Universidad de La Laguna

MASTER'S THESIS

Volumetric variations on Hippocampal Subfields due to Physical Activity: a high-resolution MRI study

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Dr Niels Janssen, componente/coordinador del Grupo de Investigación... NEUROSYS... adscrito a las Titulaciones que aparecen en el encabezamiento

CERTIFICO

- Que el Trabajo Fin de Máster (TFM) titulado "Volumetric variations on Hippocampal Subfields due to Physical Activity: a high-resolution MRI study" ha sido realizado bajo mi supervisión por Dª Sara Lozano Seoane matriculada en el Máster en Biomedicina, durante el curso académico 2017-2018.

- Que una vez revisada la memoria final del TFM, doy mi consentimiento para ser presentado a la evaluación (lectura y defensa) por el Tribunal designado por la Comisión Académica de la Titulación.

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Abstract

Faculty Psychology and Speech therapy
Department of Cognitive, Social and Organizational Psychology

MSc in Biomedicine

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by Sara LOZANO-SEOANE

Physical activity produces cognitive and structural changes in the brain. It causes effects on vascularization, neurogenesis and cognition, and these effects are related to each others. Previous studies have shown that an important part of these effects occur in the hippocampal formation, in humans and non-human animal models. But the hippocampal formation is a non-homogeneous structure divided in several subregions, due to their different cellular compositions and projections direction. The different impacts that exercise may play on the hippocampal formation's subregions in humans is not well understood.

In this study we examine the effects different types of regular physical activity have on hippocampal formation's subregions volume. To this end, we make use of high-resolution T1 and T2 MRI acquisition, along with a new automated segmentation tool based on a computational atlas. We found a positive effect of regular occupational physical activity on the volume of DG and CA4 (hilus). In previous studies, increasing volume in the hippocampus has been accompanied by an enhancement of cognitive function, and less consistently, with human neurogenesis. Our results suggest that occupational activity is key in this increasing volume.

Key words: Physical activity, Hippocampal subfields, High-resolution MRI, FreeSurfer

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Abstract

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Volumetric variations on Hippocampal Subfields due to Physical Activity: a high-resolution MRI study

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La actividad física produce cambios cognitivos y estructurales en el cerebro. Causa efectos sobre la vascularización, la neurogénesis y la cognición, y estos efectos están relacionados entre sí. Estudios previos han demostrado que una parte importante de estos efectos ocurren en la formación del hipocampo, en modelos humanos y animales no humanos. Pero la formación del hipocampo es una estructura no homogénea dividida en varias subregiones, debido a sus diferentes composiciones celulares y dirección de proyecciones. Los diferentes impactos que el ejercicio puede tener en las subregiones de la formación del hipocampo en los seres humanos no se comprenden bien.

En este estudio se examinan los efectos que tienen los diferentes tipos de actividad física regular sobre el volumen de las subregiones de la formación del hipocampo. Para ello, utilizamos la adquisición de imágenes por resonancia magnética T1 y T2 de alta resolución, junto con una nueva herramienta de segmentación automatizada basada en un atlas computacional. Se encontró un efecto positivo de la actividad física ocupacional regular sobre el volumen de DG y CA4 (hilus). En estudios anteriores, el aumento de volumen en el hipocampo ha ido acompañado de una mejora de la función cognitiva, y menos consistente, con la neurogénesis humana. Nuestros resultados sugieren que la actividad ocupacional es clave en este creciente volumen.

Palabras clave: Actividad física, subcampos del hipocampo, MRI de alta resolución, FreeSurfer

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Also, to Laura Ezama, for part of the data collection. And to the participants in the experiment.

Contents

List of Abbreviations

BDNF	**B**rain **D**erived **N**eurotrophic **F**actor
BMI	**B**ody **M**ass **I**ndex
CA	**C**ornu **A**mmonis
DG	**D**entate **G**yrus
EPI	**E**cho **P**lanar **I**maging
eTIV	**e**stimated **T**otal **I**ntracranial **V**olume
fMRI	**f**unctional **M**agnetic **R**esonance **I**maging
FSPGR	**F**ast **SP**oiled **GR**adient echo
GCA	**G**aussian **C**lassifier **A**tlas
HATA	**H**ippocampus-**A**mygdala **T**ransition **A**rea
HF	**H**ippocampal **F**ormation
IGF	**I**nsulin-like **G**rowth **F**actor
LI	**L**eisure-time **I**ndex
LTP	**L**ong-**T**erm **P**otentiation
MNI	**M**ontreal **N**eurological **I**nstitute
MRI	**M**agnetic **R**esonance **I**maging
mRNA	**m**essenger **R**ibo**N**ucleic **A**cid
NGF	**N**erve **G**rowth **F**actor
NIfTI	**N**euroimaging **I**n**f**ormatics **T**echnology **I**nformatics
PA	**P**hysical **A**ctivity
rCBV	**r**egional **C**erebral **B**lood **V**olume
rsfMRI	**r**esting **s**tate **f**unctional **M**agnetic **R**esonance **I**maging
RSN	**R**esting **S**tate **N**etworks
SBM	**S**urface **B**ased **M**orphometry
SEWL	**S**ubjective **E**xperience of **W**ork **L**oad
SI	**S**port **I**ndex
VBM	**V**oxel **B**ased **M**orphometry
VEGF	**V**ascular **E**ndothelial **G**rowth **F**actor
WI	**W**ork **I**ndex

Chapter 1

Introduction

Physical activity (PA) is considered a bodily activity that results in energy expenditure above resting levels (US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Fitness, President's Council on Physical Fitness, 1998). On the other hand, exercise is a PA that is structured to meet a specific fitness gain (Caspersen, Powell, and Christenson, 1985; Thomas et al., 2012).

Through the study of effects caused by PA and exercise, cognitive and mood changes have been of great interest (Kramer and Erickson, 2007; Hillman, Erickson, and Kramer, 2008), to the extent that PA, as well as some other lifestyle factors, has been considered as beneficial for patients that suffers from depression, Alzheimer's disease and some other conditions (Hillman, Erickson, and Kramer, 2008; Morris et al., 2017).

However, it is of notable interest for us the changes involving hippocampus. This is a brain structure localized in the inner of the temporal lobe of both hemispheres, and that is tightly related to memory function. Memory has been proven to be enhanced by physical activity both in animal models and humans.

