Anxiolytic-like effects of *Lupinus angustifolious* protein hydrolysates in Alzheimer model mice

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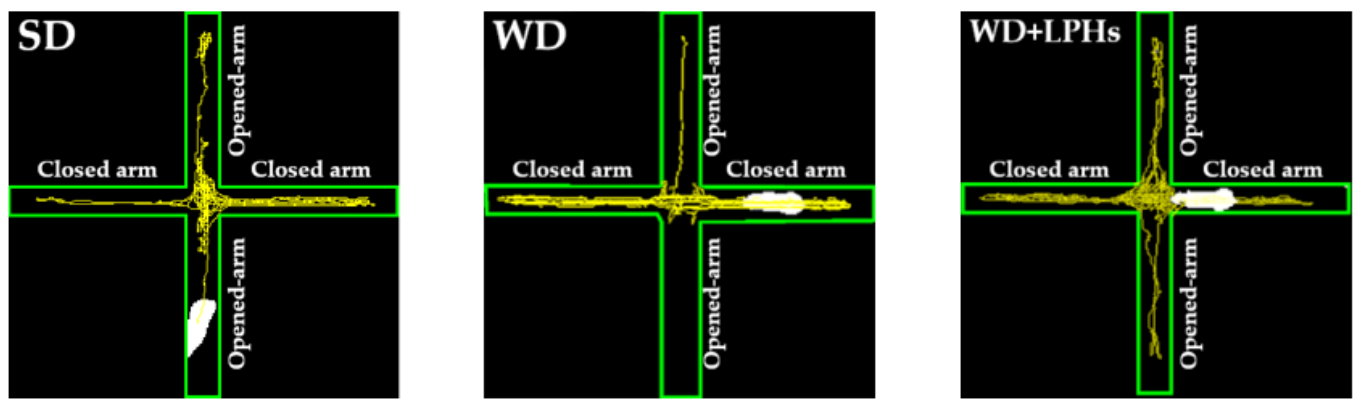

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Introduction: Alzheimer´disease

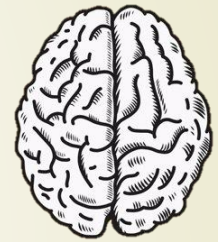
1. Alzheimer´s disease (AD) is a **neurodegenerative disorder** characterized by a quick progressive cognitive dysfunction, loss of memory, and behavior impairment.

2. Besides principal symptoms, AD produces a variety of neuropsychiatric effects such as anxiety, which generate sleep disturbances, irritability, agitation, or aggressiveness

3. **Inflammation and Oxidative stress** play a fundamental role in AD and anxiety

