Obispado. Values in bold are different from 0 with a level of significance $\alpha=0.05$. The results of the bivariate analysis for Obispado during the morning sampling (B1) are shown in Table 6.8. Benzene presented positive correlation coefficients close to linearity with ethylbenzene (0.898) and with p-xylene (0.892). Ethylbenzene presented a significant linear positive correlation with P-xylene (0.999), indicating that these compounds could have originated from common sources. Ethylbenzene and p-xylene presented a linear and significant negative correlation with $PM_{2.5}$, indicating that these hydrocarbons could have participated in photochemical reactions of secondary organic aerosol formation (SOA). Some criteria pollutants presented positive correlations close to linearity between them, such is the case of CO with $PM_{2.5}$ (0.885), with $O_3$ (0.717), and with $SO_2$ (0.798), indicating that these pollutants could have originated from emissions of vehicular sources, since CO is a tracer of combustion emissions in motor vehicles.

The correlation coefficient between CO and $O_3$ can be explained from the following atmospheric reactions:

- In the presence of solar radiation, CO reacts with OH to form the hydroperoxyl radical ($HO_2$), which leads to the formation of surface $O_3$ in the presence of $NO_2$ (Zhong *et al*., 2020).

$$(R1)\ CO + OH + O_2 \rightarrow CO_2 + HO_2 \tag{7}$$

This radical hydroperoxyl ($HO_2$) reacts with NO in order to produce $NO_2$

$$(R2)\ HO_2 + NO \rightarrow NO_2 + OH \tag{8}$$
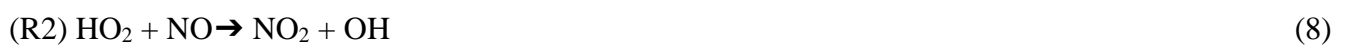
When solar radiation is intense, $NO_2$ undergoes photolysis in order to produce NO and atomic oxygen and this reacts with $O_2$ in order to produce $O_3$ (Ídem).

$$(R3)\ NO_2 + h\nu\ (\lambda < 420\ nm) \rightarrow NO + O \tag{9}$$

$$(R4)\ O + O_2 + M \rightarrow O_3 + M \tag{10}$$

**Table 6.8** Pearson correlation coefficients for the morning sampling period (B1) in Obispado

| Variables | CO | $NO_2$ | $O_3$ | $PM_{10}$ | $PM_{2.5}$ | $SO_2$ | Benzene | Toluene | Ethyl-benzene | p-xylene |
|---|---|---|---|---|---|---|---|---|---|---|
| CO | 1 | 0.646 | 0.717 | 0.079 | 0.885 | 0.798 | -0.910 | -0.142 | -0.723 | -0.732 |
| $NO_2$ | 0.646 | 1 | 0.903 | 0.074 | 0.218 | 0.695 | -0.340 | 0.605 | 0.058 | 0.046 |
| $O_3$ | 0.717 | 0.903 | 1 | -0.317 | 0.380 | 0.489 | -0.365 | 0.275 | -0.098 | -0.127 |
| $PM_{10}$ | 0.079 | 0.074 | -0.317 | 1 | 0.023 | 0.623 | -0.317 | 0.395 | -0.100 | -0.052 |
| $PM_{2.5}$ | 0.885 | 0.218 | 0.380 | 0.023 | 1 | 0.579 | -0.948 | -0.563 | **-0.957** | **-0.964** |
| $SO_2$ | 0.798 | 0.695 | 0.489 | 0.623 | 0.579 | 1 | -0.792 | 0.311 | -0.451 | -0.433 |
| Benzene | -0.910 | -0.340 | -0.365 | -0.317 | -0.948 | -0.792 | 1 | 0.330 | 0.898 | 0.892 |
| Toluene | -0.142 | 0.605 | 0.275 | 0.395 | -0.563 | 0.311 | 0.330 | 1 | 0.707 | 0.721 |
| Ethylbenzene | -0.723 | 0.058 | -0.098 | -0.100 | **-0.957** | -0.451 | 0.898 | 0.707 | 1 | **0.999** |
| p-xylene | -0.732 | 0.046 | -0.127 | -0.052 | **-0.964** | -0.433 | 0.892 | 0.721 | **0.999** | 1 |

*Source: Own elaboration from the obtained results*

During the midday sampling period (B2) (Table 6.9) significant linear correlations could be observed between CO and $PM_{2.5}$ (0.957), $NO_2$ and $PM_{10}$ (0.962) and $NO_2$- $SO_2$ (0.973), indicating that these air pollutants could be originated from emissions of vehicular sources since CO is a tracer of emissions related to vehicular traffic and from high temperature combustion sources. $PM_{10}$ and $PM_{2.5}$ showed a significant linear positive correlation (0.944), indicating that particulate matter had their origin in common sources during this study. Negative linear correlations of CO with ethylbenzene (-0.963) and p-xylene (-0.998) can be explained from the following atmospheric reactions that carry out during the midday, when solar radiation is intense and that cause the production of highly reactive OH radicals which is the main trajectory of depletion of BTEX in air:

- In the presence of solar radiation, CO reacts with OH to produce $HO_2$ radical, conducting to the formation of $O_3$ in the presence of $NO_2$ (Id. Ibid):

(R1) $CO + OH + O_2 \rightarrow CO_2 + HO_2$ (11)
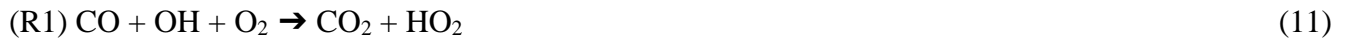
This $HO_2$ radical then reacts with NO in order to produce $NO_2$

(R2) $HO_2 + NO \rightarrow NO_2 + OH$ (12)

Negative correlations between ethylbenzene and p-xylene with $NO_2$ during the midday evidence photochemical process of depletion of BTEX in ambient air in high solar radiation conditions. $SO_2$ showed linear positive correlations with $PM_{10}$ and $PM_{2.5}$ (0.925 and 0.918, respectively), indicating that these particles could be originated from combustion processes that use high sulfur fuels as diesel. Toluene and Ozone showed a significant positive linear correlation (0.837), indicating that these compounds could be originated from photochemical reactions.

**Table 6.9** Pearson correlation coefficients for the midday sampling period (B2) in Obispado

| Variables | CO | NO2 | O3 | PM10 | PM2.5 | SO2 | Benzene | Toluene | Ethyl-benzene | p-xylene |
|---|---|---|---|---|---|---|---|---|---|---|
| CO | 1 | 0.859 | 0.013 | 0.924 | **0.957** | 0.905 | -0.151 | -0.405 | **-0.963** | **-0.998** |
| NO2 | 0.859 | 1 | -0.081 | **0.962** | 0.938 | **0.973** | -0.635 | -0.246 | -0.722 | -0.828 |
| O3 | 0.013 | -0.081 | 1 | -0.239 | -0.207 | 0.128 | 0.108 | 0.837 | -0.220 | -0.062 |
| PM10 | 0.924 | **0.962** | -0.239 | 1 | **0.994** | 0.925 | -0.451 | -0.483 | -0.788 | -0.895 |
| PM2.5 | **0.957** | 0.938 | -0.207 | **0.994** | 1 | 0.918 | -0.355 | -0.504 | -0.844 | -0.935 |
| SO2 | 0.905 | **0.973** | 0.128 | 0.925 | 0.918 | 1 | -0.525 | -0.123 | -0.826 | -0.889 |
| Benzene | -0.151 | -0.635 | 0.108 | -0.451 | -0.355 | -0.525 | 1 | -0.196 | -0.046 | 0.097 |
| Toluene | -0.405 | -0.246 | 0.837 | -0.483 | -0.504 | -0.123 | -0.196 | 1 | 0.276 | 0.380 |
| Ethylbenzene | **-0.963** | -0.722 | -0.220 | -0.788 | -0.844 | -0.826 | -0.046 | 0.276 | 1 | **0.979** |
| p-xylene | **-0.998** | -0.828 | -0.062 | -0.895 | -0.935 | -0.889 | 0.097 | 0.380 | **0.979** | 1 |

*Source: Own elaboration from the obtained results*

Table 6.9 shows the bivariate statistics during the afternoon sampling (B3). Significant positive linear correlations can be observed between $NO_2$ and $PM_{2.5}$ (0.998) and between benzene and toluene (0.976), indicating that these compounds could have originated from common sources. Positive linear correlations can be observed that, although not significant, suggest that these compounds could have originated from common sources: CO-$PM_{10}$ (0.860), CO-$SO_2$ (0.939), $NO_2$-$PM_{10}$ (0.806) and $SO_2$-$PM_{10}$ (0.840), indicating that the levels of these pollutants could be influenced by vehicle emissions derived from intense vehicular traffic on avenues surrounding the sampling site. Benzene-xylene (0.936), ethylbenzene-xylene (0.807) and toluene-xylene (0.837) presented correlations close to linearity, although not significant, indicating that these hydrocarbons could have originated at least partially from common sources. The negative correlations between CO with benzene, ethylbenzene and xylene indicate that in presence of high levels of CO, photochemical reactions of OH radical production occur, causing the depletion of BTEX in the study site.