PA has proved to perform beneficial effects and changes in our brain (Thomas et al., 2012), and some differentiations has been made as aerobic and non-aerobic exercise, but here, we seek to identify the effects of the different types of PA performed in our daily life.

Moreover, we are interested in understanding how different types of daily PA and exercise make an effect on the different Hippocampal Formation (HF) subregions. The HF is a far from homogeneous structure with great differences due to the cellular composition of its areas and the direction of its projections. Thus, effects may not be the same for all of them.

We studied the effect that different types of daily PA would have on the volume of four HF subregions: Cornu Ammonis 1 (CA1), CA2/3, CA4 and Dentate Gyrus (DG).

Until recently, limitations in instrumentation and technology did not allow us to study the HF subregions in humans, but this possibility has been recently reached by increasing the resolution of MRI scans and with new segmentation tools as the one that will be described in a section below.

1.1 Animal model studies

Most of the experiments looking for answers in the effect of PA on cognition and brain, make use of animal models. This is, among other things, given the great facility they allow to control variables and the amount of measures they can provide.

Through the study of effects caused by exercise, improvements in angiogenesis, grey matter volume, dendritic and axonic growth and neurogenesis, have been identified.

Aerobic activity has shown its effects in increasing levels of insulin-like growth factor (IGF) and vascular endothelial growth factor (VEGF). These growing factors, are relevant for physical activity induced angiogenesis and neurogenesis (Van Praag, 2009). Gene expression of IGF in the hippocampus is increased by running (Carro et al., 2000), and running also increases IGF and VEGF levels in serum (Carro et al., 2000; Fabel et al., 2003; Van Praag, 2009).

1.1.1 Neurogenesis

There are differences in the processes that occur in animals and humans, and the majority of neurogenesis studies have been conducted in animal models. As an example of these differences, striatal neurogenesis is found only in humans (Bergmann, Spalding, and Frisén, 2015), whereas, neurogenesis in the olfactory bulb is exclusive in some non-human animal models (Bergmann et al., 2012; Boldrini et al., 2018).

Increasing evidence indicates that this process has a role in learning and memory. Ablation of the new cells results in spatial memory deficits (Imayoshi et al., 2008; Van Praag, 2009). It is commonly agreed that neurogenesis in the mammalian brain occurs in the sub-granular zone of the DG in the hippocampal formation, and the sub-ventricular zone in the lateral ventricles (Thomas et al., 2012). Numerous studies suggest that physical exercise increases neurogenesis in the DG of the hippocampus (Van Praag et al., 1999).

The Neurogenesis Process

The stem neural cells are located in the subgranular zone of the hippocampal formation, that is found between the hilus and the granule cell layer of the dentate gyrus. The hilus is situated between the V-blades of the dentate gyrus, and contains inhibitory gamma-aminobutyric acid (GABA) ergic interneurons and excitatory mossy cells (Vivar and Praag, 2017).

It is thought that GABA, the principal inhibitory neurotransmitter in the brain, regulates the initial development of newly born neurons (Dieni, Chancey, and Overstreet-Wadiche, 2013; Espósito et al., 2005; Ge et al., 2006; Wadiche et al., 2005; Song et al., 2012; Tozuka et al., 2005; Vivar and Praag, 2017). During the first few days, the cells do not have clear dendritic nor axonal processes. After approximately two weeks, their axons, or mossy fibers, form synapses with the pyramidal cells in the CA3 area (Zhao et al., 2006; Vivar and Praag, 2017).

Physical Activity and Neurogenesis

Neurogenesis is positively regulated by voluntary exercise on a running wheel. Moreover, running has shown to increase cell proliferation, cell survival and differentiation in correlation with synaptic plasticity and memory function in many studies (Clark et al., 2011; Farmer et al., 2004; Marlatt et al., 2012; Vivar and Praag, 2017).

An increase in cell proliferation and cell survival in the dentate gyrus is one of the most consistent effects seen with exercise treatment (Van Praag et al., 1999; Van Praag, Kempermann, and Gage, 1999). Even newborn pups whose mothers have performed aerobic exercise during the gestation period showed a higher number of surviving cells in the hippocampus than pups born to sedentary mothers (Kim et al., 2007; Lee et al., 2006).

Growing factors BDNF and NGF are important modulators of neuronal plasticity and survival, and both have shown increased expression of mRNA for BDNF and NGF by physical activity in rats (Neeper et al., 1996). Concretely, in the experiment cited, BDNF increased mainly in CA1 and CA4 of the hippocampus, and caudal neocortex and retrosplenial cortex; and NGF increased primarily in the granule layer of the DG and CA4, and in the caudal neocortex.

Although neurogenesis decreases throughout age, running can stimulate this process along lifespan in mouse models (Kannangara et al., 2011; Kronenberg et al., 2006; Marlatt et al., 2012; Van Praag et al., 2005; Voss et al., 2013; Wu et al., 2008; Vivar and Praag, 2017).

A relationship between neurogenesis and angiogenesis has been suggested over many studies: For instance, blocking VEGF flow in the brain prevents exercise-induced neurogenesis (Fabel et al., 2003; Kramer and Erickson, 2007). This relationship has also been suggested in Pereira et al., 2007 were a correlation between the number of new neurons generated in the DG of mice and the degree of increasing in regional cerebral blood volume (rCBV), measured with contrast MRI, was identified. Furthermore, their experiment demonstrated a significant increase in rCBV after three months of aerobic exercise in a small sample of humans, and these changes in rCBV showed correlation with increases VO_2max and performance after exercise on a verbal learning task.

1.1.2 Cognitive Changes in Animal Models

Brain changes due to physical activity usually correlates with some cognitive and brain function improvements. Some studies found effects on running on the ability to pattern separation in mice Creer et al., 2010. Some other findings in rodents suggest that running also increases long-term potentiation (LTP), which is considered a physiological model of learning and memory (Bliss and Collingridge, 1993; Lynch, 2004; Vivar and Praag, 2017).

Spatial memory in rodents have been shown to be improved by voluntary and non-voluntary exercise, and this behavioural enhancement is associated to increases in adult neurogenesis and BDNF levels (Gazzaley et al., 2008; Vivar and Praag, 2017).

1.2 Human Research

Another study, that measures using MRI angiography, has demonstrated that aerobic activity levels in elderly humans is correlated to the diameter, amount and branching of brain blood vessels Bullitt et al., 2009; Thomas et al., 2012.

Human neurogenesis is constantly being questioned. Nevertheless, recent study that quantifies neurogenesis, angiogenesis and volume of the DG in autopsy samples of healthy humans in an age range of 14 - 79 years, showed thousands of immature neurons in the DG, comparable numbers of mature cells, and equivalent volume across ages. However, older individuals showed less angiogenesis, neuroplasticity, a smaller quiescent stem cells pool in the anterior-mid DG, and no variations in the posterior DG (Boldrini et al., 2018).

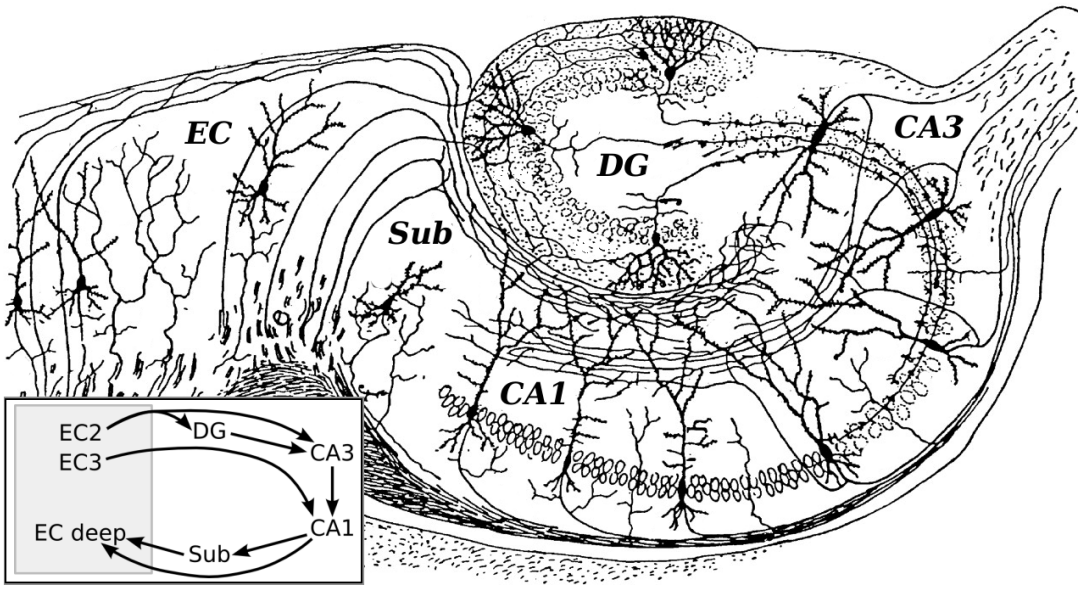

FIGURE 1.1: By original: Santiago Ramón y Cajal (1852–1934) derivative = Looie496 [Public domain or Public domain], via Wikimedia Commons

Regular exercise may reverse age-related decline (Erickson et al., 2011. In humans, physical activity benefits hippocampal-dependent memory and prefrontal cortex-mediated executive function, and may maintain the volume of white and gray matter over a lifetime. (Duzel, Praag, and Sendtner, 2016; Voss et al., 2013; Vivar and Praag, 2017). Colcombe et al., 2006, found that better physical fitness and improvements in physical fitness were related to higher volumes of prefrontal and temporal gray matter, as well as anterior white matter. These volume increases have been predictive of performance in older adults.

Molteni, Ying, and Gómez-Pinilla, 2002, investigated the interaction of diet and exercise at the behavioural and molecular levels through their effects on learning and BDNF. The exercise served to reverse the negative effects of high-fat diets on BDNF and learning levels. One year aerobic physical

training in human adults showed increased anterior hippocampal volume that lead to an improvement in spatial memory task (Andersen et al., 2007).

A better cardiorespiratory fitness is associated to greater grey matter volumes in prefrontal cortex and hippocampus, and other less consistent variations may occur in different regions of the brain. Physical activity is also associated to the same regions associated to a good cardiorespiratory fitness, including prefrontal cortex and hippocampus (Erickson, Leckie, and Weinstein, 2014).

In an experiment where association between waist circumference and gray matter volume was investigated in adult humans, Voxel Based Morphometric (VBM) analysis of volume showed a significant inverse association between waist circumference and the volume of grey matter in the brain. Inverse associations in the following structures for both hemispheres were found: frontal lobe, temporal lobes, pre-central and postcentral turns, supplementary motor area, supramarginal turn, insula, cingulate turn, caudate nucleus, olfactory groove, parahipocampus, hippocampus, gyrus rectus, amygdala, pale balloon, putamen, cerebellum, spindle and lingual turn, precuneus and thalamus (Janowitz et al., 2015).

In the previous description on the effects of physical activity, the principal participation of effects on hippocampal formation have been notable. The hippocampal formation consists of subregions: hippocampus, DG, subiculum, presubiculum, parasubiculum, and entorhinal cortex (Andersen et al., 2007).

In a cross-section of the hippocampus the trisynaptic loop described classically, can be visualized. It consists of the Perforant path, from the EC to the DG; the mossy fibers, from DG to CA3; and Schaffer collaterals, from CA3 to CA1 (Knierim, 2015). But HF's connectivity is far more complex.

The main warrant for the division of the subregions of the hippocampal formation is that they contain cells connected to the next region by distinguished pathways (Andersen et al., 2007).