**Table 6.10** Pearson correlation coefficients for the afternoon sampling period (B3) in Obispado

| Variables | CO | NO$_2$ | O$_3$ | PM$_{10}$ | PM$_{2.5}$ | SO$_2$ | Benzene | Toluene | Ethyl-benzene | p-xylene |
|---|---|---|---|---|---|---|---|---|---|---|
| CO | **1** | 0.548 | 0.713 | 0.860 | 0.596 | 0.939 | -0.031 | 0.169 | -0.812 | -0.350 |
| NO$_2$ | 0.548 | **1** | 0.919 | 0.806 | **0.998** | 0.380 | -0.066 | 0.118 | -0.682 | -0.356 |
| O$_3$ | 0.713 | 0.919 | **1** | **0.962** | 0.924 | 0.660 | -0.362 | -0.160 | -0.913 | -0.652 |
| PM$_{10}$ | 0.860 | 0.806 | **0.962** | **1** | 0.826 | 0.840 | -0.366 | -0.154 | **-0.973** | -0.669 |
| PM$_{2.5}$ | 0.596 | **0.998** | 0.924 | 0.826 | **1** | 0.423 | -0.040 | 0.150 | -0.698 | -0.342 |
| SO$_2$ | 0.939 | 0.380 | 0.660 | 0.840 | 0.423 | **1** | -0.324 | -0.146 | -0.872 | -0.576 |
| Benzene | -0.031 | -0.066 | -0.362 | -0.366 | -0.040 | -0.324 | **1** | **0.976** | 0.551 | 0.936 |
| Toluene | 0.169 | 0.118 | -0.160 | -0.154 | 0.150 | -0.146 | **0.976** | **1** | 0.357 | 0.837 |
| Ethylbenzene | -0.812 | -0.682 | -0.913 | **-0.973** | -0.698 | -0.872 | 0.551 | 0.357 | **1** | 0.807 |
| p-xylene | -0.350 | -0.356 | -0.652 | -0.669 | -0.342 | -0.576 | 0.936 | 0.837 | 0.807 | **1** |

*Source: Own elaboration from the obtained results*

Figures 6.8-6.10 show the result of the multivariate analysis (Principal Component Analysis) among the measured variables (BTEX and air criteria pollutants). For the morning sampling period (B1), two principal components were required to explain 92.79% of the total variability of the data. The attached Table in Figure 1.8 shows the loadings of the factors representing the groups of variables that were related to each other. Group F1 included CO, PM$_{2.5}$, SO$_2$, Benzene, Ethylbenzene and p-xylene, indicating that the compounds in this group are related to emissions from motor vehicle exhaust. F2 group included Ozone, NO$_2$ and Toluene, indicating that these compounds had a high influence on photochemical activity. Finally, F3 group included only PM$_{10}$, indicating that this pollutant could have a different origin, probably re-suspension of dust from the quarries and companies dedicated to the manufacture of ceramic and glass products.

The bi-plot attached in Figure 6.8 also shows the relationships between the measured variables, and it can be observed that benzene, ethylbenzene and p-xylene are in the same quadrant, indicating that these compounds could have originated from common sources. In the quadrant located on the left side of the bi-plot, it can observed that NO$_2$ and Ozone are very close to each other, so it can be inferred that these compounds were influenced by photochemical activity. SO$_2$, CO, and PM$_{2.5}$ showed proximity to each other as well as vectors with the same intensity, indicating that these compounds were influenced by vehicular combustion sources.

Figure 6.9 shows the results of the multivariate analysis with the loadings of the factors found. Two principal components were required to explain 86.48 % of the variability in the data. Group F1 showed higher factor loadings for CO, NO$_2$, PM$_{10}$, PM$_{2.5}$, SO$_2$, ethylbenzene and p-xylene, indicating that concentrations of these compounds were influenced by vehicle emissions. F2 group presented higher factor loadings for ozone and toluene, indicating that these compounds were influenced by photochemical activity. Finally, F3 group included only benzene, indicating that this hydrocarbon could have had a different source than the rest of the BTEX.

Figure 6.10 shows the results of the multivariate analysis for the afternoon sampling period in Obispado. Two principal components (F1 and F2) were required to explain 83.21% of the total variability of the data. F1 group showed higher values of factor loadings for CO, NO$_2$, O$_3$, PM$_{10}$, PM$_{2.5}$, ethylbenzene and p-xylene, indicating that all these compounds were influenced by vehicle emissions and photochemical activity, while F2 group included with higher factor loadings to benzene and toluene, likely originating from area sources such as solvent use, paints and coatings, biomass burning, and petrochemical industry.

**Figure 6.8** Principal Component Analysis for the measured variables during the morning sampling period (B1) in Obispado

|  | F1 | F2 | F3 |
|---|---|---|---|
| CO | 0.193 | **0.626** | 0.181 |
| NO$_2$ | **0.884** | 0.001 | 0.115 |
| O$_3$ | 0.271 | **0.706** | 0.023 |
| PM$_{10}$ | **0.738** | 0.112 | 0.150 |
| PM$_{2.5}$ | **0.777** | 0.208 | 0.015 |
| SO$_2$ | 0.010 | **0.989** | 0.000 |
| Benzene | **0.536** | 0.459 | 0.005 |
| Toluene | **0.993** | 0.002 | 0.005 |
| Ethylbenzene | **0.911** | 0.015 | 0.074 |
| p-xylene | **0.843** | 0.005 | 0.152 |
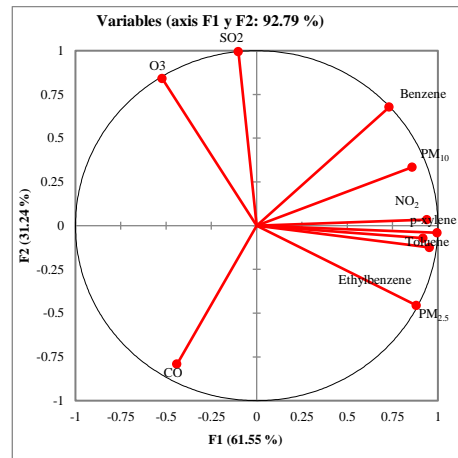
The values in bold correspond for each variable to the factor for which the squared cosine is the highest



*Source: Own elaboration from the obtained results*

**Figure 6.9** Principal Component Analysis for the measured variables during the midday sampling period (B2) in Obispado

|  | F1 | F2 | F3 |
|---|---|---|---|
| CO | 0.096 | **0.887** | 0.017 |
| NO$_2$ | **0.574** | 0.221 | 0.205 |
| O$_3$ | **0.889** | 0.003 | 0.108 |
| PM$_{10}$ | **0.705** | 0.274 | 0.021 |
| PM$_{2.5}$ | **0.528** | 0.405 | 0.067 |
| SO$_2$ | **0.897** | 0.020 | 0.083 |
| Benzene | 0.218 | **0.751** | 0.031 |
| Toluene | 0.373 | 0.220 | **0.407** |
| Ethylbenzene | **0.821** | 0.000 | 0.179 |
| p-xylene | **0.755** | 0.011 | 0.234 |
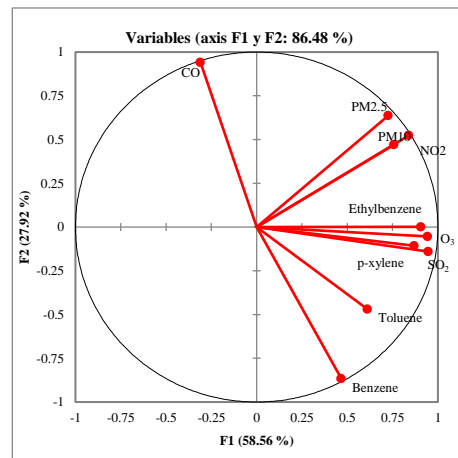
The values in bold correspond for each variable to the factor for which the squared cosine is the highest.



*Source: Own elaboration from the obtained results*

**Figure 6.10** Principal Component Analysis for the measured variables during the afternoon sampling period (B3) in Obispado

|  | F1 | F2 | F3 |
|---|---|---|---|
| CO | **0.826** | 0.166 | 0.008 |
| NO$_2$ | 0.130 | 0.216 | **0.654** |
| O$_3$ | **0.734** | 0.010 | 0.256 |
| PM$_{10}$ | 0.167 | **0.783** | 0.049 |
| PM$_{2.5}$ | 0.323 | **0.646** | 0.031 |
| SO$_2$ | 0.418 | 0.059 | **0.522** |
| Benzene | **0.550** | 0.358 | 0.092 |
| Toluene | **0.944** | 0.018 | 0.038 |
| Ethylbenzene | **0.984** | 0.002 | 0.014 |
| p-xylene | **0.965** | 0.022 | 0.013 |
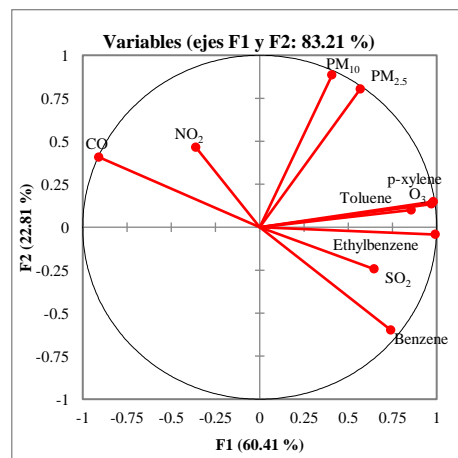
The values in bold correspond for each variable to the factor for which the squared cosine is the highest.



*Source: Own elaboration from the obtained results*

## 6.5 Conclusions

Levels of BTEX in Obispado were influenced by vehicle-type emissions and by photochemical activity. Nevertheless, the concentration levels of benzene found in the study site constitute a risk of contracting cancer in a lifetime, with possible harmful effects, especially in children. The toluene/benzene concentration ratio showed a strong influence from mobile sources (automotive exhaust emissions) and was within the range reported for other urban cities.

The ratio of Xylene/Ethylbenzene concentrations indicated that the air masses in the study site were "fresh" or originated from local emissions, which is an indicator of vehicle traffic emissions, since these compounds are constituents of gasoline and are emitted into the atmosphere from motor vehicle exhaust.

BTEX levels showed a clear diurnal pattern at the study sites with higher concentrations between noon (B2) and afternoon (B3) sampling periods. The meteorological analysis indicated that emissions were transported from Juarez and Apodaca municipalities. It should be noted that in these municipalities there are important avenues that cross the Monterrey metropolitan area from east to west and that register a large influx of vehicles most of the time. The influence of carbon monoxide (CO) on BTEX levels was evident, probably through photochemical reactions producing hydroxyl radicals, which are the main oxidants of BTEX in the troposphere. However, negative correlations were observed between atmospheric particles and BTEX, indicating the participation of these hydrocarbons in the formation of secondary organic aerosols is important in the study site. That is, the role of BTEX was more important in the formation of aerosols than in the formation of tropospheric ozone.