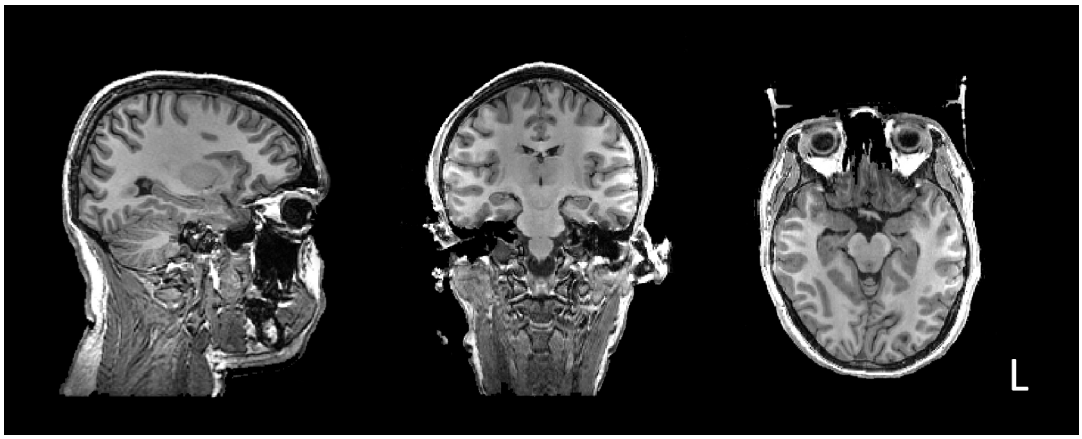

FIGURE 1.2: High-resolution T1-weighted image of a subject in our experiment.

Chapter 2

Purpose

In the hippocampal formation, the hippocampus itself is divided into CA1, CA2 and CA3. In addition, as mentioned above, neurogenesis occurs in the sub-granular area of the DG in the hippocampal formation, between the sub-granular layer and the hilus (CA4). And after two weeks of maturation, its axons reach CA3.

We hypothesized that the volume of the subfields we have selected from the hippocampal formation may show a differential effect due to the habitual physical activity of the subjects, and furthermore this effect may be different for the different types of physical activity.

The overall goal of this work is to observe the volumetric differences between people with different levels of exercise in three different factors of physical activity. To this end, we have assessed habitual physical activity, as well as collected high-resolution MRI structural data, and inspected the effects different components of habitual physical activity play on hippocampal formation subregions' volumes, corrected for estimated total intra-cranial volume (eTIV) and gender.

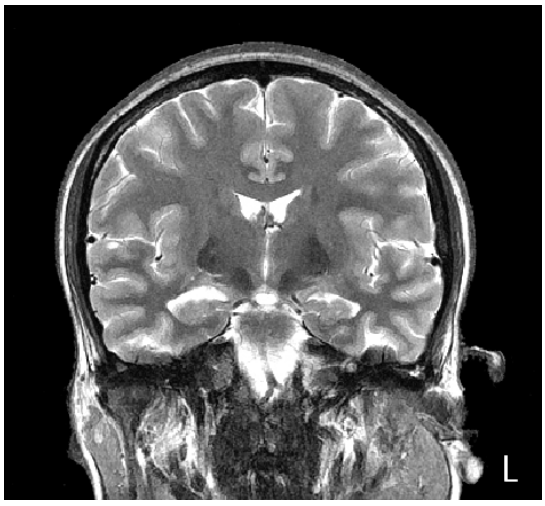

FIGURE 2.1: T2-weighted MRI. A coronal section.

Chapter 3

Methods

3.1 Overview

This is a cross-sectional study in which segmentation of the hippocampal subfields from T1 and T2-weighted MRI images, is performed using a Surface Based Morphometric (SBM) method. We examined CA1, CA2/3, CA4 and DG subfields' volumes and seek to identify the effect physical activity has on these volumes.

The work-flow implemented was as follows: First, questionnaire translation and preparation; Second, structural MRI and questionnaire assessment data acquisition; Third, MRI data preprocessing; Fourth, visual inspection for verification of skull strip without errors for every subject; Fifth, data frame set up and cleaning; and finally, data analysis.

3.2 Participants

Participants in this experiment (n = 32) consist of a sample from the Canary Islands population, Spain. All participants are native Spanish speakers and right-handed. Average age of this sample is 23.709 (SD = 7.773) as shown in Table 3.1. MRI data is obtained as part of two different fMRI studies. Participation in this experiment which involved the additional fulfillment of a questionnaire, was completely voluntary and no reward was administered. The study was conducted in agreement with the declaration of Helsinki, and all participants provided informed consent in consonance with the protocol established by the Ethics Commission for Research of the university of La Laguna (Comité de Ética de la Investigación y Bienestar Animal).

TABLE 3.1: Descriptive information on the participants

	Data set	
	N	Mean age (SD)
Male	17 (51.61%)	22.75 (7.057)
Female	15 (48.39%)	24.73 (8.598)

3.3 Data acquisition

MRI data acquisition
Structural images were obtained from a 3 Tesla MRI system (Signa Excite, General Electric Milwaukee, WI, EEUU) using a standard 8 channel gradient head coil. Head motion was constrained by placing foam pads inside the coil, and earplugs were used to minimize the scanner noise.

High resolution T1-weighted images were acquired for all the subjects using the 3D FSPGR sequence: TI 650, TR of 6.8, and TE 1.4 ms, FA = 12°, 196 slices, slice thickness 1 mm, matrix 256 × 256, voxel size = 1 × 1 × 1 mm.

High-resolution T2-weighted images were acquired for n = 16 subjects of the data set, using the following parameters: TR 9.1, TE 1.13, FA 90°, 512 × 512 × 30 voxels, and a voxel size of 0.4688 × 0.4688 × 2.2mm. The T2-weighted images were acquired anisotropically. Thus, higher in-plane resolution is obtained within each coronal slice.

A single T1-weighted MRI of the whole brain were available for each of the n = 32 subjects. And a single T2-weighted volume were available for each of n = 16 subjects from the entire sample used in the experiment.

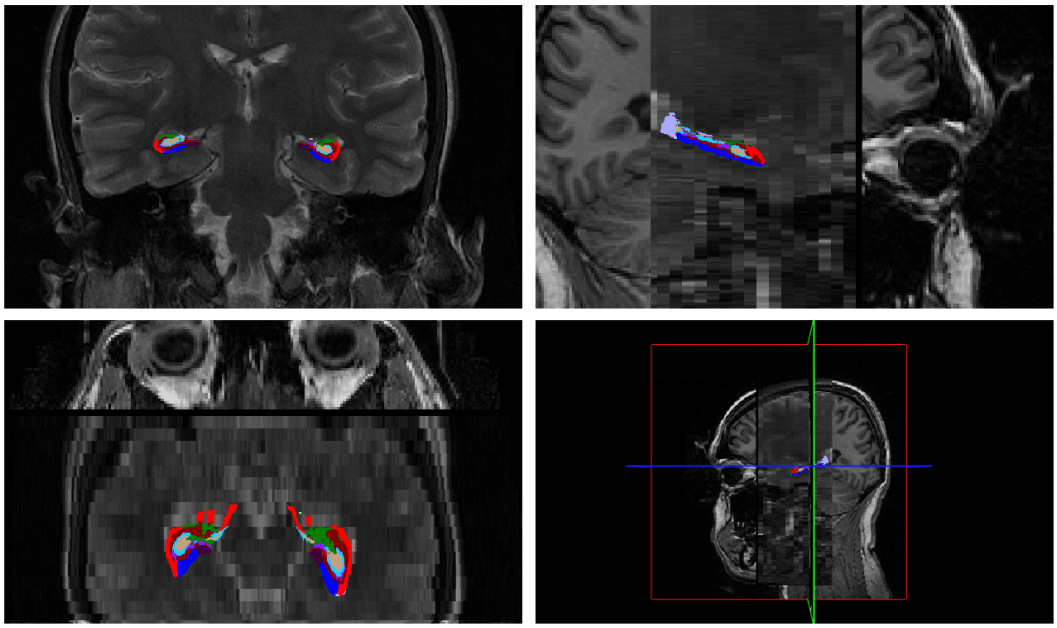

FIGURE 3.1: T2-weighted image from the data set, overlapped to the T1-weighted image of the same subject.

Questionnaire
Participants' regular physical activity was assessed through a Spanish translation of a previously validated questionnaire (Baecke, Burema, and Frijters, 1982, based on the work on Subjective experience work load (SEWL) by Josten, 1973). Three different factors of physical activity were measured, which are: **Work index (WI)**, that refers to the occupational physical activity; **Sport index (SI)**, that indexes sport during leisure time; and **Leisure index (LI)**, measuring other leisure time activities.

The questionnaire was self-assessed on-line using *Google forms* platform for it through approximately a week after the MRI scans were performed for them. Some

additional information was requested, such as height, weight, age, and hours sit per day.

3.4 MRI Processing and Segmentation

To conduct this step, FreeSurfer open source software was used (v6.0.0, http://surfer.nmr.mgh.harvard.edu). FreeSurfer consists of a kit of tools for human MRI images processing and analyzing.

Our final purpose by using this Software, is to obtain a report of the brain substructures volumes, and concretely, from some hippocampal formation subregions. FreeSurfer segments the MRI image in the native space, and contains a computational atlas based on probabilistic information estimated from a manually labeled training set (Fischl et al., 2004). Hippocampal subfields new segmentation tool available in the version used for this experiment, was constructed by manual delineation of ultra high-resolution MRI images from 15 *ex vivo* brains and 39 *in vivo* T1-weighted scans from three data sets, acquired with different resolutions. Manual delineation of the hippocampal formation subregions was performed according to thickness of the pyramidal cell layer, prior knowledge of histological and geometric features, and neighboring extra-hippocampal neuronal groups (Iglesias et al., 2015).

FreeSurfer uses the NIfTI format of MRI volumes to create a three-dimensional volume of the brain, originate a cortical surface, and segment and label the subcortical structures of the brain.

First, it creates an initial surface by performing a registration to the Talairach MNI305 atlas (Collins et al., 1994), picks seed points (corpus callosum and pons), performs correction for non-uniform intensity (Sled, Zijdenbos, and Evans, 1998), main body of the white matter estimation, removal of non-brain tissue by a hybrid watershed/surface deformation procedure (Ségonne et al., 2004), and classification of voxels as white matter or as something else, based on the intensity and on neighbouring voxels to select planes from which hemispheres separation and removal of cerebellum and brain stem would be based.