The results from this study can help to establish a basis for knowing the distribution of BTEX and its behavior in one urban site located in the metropolitan area of Monterrey, as well as its relationships with other criteria pollutants. Nevertheless, it is recommended in future works to sample at more points located in other municipalities belonging to the metropolitan area of Monterrey to have more information on the spatial behavior and distribution of BTEX sources. This study provided preliminary information on the levels of BTEX in the measured site that will allow have information in order to design a program to improve air quality in the city and/or implementation of measures or control policies of emission sources of these pollutants in a future.

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# Chapter 7 Mental health and family dynamics in university students from Tlaxcala after confinement

# Capítulo 7 Salud mental y dinámica familiar en estudiantes universitarios de Tlaxcala posterior al confinamiento

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**Abstract**

The objective of the research was to analyze mental health and family dynamics in university students from Tlaxcala after confinement. Students with degrees in Family Sciences, Gerontological Pedagogy, Special Education, and a master's degree in Family Therapy were invited to participate through their personal emails and Google Forms. 51 students responded, 84% women and 16% men, whose ages ranged between 18 and 57 years. The instruments used to assess mental health were the Beck's Depression and Anxiety Inventories and the Family Adaptability and Cohesion Evaluation Scale (FACES III), to measure family dynamics. The results showed a significant relationship between depression and anxiety ($r = .581$ **$p < .00$) and a negative and significant relationship between depression and family cohesion ($r = -.343$ *$p < .05$). A significant relationship between family adaptation and depression and anxiety was not demonstrated; but with family cohesion ($r = .588$ **$p < .00$). Greater family cohesion and less presence of depression was observed. Men presented greater depression than women, and students between 18 and 29 years old; and greater anxiety in those between 30 and 39 years of age. Greater family cohesion was observed when the head of the family is the mother, and greater family adaptation for those born in Puebla. The importance of analyzing mental health and family dynamics for the design of care strategies that provide people and their families with physical and psychosocial well-being is concluded.

**Cohesion, Dynamics, Adaptation, Confinement, Mental health**

**Resumen**

El objetivo de la investigación fue analizar la salud mental y dinámica familiar en estudiantes universitarios de Tlaxcala posterior al confinamiento. Se invitaron a participar a estudiantes de las licenciaturas en Ciencias de la Familia, Pedagogía Gerontológica, Educación Especial y de maestría en Terapia Familiar a través de sus correos personales y Google Forms. Respondieron 51 estudiantes, 84% mujeres y 16% hombres, cuyas edades fluctuaron entre los 18 y 57 años. Los instrumentos utilizados para evaluar la salud mental fueron los Inventarios de Depresión y de Ansiedad de Beck y la Escala de Evaluación de la Cohesión y Adaptabilidad Familiar (FACES III), para medir la dinámica familiar. Los resultados mostraron una relación significativa de depresión con ansiedad ($r = .581$ **$p < .00$) y una negativa y significativa de depresión con cohesión familiar ($r = -.343$ *$p < .05$). No se observó relación significativa de adaptación familiar con depresión y ansiedad; pero si con cohesión familiar ($r = .588$ **$p < .00$). Se observó mayor cohesión familiar y menor presencia de depresión. Presentaron mayor depresión los hombres que las mujeres, y los estudiantes entre 18 a 29 años; y mayor ansiedad los de 30 a 39 años. Se observó mayor cohesión familiar cuando el jefe de familia es la madre, y mayor adaptación familiar los nacidos en Puebla. Se concluye la importancia de analizar la salud mental y la dinámica familiar para el diseño de estrategias de atención que proporcione a las personas y sus familias bienestar físico y psicosocial.

**Cohesión, Dinámica, Adaptación Confinamiento, Salud mental**

**7.1 Introduction**

According to Goldberg et al. (2021), the COVID-19 pandemic has caused a crisis, not only physical, but also of mental health. The perception of risk and threats associated with infection can cause distress linked to the fear of contagion and involve anxiety and other forms of mental disorders with lasting and disabling impacts on the lives of those who suffer from them and their families. That is why, in a study carried out by Hernández (2020) whose objective was to describe the impact of COVID-19 on people's mental health, through a search for scientific information, it was concluded that COVID-19 had a negative impact on the mental health of people in the population in general, and particularly, on the most vulnerable population groups. The uncertainty associated with this disease, plus the effect of social distancing, isolation, and quarantine, could aggravate the mental health of the population.

Therefore, the pandemic, as a biopsychosocial phenomenon, affected all social classes and all continents, however, vulnerable people with risk factors were more affected in their health, both organically and psychologically. Home confinement and physical and social distancing interrupted relational processes, essential for mental health (Buitrago et al., 2021), resulting in an impact on their personal objectives, their family dynamics, their work role, and their economic stability. In this way, interpersonal relationships between family members changed, which brought with it modifications in family roles and in the composition of the household mainly causing anxiety, depression, and other mental health disorders.

In Mexico, Morales-Cheiné (2021) mentions that social distancing during confinement has apparently been associated with high levels of mental health symptoms compared to those who reported being in partial confinement; being these the ones associated with the fear of getting sick or having suffered COVID-19, suffering losses of close and loved ones, in addition to previously suffering from emotional illnesses. It was also founded a high association with acute stress, generalized anxiety, sadness, and anger in such conditions. Besides, it was reported rates of avoidance, sadness, distancing, anger, and anxiety as an effect of acute stress and high rates of generalized anxiety as an effect of fear of losing health. In Mexico as in the world, health anxiety has been strongly associated with indices of acute stress, avoidance, distancing, anger, and sadness during the pandemic.

Following the declaration of an emergency in China due to COVID-19, an increase in negative emotions such as anxiety, depression, and reaction to stress in the general population was revealed. Health anxiety was characterized by catastrophic interpretations of bodily sensations and changes, dysfunctional beliefs about health and disease, and poor adaptive mechanisms, which influenced their ability to make rational decisions, their behavior and the normal functioning of the person. Depression occurs in the affected person with sad mood, loss of the ability to be interested and enjoy things, a decrease in their vitality that leads to a reduction in their activity level and exaggerated fatigue. In addition, it was shown that, in the initial phase of the pandemic, people presented mild, moderate, and severe depressive symptoms, manifesting themselves in fatigue, sleep disorders, appetite disorders, decreased social interaction and loss of interest in the development of their personal, family, work and social activity (Huarcaya-Victoria, 2020).

In a research carried out by Barrios et al. (2021) in Bogotá, it is mentioned that mental health is one of the main challenges of the pandemic, not only focused on the individual but on the family and society, so it was analyzed how mental health has been affected in families during the confinement of Covid-19 through a review of 36 scientific articles in electronic databases and where it was concluded that, in the general population, great economic and psychological impact was caused, being the main causes no to be able to share with their peers, due to changes in their life habits, virtual classes, parents playing the role of guardians, something for which they were not prepared, thus causing anxiety and stress. Mental health is a dynamic process of well-being, a product of the interrelationship between the environment and the individuals that make up society (MINSA, 2020). It involves the process of seeking meaning and harmony, as well as the capacity for self-care, empathy and trust that is put into play in the relationship with other people.

For this reason, one of the sectors that most impacted the pandemic was the family, due to the need it had to reorganize its functions, establish care roles, restructure the position of its members in their mutual relationships and their belief system, in addition to the spaces, times and rhythms of family, work and household activities. This caused it to be affected in its emotional and physical well-being, as well as in its economy and in its network of social and family relationships. In addition to this, the confinement made all its members had the need to carry out their work, school, and recreational activities at home. So, its members had to adapt to the new situation and, at the same time, maintain a certain structure and organization trying to avoid very intense changes that limited their response capacities (Femat and Ortiz, 2020).

Thus, in a study carried out by Solano and Vásquez (2014) whose objective was to address social representations about health and mental illness with the relatives of people with mental disorder, through the analysis and interpretation of what was said by the relatives of patients with mental illness in three hospital institutions in Bogotá. It was concluded that the representation of "family" constitutes the structure of the meanings of family relationships to face mental illness. Mental illness as a social representation comprises a dehumanization that affects family relationships and inevitably transforms pre-established dynamics. However, the family, as a universal institution, is quite dynamic in its composition since it adjusts to circumstances and crises such as ruptures or diseases like the COVID-19 pandemic.

Therefore, for González (2007), the family is considered as the basic unit of society that has the capacity to face the changes of the social environment and its own group, promoting individual development and growth according to the demands of each stage of life. A family is a system where its members establish relationships of intimacy, reciprocity, dependence, affection, and power, conditioned by blood, legal or long-term tacit commitment ties, which includes at least one generation with a common residence most of the time. In this process of being a family, a series of situations arise that show it as healthy or pathologizing. Among these are the changes that both non-normative and normative undergo, when they conform to what is established by each culture and social group.

In this way, it is understood that the relationship in the family nucleus is fundamental for their health. Family health is understood as the stability of the internal relational dynamics in the fulfillment of functions as a family. Therefore, from its socializing role, the family is influencing health and disease situations of its members in an internal family dynamic in which if a member of the family gets sick, his state affects to a greater or lesser extent the rest of the members, the degree of affectation by the disease of one of its members will depend on factors such as the degree of internal cohesion of the system and the level of each of its sub-systems; self-knowledge and socio-cultural meanings attributed to the discomfort experienced; and information on the relationship between discomfort and illness. In this sense, socialization constitutes a basis for family health. Therefore, human relationships in the family nucleus are essential for their well-being (Idem).

So, the family as a system, plays a preponderant role in the generation of health alteration, as well as its rehabilitation process, this will depend on whether the family is functional or dysfunctional, in addition to having adequate resources of social support. Thus, family functioning will be observed, to the extent that the family fulfills its functions and is able to face and overcome each of the crises it goes through; while, the good or bad functioning of the family, will be a determining factor in the appearance or conservation of the health of its members. The family will function to the extent that it can facilitate and promote its adaptation in an specific situation. Family functioning is understood as the interaction of affective bonds between family members (cohesion) and that they may be able to change their structure to overcome family evolutionary difficulties (adaptability) (Cóndor Macas, 2019).

Since Olson's Circumplex Model (1991), family functioning is considered as the interaction of affective bonds between family members (cohesion) and that it may be able to change its structure to overcome family evolutionary difficulties (adaptability), in addition, it establishes that the problem is not only of the individual, but of his entire family system.

Highlighting the mental health implications brought about by the COVID-19 pandemic and the role of the family in the search for effective strategies to face and overcome the critical situation that resulted from it, in which transformations were carried out in its organization and structure that allowed its members individual and family development and growth, the objective of this research was to analyze mental health and family dynamics in university students of Tlaxcala after confinement.

## 7.2 Methodology

### 7.2.1 Participants

The present research is quantitative relational. The sample was unintentional non-probabilistic. The participants were 51 students of the bachelor's degrees in Family Sciences, Gerontological Pedagogy, Special Education and master's degree in Family Therapy of the Autonomous University of Tlaxcala, who agreed to participate in the research of a total of 208, 25% of fourth semester, 29% of eighth, 16% second, 16% first and 14% of sixth semester,  of which were 84% women and 16% men, whose age ranges ranged: between 18 and 19 years (19%). between 20 and 29 years (63%), between 30 and 39 years (14%), between 40 years and older (4%). 76% were from Tlaxcala, 14% from the state of Puebla, 6% from Mexico City, 4% from other states. Currently, 98% live in Tlaxcala and 2% in Puebla. Regarding their marital status, it was found that 84% were single, 10% married and 6% live in a free union. As for the number of people living in the house, it was observed that 31% live with four people, while 25% with five, 16% with three people, 10% with six, 8% with two, 2% with one person and 2% with 8. In 45% the head of the family is the father, while 26% is the mother and 29% another member. In addition, 31% depend on the father, 31% of both, 20% of the mother and 18% of another person. 80% have no dependents, while 14% of their dependents are their children, 4% have a different one and 2% are their parents. 57% do not work, while 43% work. The members who work in his family were, 43% two, 29% one, 20% three and 8% four. Regarding the monthly family income, 35% receive from $3,000 to $6,000 pesos*, 29% more than $6,000 pesos, 20% less than $3,000 and 16% do not know the family income.

### 7.2.2 Materials and procedure

The instruments used to collect the information were: A sociodemographic file where data was requested such as bachelor's degree and coursing semester, gender, age, marital status, place of birth, current residence, number of people they live with, who is the head of family, who they depend on, dependents, if they work, family members who work and monthly family income.

To evaluate mental health, the Beck Depression Inventory (validated in Mexico by Jurado, Villegas, Méndez, Rodríguez, Loperena and Varela, 1998) was used with a Cronbach's Alpha of .87 and which is constituted by 21 questions with four response options 0, 1, 2 and 3; being the minimum score of 0 and the maximum of 63,  and which are interpreted: normal ups and downs, mild mood disturbance, states of intermittent depression, moderate depression, severe depression and extreme depression.  In addition, the Beck Anxiety Inventory (validated in Mexico by Padrós, Montoya, Bravo and Martínez, 2020) with a Cronbach's Alpha of .81, is integrated of 21 questions, with a rating range of 0 to 63, each item is scored from 0 to 3 and interpreted in very low anxiety, moderate anxiety, and severe anxiety.

To measure family dynamics, the Family Cohesion and Adaptability Assessment Scale (FACES III), certified and validated in Mexico by Ponce, Gómez, Terán, Irigoyen and Landgrave in 2002, was used, with a Cronbach's Alpha of .70. It consists of a Likert scale that integrates 20 questions, 10 to evaluate family cohesion and 10 to evaluate family adaptability, distributed alternately in questions numbered as even and odd. Questions have a score value of 1 to 5: never 1; almost never 2; sometimes 3; almost always 4; always 5.

### 7.2.3 Procedure

To carry out the research, the invitation was made to the students of the bachelor's and master's degrees through their personal email, where they were notified of the project "Family developments and pandemic processes of accompaniment to Tlaxcala families before the new normality" in which they would participate, as well as the presentation of the members of the Academic Body "Families, Intersectionality and Intervention" (UATLX-CA-212); in addition, the reasons for requesting their collaboration and the data confidentiality. The Google Forms link was also sent to them by this means to respond to the instruments already indicated, because the students did not return completely in person.

**7.3 Results**

For data processing, frequency analyses were performed to obtain descriptive measures of mean and standard deviations. In addition, Pearson correlation was carried out to analyze the relationship between depression, anxiety, cohesion, and family adaptation, as well as the variables with sociodemographic data. In the same way, the Student's t was used for independent samples in order to determine the differences between depression, anxiety, cohesion and family adaptation with sociodemographic data. The results are shown below:

**Table 7.1** Means and standard deviation of depression, anxiety, family cohesion and adaptation

|  | N | M | DE |
|---|---|---|---|
| Anxiety | 51 | 21.43 | 14.565 |
| Depression | 51 | 11.04 | 9.529 |
| Family cohesion | 51 | 39.35 | 7.263 |
| Family adaptation | 51 | 27.69 | 6.541 |

*Source: Own elaboration*

Table 7.1 shows in the resulting means and standard deviations, the presence of greater cohesion in the studied sample, than family adaptation, anxiety, and depression. In the same way, greater family adaptation was observed than anxiety and depression. And likewise, they manifested greater anxiety than depression. This may be indicative of the presence of increased connection between family members despite the lockdown due to the COVID-19 pandemic. According to these results, it is possible that the family, faced with such a situation of change, facilitated, and promoted adaptation among its members, which probably caused the least presence of anxiety and depression in them.

**Table 7.2** Pearson's correlation between depression, anxiety, cohesion, and family adaptation

|  | Depression | Anxiety | Family cohesion | Family adaptation |
|---|---|---|---|---|
| Depression | 1 | .581** | -.343* | -.163 |
| Anxiety | .581** | 1 | -.028 | .057 |
| Family cohesion | -.343* | -.028 | 1 | .588** |
| Family adaptation | -.163 | .057 | .588** | 1 |
| **p < .01    *p < .05 | | | | |

*Source: Own elaboration*

Table 7.2 shows that there is a significant relationship of depression with anxiety (** $p < .00$), which would indicate that students who presented depression, possibly also presented anxiety. In the same way, a negatively significant relationship with family cohesion was observed (* $p < .05$), which would indicate that the greater the degree of family cohesion in the sample studied, the lower the presence of depression and the lower the degree of cohesion, the greater the presence of depression, taking into account that cohesion implies the degree of separation or connection that the individual has with respect to his family.

Therefore, it was observed that family adaptation was not significantly related to depression and anxiety, noting that the flexibility and aptitude that the family has in the face of the changes that were presented by the COVID-19 pandemic, is not indicative of the presence of depression and anxiety in the participating students.

In the same way, it was possible to realize a significant relationship of cohesion with family adaptation, which would probably indicate that family members, despite the confinement, showed more union and greater capacity to adapt to the changes due to COVID-19. On the other hand, regarding the relationship of the sociodemographic data and the variables, it was possible to realize that depression was significantly related to how many people live with ($r = .393$ ** $p < .00$) and negatively with who is the head of the family ($r = -.330$ * $p < .05$). As anxiety, it was significantly and negatively related to age ($r = -.395$ ** $p < .00$), negatively and significantly to place of birth ($r = -.279$ * $p < .05$), significantly to how many people live with ($r = .378$ ** $p < .00$) and to who is the head of the family ($r = -.308$ * $p < .05$). Regarding family cohesion, it was significantly related to age ($r = .296$ * $p < .05$).

Concerning the differences between depression, anxiety, cohesion and family adaptation with sociodemographic data, the results were:

Depression showed significant differences (*p<.05) with gender (F (4,454) p=.040) observing that men had greater depression (M=11.88) than women (M=10.88). In the same way, it showed significant differences (**p<.00) with the educational programs that are being studied (F (20,193) p=.000) where it was noted that the students of Family Sciences presented greater depression (M=12.89) than the students of Family Therapy (M=6.00). Also, significant differences were observed (*p<.05) of who are their dependents (F (12,304) p=.010), showing that students whose dependents are their children presented greater depression (M=14.14) compared to those who are other dependents (M=9.50). In addition, significant differences were observed (*<p.05) with the monthly income they receive (F (4,597) p=.042) which showed that the families of students who have a monthly income between $3,000 and 6,000 pesos presented greater depression (M=10.94) compared to those with an income of less than $3,000 pesos (M=10.60).

In the same way, depression showed significant differences (*p<.05) with age (F (10,758) p=.005) where it could be realized that students between 18 and 19 years old presented greater depression (M= 12.50) compared to those between 30 and 39 years old (M=7.86). Likewise, significant differences were observed (F (9,666) p = .004) between students aged 20 to 29 years, who presented greater depression (M = 11.88) than those aged 30 to 39 (M = 7.86), and students aged 40 and over (M = 1.50). There were also significant differences (*p<.05) regarding the number of people with whom they live (F (65,333) p=.001), with those living with seven members presenting greater depression (M=19.50) than those living with two (M=5.50). In addition, significant differences were shown in these areas (F (7,739) p=.009), finding that those living with five members presented greater depression (M=12.07), than those living with four people (M=7.18). Similarly, significant differences were observed (*p<.05) according to the semesters they were studying where it was shown that the second semester students (F (6,011) p=.028) (M=9.88); those in the fourth (F (6,442) p=.020) (M=13.00); those of sixth, (F(11.278) p=.005) (M=11.86); and those in the eighth (F(7,164) p=.014) (M=12.27), presented greater depression than those who attended the first semester (M=6.00).