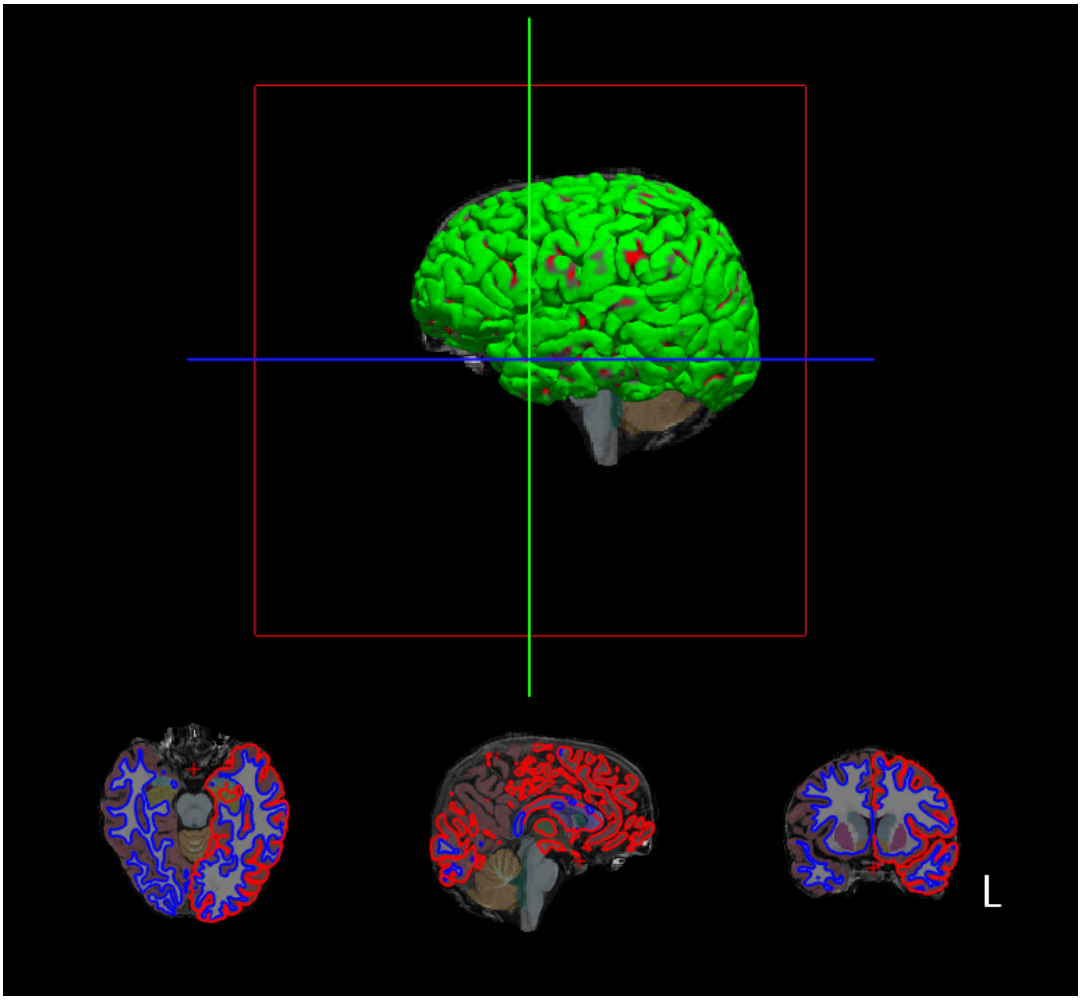

FIGURE 3.2: At the top, the three-dimensional representation of the pial surface made on FreeSurfer, the gyri are in green and the sulci in red; Underneath, the pial surface is marked by the red margin, and white surface, by the blue one.

Second, from the surface created, a registration to align the data resulting from the previous step to the main atlas of FreeSurfer is performed; a normalization based on an estimation of the most certain segmentation voxels; a computation of a non-linear transformation to align to the GCA atlas; labels sub-cortical structures based on GCA model, performs a second intensity correction based only on the brain volume (this step is performed after the skull strip), applies a brain mask, does a white matter segmentation, separating white matter from anything else (using intensity, smoothness and neighbourhood), white matter and grey matter segmentation, and labeling of sub-cortical structures. The mid brain is cut from the cerebellum, and the hemispheres are cut from each other.

MRI data processing was performed using `recon-all` commando and additional `-hippocamp al-subfields-T1` and `-hippocampal-subfields-T1&T2` flags. The resulting hippocampal segmentation can be visualized in Figure 3.1 in page 10.

```
recon-all -s <subject_name> -hippocampal-subfields-T1T2 <file name of a
dditional scan> <analysisID>
recon-all -all -s <subject_name> -hippocampal-subfields-T1
```

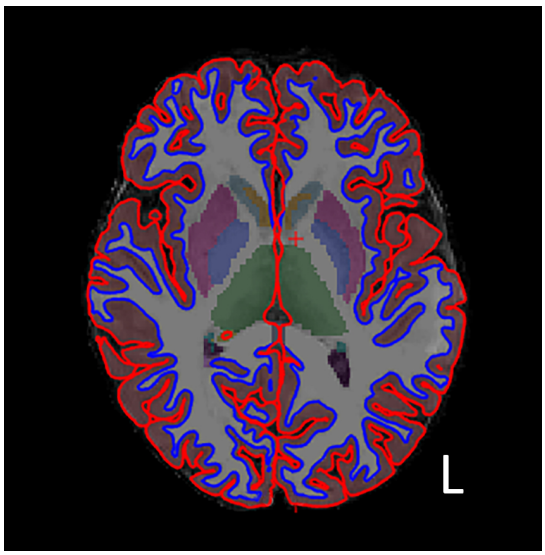

FIGURE 3.3: Segmentation based on labeling of each voxel.

In vivo segmentation allows to distinguish: parasubiculum, presubiculum, subiculum, CA1, CA2/3, CA4, GC-ML-DG, molecular layer, fimbria, hipocampal fissure, HATA, hipocampal tail, whole hippocampus.

Visual inspection for verification of skull strip without errors for every subject was accomplished using Tkmedit tools.

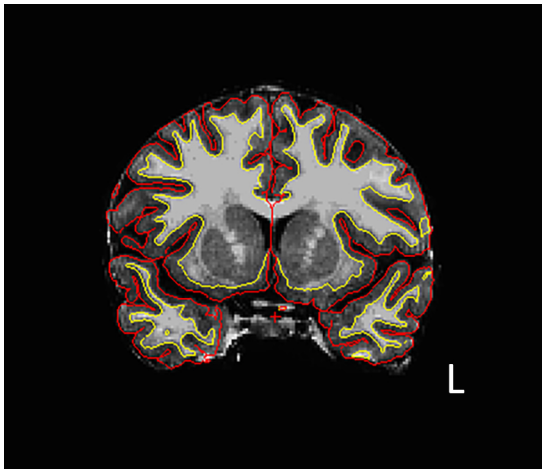

FIGURE 3.4: Non-brain tissue removal inspection. On the left skull removed, and original T1 overlapped on the right.

3.5 Data frame set up and cleaning

Set up and cleaning of the data frame, as well as data analysis - that will be detailed on the next section of the work -, was performed using open source R software (version 3.4.3, R Core Team, 2013) and RStudio (version 1.1.423, RStudio Team, 2015).

Freesurfer outcome set up

Data text documents reporting volumes of the segmentations computed, were collected for hippocampal volumes in the segmentations carried out on T1-weighted, segmentations of the half with both T1 and T2 images, cortical segmentation from the right hemisphere, cortical segmentation from the left hemisphere and sub-cortical segmentation separately. These data table were acquired as specified on FreeSurfer wikis. Volumes of the hippocampal formation subregions were obtained through:

```
quantifyHippocampalSubfields.sh <T1>-<analysisID> <output_file> <OPTION
AL_subject_directory>}
```

Data tables for cortical volumes from both brain hemispheres were collected by:

```
aparcstats2table --subjects subj01 subj02 subj03 subjN --hemi rh --meas
volume --tablefile aparc_stats.txt
```

and

```
aparcstats2table --subjects subj01 subj02 subj03 subjN --hemi lh --meas
volume --tablefile aparc_stats.txt
```

And data tables for sub-cortical volumes, using:

```
asegstats2table --subjects subj01 subj02 subj03 subjN --meas volume --t
ablefile aseg_stats.txt}
```

R packages `tidyr`, `dplyr` and `stringr` were used for data frame setting up. First, both hippocampal subfields tables were combined by rows, using `rbind` function. Structures and their volumes were disposed in rows and the data set was composed by 32 rows and 27 columns. Both two cortical and the sub-cortical tables were combined by columns, using `cbind` function, and obtaining a table of 32 rows and 137 columns. All tables of volumes were combined by columns, and then disposed in a 5120 table, being columns names: "Subject", "Hemisphere", "Structure" and "Volumes", after several transformations.