Respecting anxiety, significant differences were found (*p<.05) with age (F (5,684) p=.049) and students between 30 and 39 years old (M=7.86) had higher levels of anxiety compared to those aged 40 and over (M=1.50). Moreover, significant differences were observed regarding the number of family members with who the students live with (F (9,649) p=.006), the results reflected that those living with six members presented greater anxiety (M=22.20) than those living with four people (M=7.18).

As to family cohesion, significant differences were observed (*p<.05) with respect to who is the head of the family (F (5,585) p=.024) in which greater cohesion was observed when the mother is the head of the family (M=39.38); and (F(6.153) p=.018) and when another member is the head of the family, (M=41.47), unlike when the head of the family is the father (M=37.96).

For family adaptation, significant differences were found (*p<.05) with the place of birth of the students (F (4,691) p=.036); (F (28,764) p=.001) observing that those born in Puebla had greater family adaptation (M=30.14) than those born in Tlaxcala (M=27.05) and in other states (M=27.50). Significant differences were also observed (F (21,604) p=.001) between those born in Mexico City, who presented greater familiar adaptation (M=30.14), than those born in other states (M=27.50).

## 7.4 Conclusions

Mental health is the dynamic process of well-being that involves the search for meaning, harmony, capacity for self-care, empathy, and trust as a product of the relationship with others (MINSA, 2020). Therefore, adequate mental health is essential for the proper functioning of society because it reinforces in people, the ability to maintain a healthy and safe behavior for themselves and for others; in addition, to facilitate the performance of essential family, community, and social functions. However, due to the COVID-19 pandemic, mental health was put at risk in conditions such as social distancing, loss of loved ones, economic and academic difficulties, not only in Mexico, but in the world (Morales-Cheiné, 2021). Staying at home for many days and stopping daily activities had implications for family life and people's mood, manifesting themselves in stress, overwhelm, anxiety, anguish, and irritability, and at other times, difficulties sleeping, apathy, feeling sadness and depression.

Hence the importance of identifying in people, the direct effects of COVID-19 infection on mental health, sometimes manifesting in depression or anxiety, which makes it necessary to create conditions and efficient strategies for prevention and attention to the most relevant social determinants, as well as monitoring the conditions of risk to mental health (Guide for relatives of people with mental health problems).

For this reason, there is a need to analyze the mental health and family dynamics of Tlaxcala students after confinement, where the results showed a greater presence of family cohesion and less depression; family adaptation was also greater than the presence of anxiety and depression, and anxiety was greater than depression. These data could indicate a greater emotional bond with the family and greater adaptation to the changes that occurred due to the confinement derived from the pandemic.

In the same way, it was observed that family cohesion and depression are negatively related, which would indicate that in the presence of greater cohesion in the family, the lower the presence of depression and conversely. Given these results, it is relevant to note that the students showed greater family cohesion and less depression, which would indicate a greater emotional bond between family members that avoided the presence of depression. It was also observed that family adaptation was not significantly related to anxiety and depression, noting that the family showed flexibility in the face of the changes that were presented by the COVID-19 pandemic, which allowed these disorders possibly not to occur in the students.

It was also possible to realize that the presence of depression was related to the number of people with whom they live and negatively with who the head of the family is. Regarding the presence of anxiety, it was found that it was related to the number of people with whom they live, and negatively with age, with the place where they were born and with who the head of the family is. In terms of family cohesion, it was related to the age of the students. Considering these data, Pérez et al. (2022) mention that family functioning, from adaptation and family cohesion, was a fundamental part during the confinement due to the COVID-19 pandemic, where they observed a process of evolution during this time; going through stages of greater level of conflict, overload, and tension, until reaching a more or less functional reorganization that allowed families to adapt. This made possible to relate family cohesion and adaptability in the mental health of family members.

In addition, from the data obtained, it could be noted that, the presence of depression was greater for men and for family science students, as well as for those whose dependents were their children and when the monthly family income was between $ 3,000 and 6,000; in the same way, in students between 18 and 19 years old and between 20 and 29 years old, and in those who live with seven and five people, and for those who study second, fourth, sixth and eighth semesters. As for the presence of anxiety, it was found that for students between 30 and 39 years old it was higher, and for those living with six people. Greater family cohesion was also observed when the mother was the head of the family or another relative; and greater family adaptation in students who were born in Puebla and Mexico City.

Therefore, it is important to consider that the COVID-19 disease affected the population of many countries in the world. However, each person can contribute to reducing risks at the individual, family, community, and social levels (WHO, 2020). For this reason, it is relevant to highlight the leading role of the family in the physical, psychological, and social development of its members for the recovery from diseases. This is mainly because it contributes to the conservation and protection of health, in addition to satisfying material and affective needs for the increase of confidence, security and self-esteem, essential for the psychological well-being of the group. Therefore, it is necessary to design care strategies that focus on family health to enhance the quality of life of its members, constituting itself as a dynamic process that allows the development of its members (Jiménez-Aguilar and Romero-Corral, 2021).

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# Chapter 8 Effects of a training program for fitness instructors based on STD and BPN on the attitude of users of a private university gym

# Capítulo 8 Efectos de un programa de entrenamiento para instructores de fitness basado en la STD y la BPN en la actitud de los usuarios de un gimnasio privado

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**Abstract**

Self-determination theory SDT (Ryan & Deci, 2000) and the satisfaction of Basic Psychological Needs BPN are valuable approaches to promote improvements in the pedagogical forms used by fitness instructors. Both approaches positively influence practitioners' satisfaction and behaviors related to physical activity. Objective: assess the effects of a fitness instructor training program based on STD and BPN on attitudes of the users of a private university gym. Sample: 441 gym users. Method: Pre and post measurements of attitudes toward fitness instructors of group classes and strength area were performed. A training program based on SDT and BPN was implemented between both measurements and a comparative analysis of attitude was performed through the Student's t-test for related samples. Results and conclusions: The comparative analysis of attitudes showed a significance index of .565 and .000 for group classes and strength samples, respectively. The implementation of a training program for strength instructors based on SDT and BPN promotes changes in their interaction style that leads to a better attitude of the users of their services.

**Self-determination, Attitudes, Physical activity, Fitness, Comparative-analysis**

**Resumen**

La teoría de la autodeterminación TAD (Ryan & Deci, 2000) y la satisfacción de las Necesidades Psicológicas Básicas NPB son enfoques valiosos para promover mejoras en las formas pedagógicas utilizadas por los instructores de fitness. Ambos enfoques influyen positivamente en la satisfacción y conductas de los practicantes, relacionados con la actividad física. Objetivo: evaluar los efectos de un programa de formación de instructores de fitness basado en la TAD y NPB, sobre las actitudes de los usuarios del gimnasio de una universidad privada. Muestra: 441 usuarios del gimnasio. Método: Se realizaron mediciones pre y post de las actitudes hacia los instructores de fitness de las clases grupales y del área de fuerza. Entre ambas mediciones se implementó un programa de capacitación basado en la TAD y NPB y se realizó un análisis comparativo de actitudes mediante la prueba t de Student para muestras relacionadas. Resultados y conclusiones: El análisis comparativo de actitudes mostró un índice de significancia de .565 y .000 para las muestras de clases grupales y fuerza, respectivamente. La implementación de un programa de entrenamiento para instructores de fuerza basado en la TAD y NPB promueve cambios en su estilo de interacción que conducen a una mejor actitud de los usuarios de sus servicios.

**Autodeterminación, Actitudes, Actividad física, Fitness, Análisis comparativo**

**8.1 Introduction**

The regular practice of physical activity provides important health benefits for people of every age group. According to various health organizations such as the World Health Organization (WHO, 2022) and the American College of Sports Medicine (ACSM, 2014), physical activity performed on a regular basis and with the recommended doses, brings cardiovascular, musculoskeletal, psychological and functional health benefits and improves the general well-being of practitioners. Health institutions like as ACSM, American Heart Association (AHA) and WHO have made specific recommendations to people of different age groups to specify the right doses of physical activity to obtain health benefits. The WHO´s current weekly recommendation for adults between 18 and 64 years of age, consists of a minimum of 150-300 minutes of moderate- intensity aerobic physical activity; or a minimum of 75-150 minutes of vigorous-intensity aerobic physical activity, or an equivalent combination of both; plus, at least two days of muscle strengthening activities of moderate or higher intensity focused on all major muscle groups (WHO, 2022).

Despite the great benefits of physical activity mentioned above, currently one in four adults and four out of five adolescents worldwide do not undertake enough physical activity to obtain health benefits, generating serious implications in terms of population health, human lives, and the economy of countries. In Mexico, according to the INEGI (National Institute of Statistics, Geography and Informatics) data contemplated in its Module of Sports Practice and Physical Exercise (MOPRADEF) module in 2020, only 38.9% of adults over 18 years perform some type of physical activity in their spare time (INEGI, 2021).

When we talk about lifestyles, we refer to "the set of decisions that people make about their health and over which they have relative control. Good decisions and personal habits favor health" (Giraldo et al., 2010); On the other hand, García-Laguna et al. (2012) refer that " among the most influential factors in the health of young college students are the consumption of tobacco and alcohol, nutrition and physical activity", thus confirming the importance of prioritizing research, design and implementation of strategies aimed at promoting it with the objective of improving health, specifically in this age group.

### 8.1.1 Factors determining physical activity

The practice of physical activity can be influenced positively or negatively by various factors and are considered as a starting point for the design and implementation of strategies for health promotion. These factors can be grouped into three main categories: 1) Personal factor refer to aspects inherent to the person him/herself: age, genetics, sex, race, level of education, incomes, and psychological aspects. 2) Environmental aspects are related to the physical space where one lives in terms of geography, urbanization, accessibility for physical activity practice and climate, among others. 3) Social factors involve the relationships we have with our social groups and whose level of influence is likely to change at different stages of life. Several studies support the importance of social factors as a determinant of physical activity, pointing specifically to social support, which is understood as "the perceptions of pressure (real or imaginary) exerted for another to change a behavior, attitude, or belief" (Gammage and Lamarche, 2014).

In published reports, didactic and communication tools of exercise leaders are considered as one of the most influential sources of social support, particularly in terms of the initiation and permanence of people in physical activity and exercise (Burke et al. 2006; Gammage and Lamarche, 2014; Joseph-Polyte et al., 2015, Salgado et al., 2021). These authors coincide in referring to the fitness instructor as a powerful and influential variable in the positive or negative experiences of the participants while exercising, and its consequent engagement and permanence in physical activity, for the most part in the initial stages.

### 8.1.2 The fitness instructor as a social determinant for physical activity

In the recent years, the fitness instructor has become a key figure in terms of motivation to physical activity due to the influence their role has shown on the participation of the people they serve. According to several reports, these leaders possess characteristics that are relevant in terms of the permanence in training programs of their customers. These characteristics include enthusiasm, ability to provide feedback, motivate, and understand capabilities of each person they attend. Moreover, previous research also refers that the fitness instructor communication style, might have an impact not only on the adherence to the activity itself but also on motivation and physical performance (Gammage and Lamarche, 2014; Joseph-Polyte et al., 2015; Madeson et al., 2010).

On the other hand, recent reports also point out that fitness instructors´ didactics and communication skills may have influence the participants' feelings of wellbeing, enjoyment, and motivations towards physical activity. Maher, et al. (2015) point out that when the instructors promoted the feeling of competence in their activities, participants reported higher levels of satisfaction. Similarly, Ryan & Deci (2018) talk about the growing evidence supporting the coach or instructor as a key element on facilitating the wellbeing development on individuals and groups.

### 8.1.3 Self-determination theory and basic psychological needs

Self-determination theory SDT (Ryan & Deci, 2000) has become a useful and valuable approach for instructors and leaders in charge of leading activities like exercise due to the importance it gives to a routine that is focused on high quality motivation and psychological well-being. Through the understanding and satisfaction of basic psychological needs, the participants´ motivation and personal growth can be enhanced, increasing the possibility of permanence in the activity. Hence, Deci & Ryan point out that SDT and the satisfaction of psychological basic needs are essential components of basic training for leaders like fitness professionals.

The self-determination theory (Ryan & Deci, 2000) states that people's behaviors (physical activity in this case) are influenced by three basic psychological needs: autonomy, competence, and relatedness to others:"…basic psychological needs are defined as the essential nutrients of effective behavior and well-being... the satisfaction of these needs is critical for the self-regulation of everyday behavior, for positive experiences and life satisfaction" (Ryan & Deci, 2018, p. 3). In the same regard, Moreno-Murcia (2016) refers that the initial motives to participate in physical exercise programs are closely related to intrinsic motivation, particularly with enjoyment and competence "it seems particularly important to generate motivational climates aimed at satisfying the needs of autonomy, competence and relationship" (p. 154).

The term autonomy refers to an individual's perception that his or her actions are voluntary and reflexively chosen by him or her: "The concept of autonomy refers to the idea that a person's actions are voluntary or volitional; to be autonomous means to feel self-regulation and authentic action to own and take ownership of what one does" (Ryan & Deci, 2018, p. 4). Some strategies suggested for fitness instructors and coaches to promote autonomy (Ryan & Decy, 2018) are to explain why and what for of the things being done, to provide options that can be freely chosen and avoid control and conditionality in the interaction.

The need for competence relates to the feeling of confidence and capacity in the activities in which people engage. To be promoted, fitness instructors must have the ability to set realistic goals in the activities they lead to validate their capabilities and even improve them. Among the strategies recommended to promote competence are providing supportive information, including relevant, clear, and positive feedback and the creation and establishment of optimal challenges (Ryan & Deci, 2018).

The relational psychological need points to build quality bonds based on unconditional support, genuine interest, authenticity, and transparency. Being able to promote the satisfaction of this need, is an important ability in coaches and fitness instructors that improves the permanence of customers in the activity.

After all the previously exposed, the SDT and the satisfaction of BPN seem to be promising tools to promote improvements in the pedagogical forms used by the instructors. This theoretical acknowledgement could have a significant impact on feelings, beliefs, and actions of the users they serve; in other words, the adoption of strategies based on TAD and Basic Psychological Needs could positively influence the practitioners' attitudes towards the instructor and the physical activity itself.

The term attitude refers to "the degree of positive or negative psychological affect associated with a psychological object" (Edwards, 2009, p. 2). It has three components: cognitive (representations and perceptions), affective (feeling of liking or disliking), and behavioral (the way of reacting to the object). An attitude of liking towards the fitness instructor is related to positive behaviors for improving health and well-being, such as a higher level of physical activity (amount of time of physical activity accumulated per week; Salgado-Núñez et al., 2021).

After all that has been exposed, it is necessary to know the effects in terms of user's attitudes and of the training program for fitness instructors whose objective is to improve the quality of their interactions. Furthermore, it is necessary to better understand the impact of strategies specifically aimed to train fitness instructors in psychology and motivation theories like the Self-determination Theory. Therefore, the aim of the study presented in this chapter is to assess the effect of a fitness instructor training program based on STD and LBW on the attitude of users of a private gym at a community college. To cover the proposed purpose, this chapter is made up of the following sections; First, the problem to be solved and the usefulness of the information presented and the study carried out are presented, secondly, the process that was carried out to develop the study is presented, thirdly, the results of the information collected from according to the proposed purpose, fourthly, the contrast of the results found in the present study with the results of other studies and as the fifth and last section, the conclusions derived from the findings according to the proposed purpose are presented.

## 8.2 Materials and methods

This study took place at a private University´s physical activity facility in Guadalajara, Jalisco México over a period of 8 weeks and during the autumn of 2019.

Participants: The sample was obtained incidentally; it consisted of a total of 441 fitness class users and strength training services: 263 users of the fitness classes area and 178 users of the strength training area. The training program about TAD and Basic Psychological needs was implemented to the 9 instructors in charge of leading the fitness classes and strengthening and training programs at the university gym.

Eligibility criteria: The participants in the study to whom the attitude questionaries were applied, were selected in a non-probabilistic way under the following criteria: (1) They had to be users of the Physical Activity Dome and (2) they had just completed a fitness class (regardless the modality) or exercise in the strength area.

The instructors who received the training program were the team in charge of leading the fitness classes and strength training at the university´s physical Activity Dome (gym). The training sessions were part of the training program designed by the Physical Activity Coordination during the fall of 2019.The instructors were informed and manifested their agreement about being part of this investigation.

For the purposes of this paper, the following terminology will be used:

Fitness Instructor: term used to refer indistinctly to both the instructors in charge of providing group training sessions called physical classes and the personal trainers in charge of attending to users in the strength and cardiovascular area.

Users: university students, graduates, employees, and members of the community who attend the Physical Activity Dome for physical and/or strength and cardiovascular classes indistinctly.

Attitude: positive or negative evaluation of users toward fitness instructors.

### 8.2.1 Materials

To evaluate the attitude of users, two validated questionnaires were applied: Attitude Toward Fitness Instructors Questionnaire (CA-ICF) and Attitude Toward Strength Instructors Questionnaire (CA-IF) instruments (Salgado-Núñez, Célis-Rivera & Cruz-Castruíta, 2020). The data obtained were entered into an Excel database and processed to obtain statistical data in the SPSS 26 software.

### 8.2.2 Design and procedures

This is a pre-experimental, comparative study, with pre and post measurements. The methodological part of this research included an Initial measurement of attitudes toward fitness instructors through the application of CA-ICF and CA-IF questionnaires on week 1 and a final measurement of attitudes on week 8, after the implementation of the instructors' training program. Thus, the study was conducted as it follows:

Initial measurement of attitudes toward fitness instructors through the application of CA-ICF and CA-IF questionnaires. According to the inclusion criteria described above, in the first stage of this study (October 2018) CA-ICF questionnaires were applied to 150 users of fitness classes area and CA-IF to 102 users of the strength training area. The results obtained from both instruments were captured in an Excel database and processed to obtain statistic data through SPSS 26 software.

A training program was designed focused on the unification of concepts around motivation basics, SDT and Psychological Basic Needs. The training was applied to the nine fitness instructors over a period of eight weeks. The topics included were:

- Motivation, SDT and Psychological Basic Needs.

- Identification of the current instructor style, at a personal and team level

- The autonomy concept applied to fitness

- Identification of behaviors that influence the user's competence.

- Constructing a model of service orientated to promote competence in users of the fitness services.

The training session was implemented in three modalities: individual, group and through digital resources such as WhatsApp and online forms; during a period of 8 weeks, a total of 20 training sessions were implemented: 2 group sessions (one at the beginning and one at the endo of week 8th), and 2 individual sessions for each instructor. Additionally, a total of 4 infographics (1 every two weeks) with theoretical information about DT, BPN and motivation were sent via WhatsApp. During the last week and prior to the final group session, an online questionary was sent out with questions related to the topics covered in the training. Instructors training about SDT and Basic Psychological Needs. The information obtained was used to better understand the appropriation level of the concepts and determine the content of the final group session.

Final measurement of attitudes toward fitness instructors through the application of CA-ICF and CA-IF questionnaires. According to the inclusion criteria described above, CA-ICF questionnaires were applied to 120 users of fitness classes area and CA-IF to 115 users of the strength training area. The results were captured into an Excel database and processed and analyzed using SPSS 26 software.

For data analysis, a comparative test was run to determine the difference in terms of attitude, between the first and the last application of the CA-IFC and CA-IF tests. The results obtained from the application of both instruments were subjected to student's t-test for related samples through the SPSS 26 software.

## 8.3 Results

### 8.3.1 Initial measurement of attitudes toward fitness instructors through the application of CA-ICF and CA-IF questionnaires

The total sample consisted of 252 people, of which 158 (61%) were female and 101 (39%) were male all of them users of the Physical Activity Dome at ITESO University. The age range of the participants was between 18 and 62 years old. Of the total sample, the CA-IFC questionnaire was applied to 150 users (107 women and 43 men) of the physical classes area. The CA-IF questionnaire was applied to 102 users (51 women and 58 men) of the strength and cardiovascular area.