Questionnaire data set up
Answers to the questionnaire were pondered, and the three habitual physical activity indexes were calculated based on different scale scores as regarded in Baecke, Burema, and Frijters, 1982 and further explanations in *AppendixB*. The contents of the questionnaire can be consulted in *AppendixA*. Indexes calculations were also performed using `mutate` function from `dplyr` package.

All tables mentioned above (volumes and indexes) were merged together and the final data frame was written.

3.6 Statistical analysis

A linear model was applied to identify the effect the three different habitual physical activity indexes have on the volumes of the hippocampal formation subregions that were selected for the experiment (CA1, CA2/3, CA4 and DG). It was corrected for Gender and eTIV.

```
lm(volume ~ (Work index + Sport index + Leisure index) + Gender + eTIV,
data)
```

Both hemispheres were analyzed collectively. Thus, 64 hemispheres were available for each structure analysis. Cook's distance correction (Cook and Weisberg, 1982) was performed for each of the linear models, using influence.measures R suit of functions which serves for leave-one-out deletion diagnostics. Five outliers were removed for the linear model applied to CA1, 7 for CA3, 5 for CA4 and 7 for DG.

Chapter 4

Results

We created subsets of our data for each structure selected: CA1, CA2/3, CA4 and DG, and applied the linear model previously specified to each of these datasets.

TABLE 4.1: Correlations between independent variables

	eTIV	Age	BMI	WI	SI	LI
eTIV	1	-0.152	0.022	0.124	0.183	0.053
Age	-0.152	1	0.231	0.390	0.124	0.120
BMI	0.022	0.231	1	-0.046	0.100	0.071
WI	0.124	0.390	-0.046	1	0.007	0.082
SI	0.183	0.124	0.100	0.007	1	0.345
LI	0.053	0.120	0.071	0.082	0.345	1

4.1 Effect of Physical Activity Indexes on Hippocampal Subregions

As can be seen on Table 4.2, none of the physical activity indexes were a good predictor of CA1 volumes in our sample. Gender differences hadn't got any significant effect either, and a significant effect of eTIV was found for this volume.

TABLE 4.2: Linear model for CA1

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	188.6133	96.9357	1.95	0.0570
WI	8.5396	8.1496	1.05	0.2995
SI	1.0784	9.8334	0.11	0.9131
LI	8.2664	18.9895	0.44	0.6651
Gender (female)	15.2027	15.9126	0.96	0.3437
eTIV	0.0002	0.0001	4.51	0.0000 ***

Physical activity indexes were not good predictors of CA2/3 volumes either. There was no significant effect of gender, and eTIV was good predictor of the volume in this hippocampal subfield (Table 4.3).

TABLE 4.3: Linear model for CA2/3

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	62.1215	44.2823	1.40	0.1664
WI	-1.9319	3.7373	-0.52	0.6073
SI	-7.3703	4.5225	-1.63	0.1090
LI	9.1953	8.0551	1.14	0.2587
Gender (female)	-4.0537	7.3270	-0.55	0.5824
eTIV	0.0001	0.0000	4.37	0.0001 ***

Work index had a significant effect on CA4 volume and the other indexes did not (Table 4.4). Again, there was no significant effect of gender and eTIV was a good predictor of the subregion volume.

TABLE 4.4: Linear model for CA4

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	46.7987	43.6046	1.07	0.2880
WI	8.7055	2.9836	2.92	0.0052 **
SI	-1.9674	3.5524	-0.55	0.5820
LI	1.8902	7.2572	0.26	0.7955
Gender (female)	1.1223	6.7571	0.17	0.8687
eTIV	0.0001	0.0000	5.32	0.0000 ***

Work index was a good predictor of DG volume in our sample, whereas the other indexes did not. Gender did not have a significant effect on the volume of DG region, and eTIV did.

TABLE 4.5: Linear model for DG

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	53.4321	41.0265	1.30	0.1986
WI	10.4619	3.7066	2.82	0.0068 **
SI	-3.8789	3.6770	-1.05	0.2964
LI	10.1883	6.8247	1.49	0.1416
Gender (female)	6.4569	6.7694	0.95	0.3447
eTIV	0.0001	0.0000	5.71	0.0000 ***

Chapter 5

Discussion and Conclusions

In this study, we looked for the relationship between volume in some HF subregions and physical activity. We wanted to know specifically, whether different types of physical activity would have an impact on the hippocampal subfields differentially.

Previous studies found that vascular hippocampal activity after aerobic exercise (Maass et al., 2015); Exercise selectively increases DG CBV in humans and correlates with aerobic fitness and cognition (Pereira et al., 2007). Furthermore, in the study of Pereira et al. (2007), CBV correlated to neurogenesis in mice. As CA4 in our segmentation comprises the hilus (Iglesias et al., 2015), and subgranular zone is between the hilus and granule cell layer from DG, so we hypothesized that this two HF subregions would present increasing volume due to a higher level of PA.

We considered to study different types of PA, trying to comprise all daily PA, and to seek which of these activities would be more important for volumetric variations. Here we performed an automated segmentation of the HF subregions in high resolution MRI images, and assessed daily PA throughout a questionnaire that distinguished three types of daily PA: occupational PA, sports during leisure time, and other leisure time activities that are not sports. We found that a higher occupational PA level, measured as WI, had a positive effect on HF volume in DG, as well as CA4 subregions.

Given that DG is a subregion specially vulnerable to structural changes, as we could see in many examples in the introduction to this work (e.g.: Pereira et al., 2007), we expected some volumetric changes in it. And due to the close location of the hilus to the sub-granular zone, where neurogenesis is thought to occur, we thought some variation could be seen in this area too. Thus, we had two hypotheses: First, PA would affect DG, and CA4 (hilus) subregions and not CA1 and CA2/3. Second, the three different types of PA would affect the same the HF subregion's volumes.

Our results are in agreement with the hypothesis that DG and CA4 would have increased volumes due to PA, but in the division we used of PA, types of PA influenced the volumetric changes differentially. Not all activity is the same. In fact, among the indexes, PA required in the working time (WI) was the better predictor of these subregions volume.

Pereira et al. (2007), performed some experiments whose findings are key for our work. They found that CBV of the DG provided a correlate of exercise-induced neurogenesis in mice, and that CBV changes in DG of humans correlated with their individual changes in VO_2max and their cognitive performance on a learning task.