As of the 150 CA-ICF questionnaire applications, 147 were valid. A mean score of 157.65 and standard deviation of 10.001 was obtained, with minimum values of 111 and maximum values of 170. The normalization of the results obtained is presented below in Figure 8.1 and the corresponding table of categories in Table 8.1. Of the 102 CA-IF questionnaire instruments applied, 63 were valid. A mean score of 108.28 and standard deviation of 10.001 were obtained with minimum values of 25 and maximum values of 132. The distribution curve of the normalized scores as well as the corresponding results categories are displayed below as figure 8.2 and table 8.2, respectively.

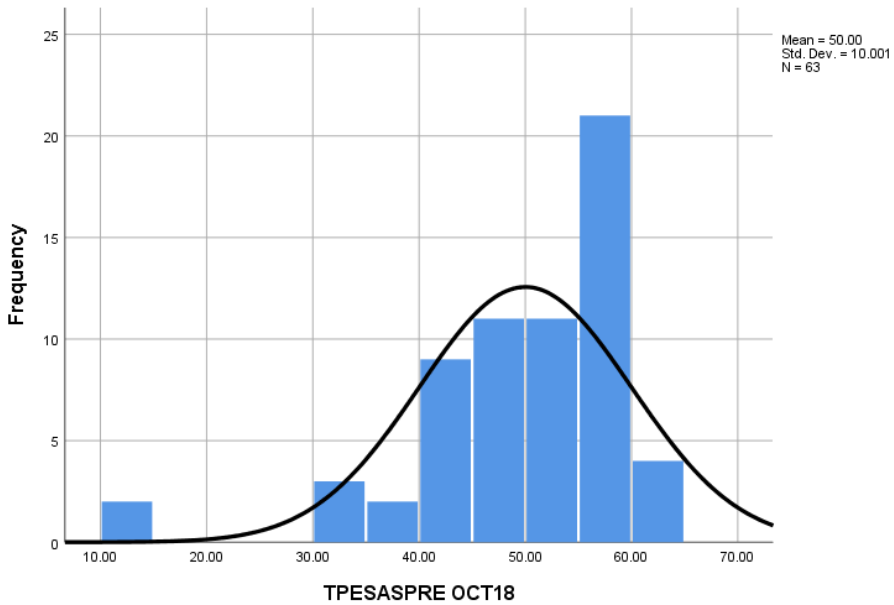**Figure 8.1** CA-ICF normalized results
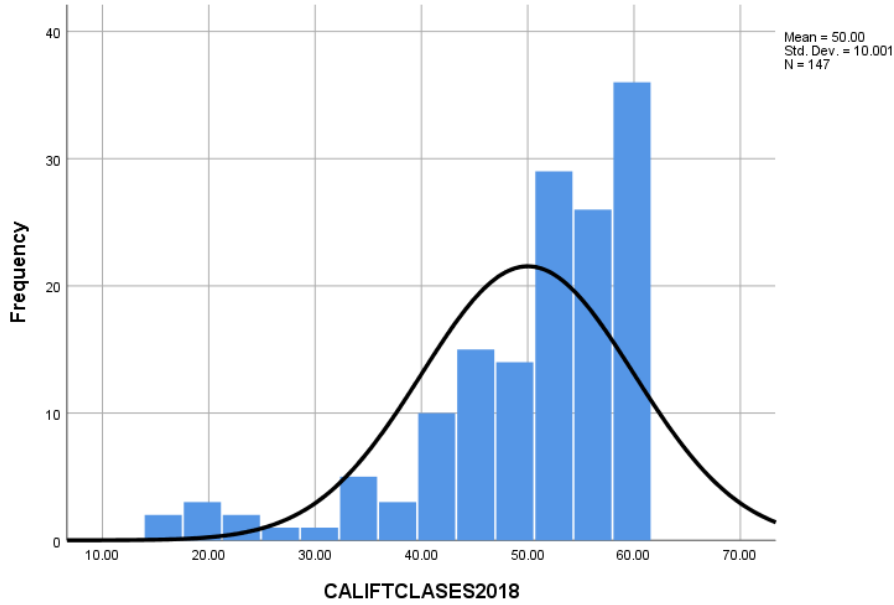


Note: CALIFTCLASES2018: Sum of the attitude results obtained in the sample of classes area users'
N = number of participants in the sample

**Table 8.1** CA-ICF Categories of attitudes toward fitness class instructors

| CA-ICF Rating | Attitude Toward Instructor |
|---|---|
| T=40 | "Unfavorable" |
| T= >40 and <50 | "With areas of improvement" |
| T= 50 | "Functional" |
| T=60 | "Good" |
| T=>70 | "Excellent" |
| Note: $T$ = obtained result | |

**Figure 8.2** CA-IF normalized results



Note: TPESASPREOCT18: Sum of the attitude results obtained in the sample of strength area users
N = number of participants in the sample

**Table 8.2** CA-IF Categories of attitudes toward strength area instructors

| CA-IF Rating | Attitude Toward Instructor |
|---|---|
| T= 40 | "Unfavorable" |
| T= >40 & <50 | "With areas of improvement" |
| T= 50 | "Functional" |
| T= 60 | "Good" |
| T= >70 | "Excellent" |
| Note: *T* = obtained result | |

### 8.3.2 Instructors training about TAD and basic psychological needs

The training sessions were implemented during a period of 8 weeks and all the instructors completed all the training activities.

### 8.3.3 Final measurement of attitudes toward fitness instructors through the application of CA-ICF and CA-IF questionnaires

Regarding to the last measurement through the CA-ICF, 116 out of 120 questionnaires applied, resulted valid. A mean of 157.01 and standard deviation of 10.004 was obtained, with minimum values of 23 and maximum of 170. On the other hand, from the application of CA-IF questionnaires, 115 resulted valid out of 116. A mean value of 149.10 and a 10.005 standard deviation were obtained, with a minimum of 85 and a maximum of 170. The normalized results are displayed below (Figure 8.3-8.4).

**Figure 8.3** CA-ICF Final measurement normalized results
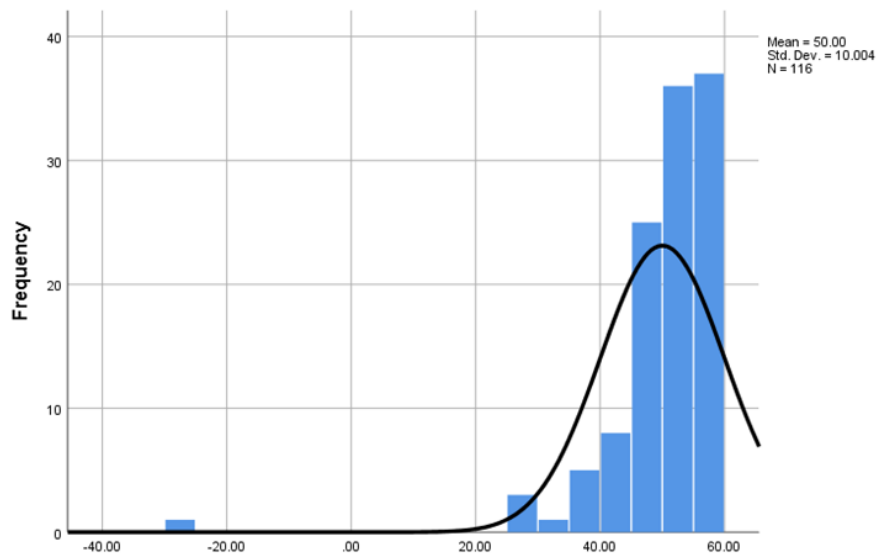


Note: POSTCLASES2019: Sum of the attitude results obtained in the sample of classes area users in 2019
N = number of participants in the sample

**Figure 8.4** CA-IF Final measurement normalized results
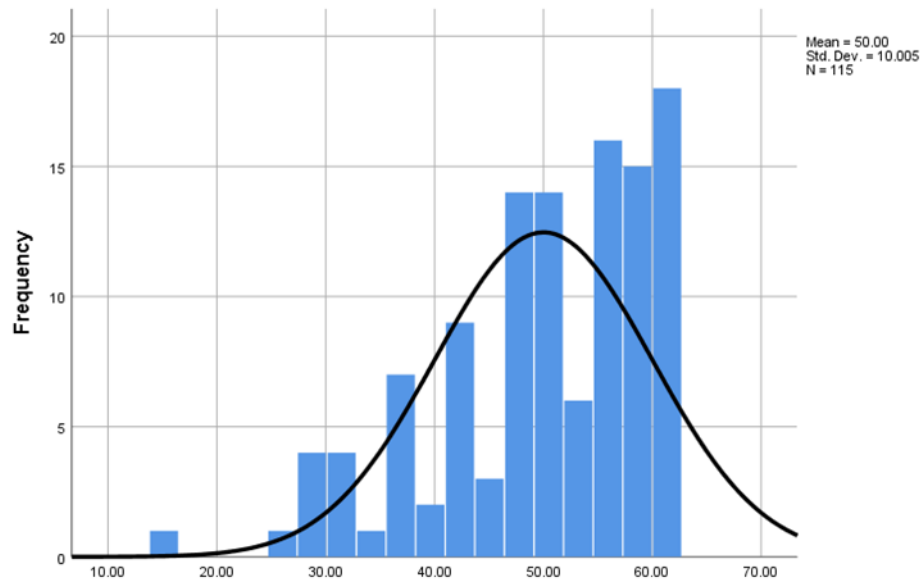


Note: POSTPESAS2019: Sum of the attitude results obtained in the sample of strength area users in 2019
N = number of participants in the sample

## 8.3.4 Comparative analysis of the results obtained through the initial and final measurements of attitudes toward fitness instructors

Regarding the comparative analysis of the results obtained through the application of the t student test, a correlation level of .230 and a significance index of .565 was found. The results of this analysis are presented in Table 8.3 and 8.4.

**Table 8.3** CA-ICF Correlation analysis for paired samples results

| Paired samples statistics | | | | | |
|---|---|---|---|---|---|
| | | Mean | N | Std. Deviation | Std. Error Mean |
| Pair 1 | OCT18PRE | 155.9655 | 116 | 14.41131 | 1.33806 |
| | OCT19POST | 157.0172 | 116 | 17.05693 | 1.58370 |
| **Paired samples correlations** | | | | | |
| | | N | Correlation | Sig | |
| Pair 1 | OCT18PRE & OCT19POST | 116 | .230 | | .013 |

**Table 8.4** CA-ICF t-student analysis results

| Paired Samples Test | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Paired Differences | | | | | | | | | |
| | | | | | 95% confidence interval of the difference | | | | |
| | | Mean | Std. Deviation | Std. Error Mean | Lower | Upper | t | df | Sig. (2.tailed) |
| Pair 1 | OCT18PRE & OCT19POST | -1.05172 | 19.63575 | 1.82313 | -4.66300 | 2.55955 | -.577 | 115 | .565 |

Regarding to the CA-IF, a correlation level of .199 and a significance index of .000 were obtained from the comparative analysis. The results are displayed below as Tables 8.5 and 8.6.

**Table 8.5** CA-ICF Correlation analysis for paired samples results

| Paired samples statistics | | | | | |
|---|---|---|---|---|---|
| | | Mean | N | Std. Deviation | Std. Error Mean |
| Pair 1 | OCT18PESASPRE | 108.2857 | 63 | 21.76223 | 2.74178 |
| | OCT19PESASPOST | 151.1270 | 63 | 17.88268 | 2.25301 |
| Paired samples correlations | | | | | |
| | | N | Correlation | Sig | |
| Pair 1 | OCT18PESASPRE & OCT19PESASPOST | 63 | .199 | .118 | |

**Table 8.6** CA-IF Results of t-student analysis

| Paired Samples Test | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Paired Differences | | | | | | | | | |
| | | | | | 95% confidence interval of the difference | | | | |
| | | Mean | Std. Deviation | Std. Error Mean | Lower | Upper | t | df | Sig. (2.tailed) |
| Pair 1 | OCT18PESASPRE & OCT19PESASPOST | -42.84127 | 25.27028 | 3.18376 | -49.20551 | -36.47703 | -13.47703 | 62 | .000 |

## 8.4 Discussion

The main objective of this work is to determine whether the implementation of a training program for fitness instructors based on SDT and BPN modifies the users' attitudes. In this aspect, from the comparative analysis performed from the CA-IFC, a correlation level of .230 and a significance index of .565 was found, which does not represent a statistical significative difference. However, from the comparative analysis performed over the CA-IF results, an index of .000 was obtained, which represents a significant difference and an important improvement in the variable attitude towards strength instructors. These results could be explained by considering two main factors: 1) the social context where the interaction instructor-student takes place (group-individual), and 2) the academic profile of the instructor in each area. As for fitness instructors in the area of physical classes, the initial results show a higher attitude rating compared to that of strength instructors. That is, users of physical classes manifest a greater liking towards their instructors prior to training, possibly because the group context in which they develop is considered as one of the social factors that positively influence the user's enjoyment and their permanence in exercise (Burke et al. (2006), Brown et al. (2014), Farren et al. (2017), Gammage & Lamarche (2014)). Hence, the nature of this contexts strengthens the work of the instructor as it provides itself a sense of social support and belonging thus satisfying one of the basic psychological needs contemplated in TAD, the relational one (Deci and Ryan, 2000); Conversely, in the case of strength instructors, they provide their services in a one-to-one modality, facing a greater challenge in terms of social support and increasing the burden on the quality of the relationship they build, having to implement clear actions aimed at the formation of bonds of communication, care and genuine interest towards the users they serve.

Regarding to the academic profile, the are several reports referring that group instructors present a higher index of attendance to courses and workshops compared to the strength instructors, because most of them teach classes of different modalities that require periodic updates and qualifications. (Boned,et al 2015; De Lyon, 2017; Dorado Andia, 2018; Estrada-Marcén, et al 2019; Jankauskiene, 2018; Juan¬-Llamas, 2015); this continous education gives them an access to a variety of exercises that, when offered as voluntary options at different levels of complexity and intensity, favor the competence and autonomy of the participants in their classes. On the other hand, strength instructors usually have a bachelor's degree or in some cases some specialized technical training in strength  but just a few specializations or updates: "in relation to the necessary academic training, they indicated that the universities or study centers to which they belonged did not provide them with the necessary tools to work professionally in a gym; ... the results indicate that, in general, the instructors do not have multidisciplinary academic training that merits the performance of some of the functions they occupy" (Portes Junior et al, 2014).

The results obtained regarding the change in attitudes towards strength instructors, are consistent with other authors findings such as Edmunds (2008); Ntoumanis, et al (2017) & Wilson(2006), in the fact that it is not only possible but effective to train instructors to adopt a STD bases teaching style: "compared to the control group, participants in the SDTc exhibited a significant increase in positive affect derived whilst exercising over the 10-week exercise program" (Ntoumanis, et al 2017). In this same regard, theses authors refer in their conclusions that: "These findings concur with prior research indicating that teachers and health professionals can be effectively trained to adopt a self-determination centered teaching style" p. 385. Thus, we consider that the training implemented in this study constitutes a useful material for fitness instructors, especially for strength area providing theoretical information and practical strategies likely to be applied in their work settings.

The findings of this study are relevant since they do not only highlight the important role of instructors as health promoters but also confirm the need of training these professionals in basic aspects of motivation applied to physical activity, as well as communication styles. Its implementation, mainly in strength instructors could not only favor the user's experience in physical activity but also their permanence in it, which will contribute to the construction of a more active and therefore healthier society.

Between the limitations of the current study, we find necessary the collection of detailed information about the academic and professional profile of the instructors since this information would allow us to find a correlation between the previous knowledge and changes obtained after the training. We also consider important to measure attitudes towards instructors separately since the general results could hide specific improvements in certain members of the team of instructors.

## 8.5 Conclusions

Physical activity has enormous benefits for health when practiced at the appropriate amount and frequency. The practice of physical activity can be positively or negatively influenced by several factors, and particularly fitness instructors as a relevant social factor, have shown to influence the motivation of the people they serve. It is important to train fitness instructors in psychology and motivation theories as it has demonstrated to remarkably impact not only the enjoyment but also the amount and permanence in physical activity. Several reports have proved that SDT and the satisfaction of BPN are not only solid psychological theories applied to the physical activity field but also practical frameworks to build better experiences and relations between instructor-client. This is demonstrated by two main factors/conclusions: 1. The implementation of a training program for strength fitness instructors based on SDT and BPN promotes changes in the interaction between instructor-user 2. These changes lead to a better attitude of the users of their services, which has been shown to be a causal factor for increased time spent in physical activity (Salgado-Núñez et al., 2021). However, further research is needed to better understand the needs and effects of training and education of fitness instructors to maximize the benefits of their work and ultimately to contribute to a more active and healthier population.

## 8.6 Funding

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Indicate (3-5) keywords in Times New Roman and Bold No.12.

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Explanation of the topic in general and explain why it is important.

What is its added value with respect to other techniques?

Focus clearly on each of its characteristics.

Clearly explain the problem to be solved and the central hypothesis.

Explanation of the sections of the Chapter.

Development of Sections and Sections of the Chapter with subsequent numbering.

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**Inclusion of Graphs, Figures and Tables-Editables**

In the content of the Chapter, all graphs, tables and figures must be editable in formats that allow modifying size, type and number of letters, for editing purposes, these must be in high quality, not pixelated and must be noticeable even if the image is reduced to scale.

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| Variable | Description | Value |
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| $P_1$ | Partition 1 | 481.00 |
| $P_2$ | Partition 2 | 487.00 |
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| $P_4$ | Partition 4 | 483.50 |
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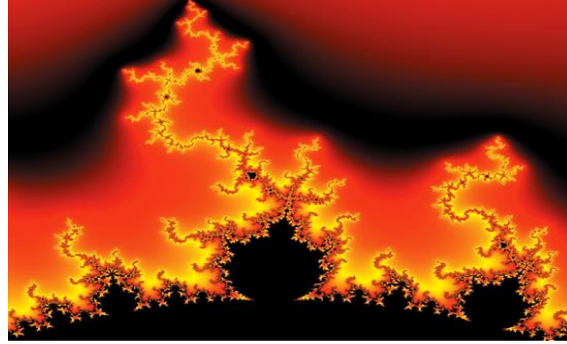


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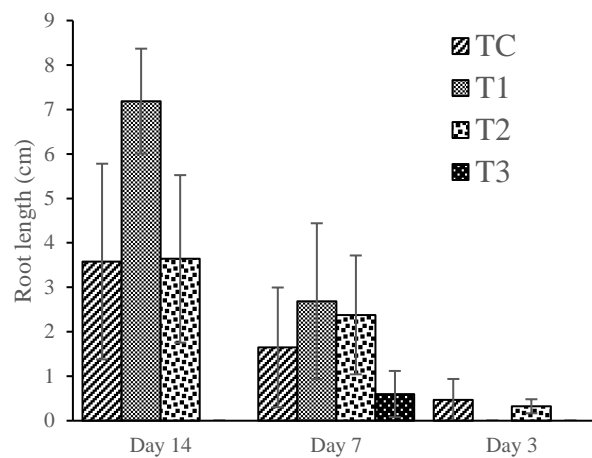


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$$\int_{lim^{-1}}^{lim^1} = \int \frac{lim^1}{lim^{-1}} = \left[\frac{1\,(-1)}{lim}\right]^2 = \frac{(0)^2}{lim} = \sqrt{lim} = 0 = 0 \to \propto \tag{1}$$

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Give the meaning of the variables in linear wording and it is important to compare the criteria used.

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The results should be per section of the Chapter.

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**Conclusions**

Clearly explain the results obtained and the possibilities for improvement.

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