Some other studies have found increases in volume on HF due to exercise and changes in fitness. Volumetric changes that were related to improvements in spatial memory (Erickson et al., 2011). But there is very little literature focusing on the effect of PA on HF subregions.

Our results suggest that PA during working time may produce important changes on DG and hilus. We think that this PA index and not the others, could have an effect on these subregions due to its routine implication in subjects life.

On the other hand, we find some problems on our study regarding to the assessment of PA. The questionnaire we used was validated in 1982 with a Deutsch population, and our experiment was performed in 2017-2018 with a Spanish population. Furthermore, this sort of assessment may give rise to some biases, such as social desirability bias. Future experiments on this matter could be performed using objective measures of PA. Also, it could be of great interest to investigate the resting state networks (RSN) of HF subregions regarding different levels in differentiated components of daily life PA.

In conclusion, this study seeked to identify the effects of different types daily life PA on some HF subregions (CA1, CA2/3, CA4 and DG). We found that only occupational PA was good predictor of any HF subregions, and that these were DG and CA4 (or hilus). The effect of this type of activity suggests that PA performed in our work time is closely related to our brain structure and perhaps even health.

Appendix A

Questionnaire

1. ¿A qué se dedica?
2. En el trabajo permanezco sentado:

1. Nunca
2. Rara vez
3. Algunas veces
4. Frequentemente
5. Siempre

3. En el trabajo permanezco de pie:

1. Nunca
2. Rara vez
3. Algunas veces
4. Frequentemente
5. Siempre

4. En el trabajo camino:

1. Nunca
2. Rara vez
3. Algunas veces
4. Frequentemente
5. Siempre

5. En el trabajo levanto cargas pesadas:

1. Nunca
2. Rara vez
3. Algunas veces
4. Frequentemente
5. Muy frequentemente

6. Después del trabajo me encuentro cansado:

1. Nunca
2. Rara vez
3. Algunas veces
4. Frequentemente
5. Muy frequentemente

7. Sudo en el trabajo:

1. Nunca
2. Rara vez
3. Algunas veces
4. Frequentemente
5. Muy frequentemente

8. En comparación con otros de mi edad, creo que mi trabajo es físicamente:

1. Nunca
2. Rara vez
3. Algunas veces
4. Frequentemente
5. Muy frequentemente

9. Practica algún deporte?

9.1 ¿Qué deporte practica más frequentemente?
9.2 ¿Cuantas horas a la semana?

1. < 1

2. 1 - 2
3. 2 - 3
4. 3 - 4
5. > 4

9.3 ¿Cuántos meses al año?

1. < 1
2. 1 - 3
3. 4 - 6
4. 7 - 9
5. > 9

9.4 ¿Practicas algún otro deporte? ¿Cuál?
9.5 ¿Cuantas horas a la semana?

1. < 1
2. 1 - 2
3. 2 - 3
4. 3 - 4
5. > 4

9.6 ¿Cuántos meses al año?

1. < 1
2. 1 - 3
3. 4 - 6
4. 7 - 9
5. > 9

10. En comparación con otras personas de mi edad, creo que mi actividad física en el tiempo de ocio es:

1. Mucha menos
2. Menos
3. Igual
4. Más
5. Mucha más

11. En mi tiempo libre sudo:

1. Nunca
2. Rara vez
3. Algunas veces
4. Frequentemente
5. Muy frequentemente

12. En mi tiempo libre practico deporte:

1. Nunca
2. Rara vez
3. Algunas veces
4. Frequentemente
5. Muy frequentemente

13. En mi tiempo libre veo la televisión:

1. Nunca
2. Rara vez
3. Algunas veces
4. Frequentemente
5. Muy frequentemente

14. En mi tiempo libre camino:

1. Nunca
2. Rara vez
3. Algunas veces
4. Frequentemente
5. Muy frequentemente

15. En mi tiempo libre voy en bicicleta:

1. Nunca
2. Rara vez
3. Algunas veces
4. Frequentemente
5. Muy frequentemente

16. ¿Cuántos minutos camina y/o va en bicicleta al día para ir y volver del trabajo, colegio y compra?

1. < 5
2. 5 - 15
3. 15 - 30
4. 30 - 45
5. > 45

Appendix B

Questionnaire Scores and Indexes

The questionnaire scores were calculated following the instructions of Baecke, Burema, and Frijters, 1982, that we display in Table B.1. Final scores for each question is shown in the "Final score column", but for question 9. The results from "Final score" for the six sections of question 9 were multiplied: intensity $\times$ hours $\times$ months for each sport, and results from this two multiplications were summed up. The result of this sum was then scaled in 1/2/3/4/5: if result was 0, then a score of 1 would be assigned. A score 2 was assigned to a result of 0.01 - $<$4; 3 to a result between 4 - $<$8; 4 to a result between 8 - $<$12; and 5 to a result of $\geq$12.
Work index was computed by the next formula that uses scores for questions 1 - 8: WI = [S1 + (6 - S2) + S3 + S4 + S5 + S6 + S7 + S8]/8; Sport index using this formula: [S9 + S10 + S11 + S12]/4; and Leisure-time index using the following one: [(6 - S13) + S14 + S15 + S16]/4.

TABLE B.1: Questionnaire Scores

Question	Answer	Final score
1	Type of job	1/3/5
2 - 4	never/seldom/sometimes/often/always	1/2/3/4/5
5 - 7, 11 - 15	never/seldom/sometimes/often/very often	1/2/3/4/5
8	much lighter/lighter/as heavy/heavier/much heavier	1/2/3/4/5
9	Sport 1 Intensity	0.76/1.26/1.76
	Sport 1 hours/w: <1/1-2/2-3/3-4/>4	0.5/1.5/2.5/3.5/4.5
	Sport 1 months/y: <1/1-3/4-6/7-9/>9	0.04/0.17/0.42/0.67/0.92
	Sport 2 Intensity	0.76/1.26/1.76
	Sport 2 hours/w: <1/1-2/2-3/3-4/>4	0.5/1.5/2.5/3.5/4.5
	Sport 2 months/y: <1/1-3/4-6/7-9/>9	0.04/0.17/0.42/0.67/0.92
10	much less/less/the same/more/much more	1/2/3/4/5
16	minutes: <5/5-15/15-30/30-45/>45	1/2/3/4/5

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