4. Previous studies have demonstrated that a **high-fat diet**, in addition to exacerbating AD, aggravates anxiety

Inflammation
Oxidative stress



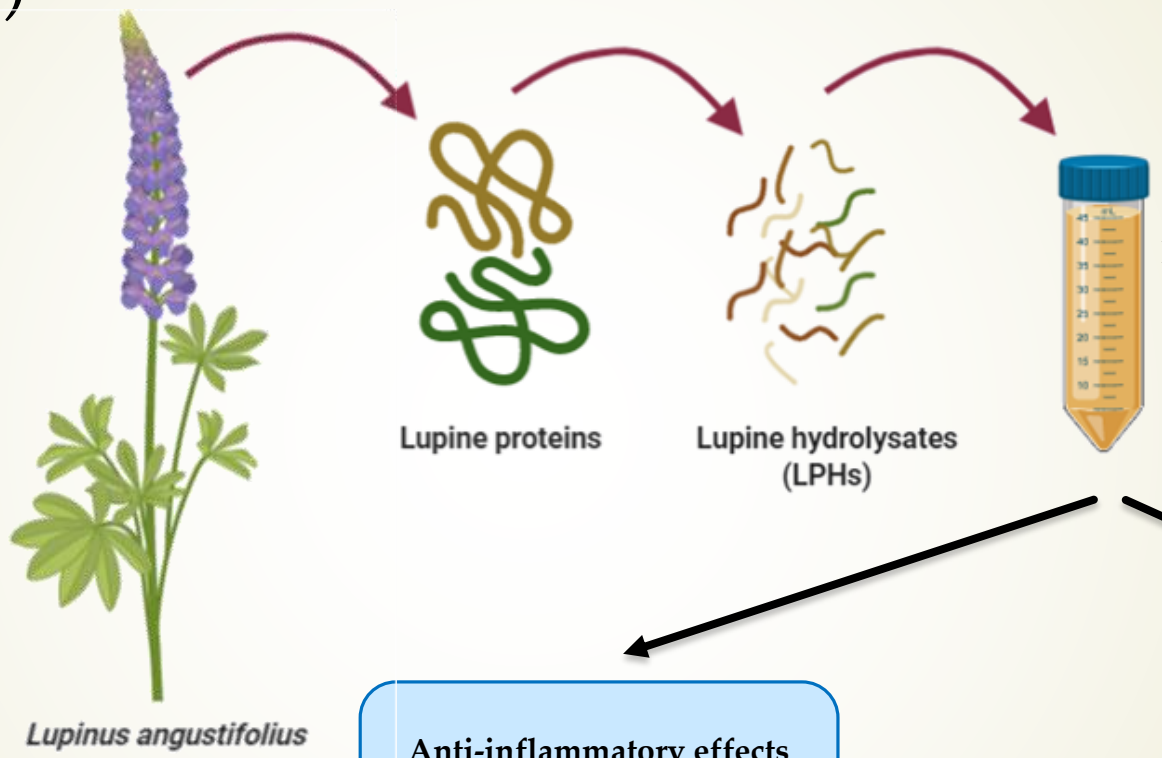
Neurodegeneration

Anxiety

Irritability, agitation, aggressiveness…

Introduction: Lupine protein hydrolysates (*Lupinus angustifolius*)

We have previously demonstrated that *Lupinus angustifolius* protein hydrolysates (LPHs) have anti-inflammatory and antioxidant effects, key risk factors for AD and anxiety

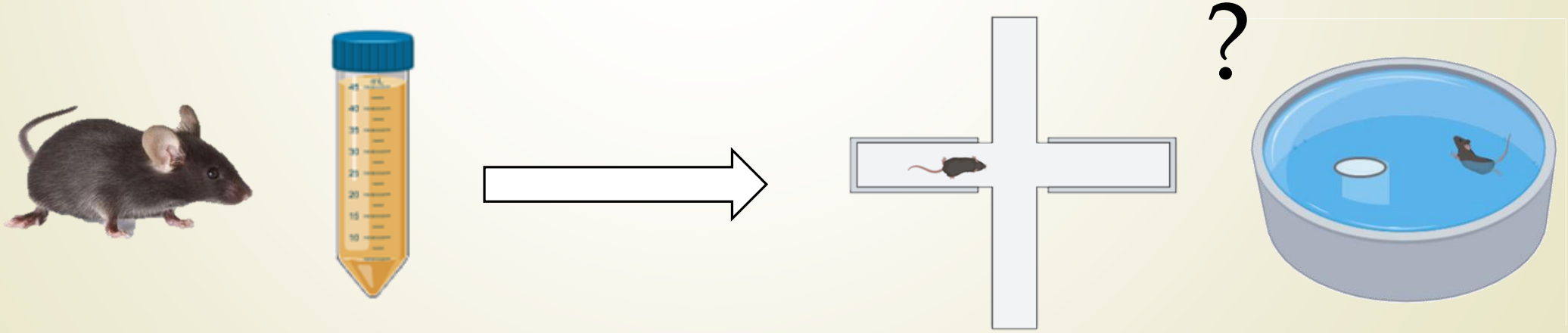


Cruz-Chamorro *et al*, Food Res Int. 2019

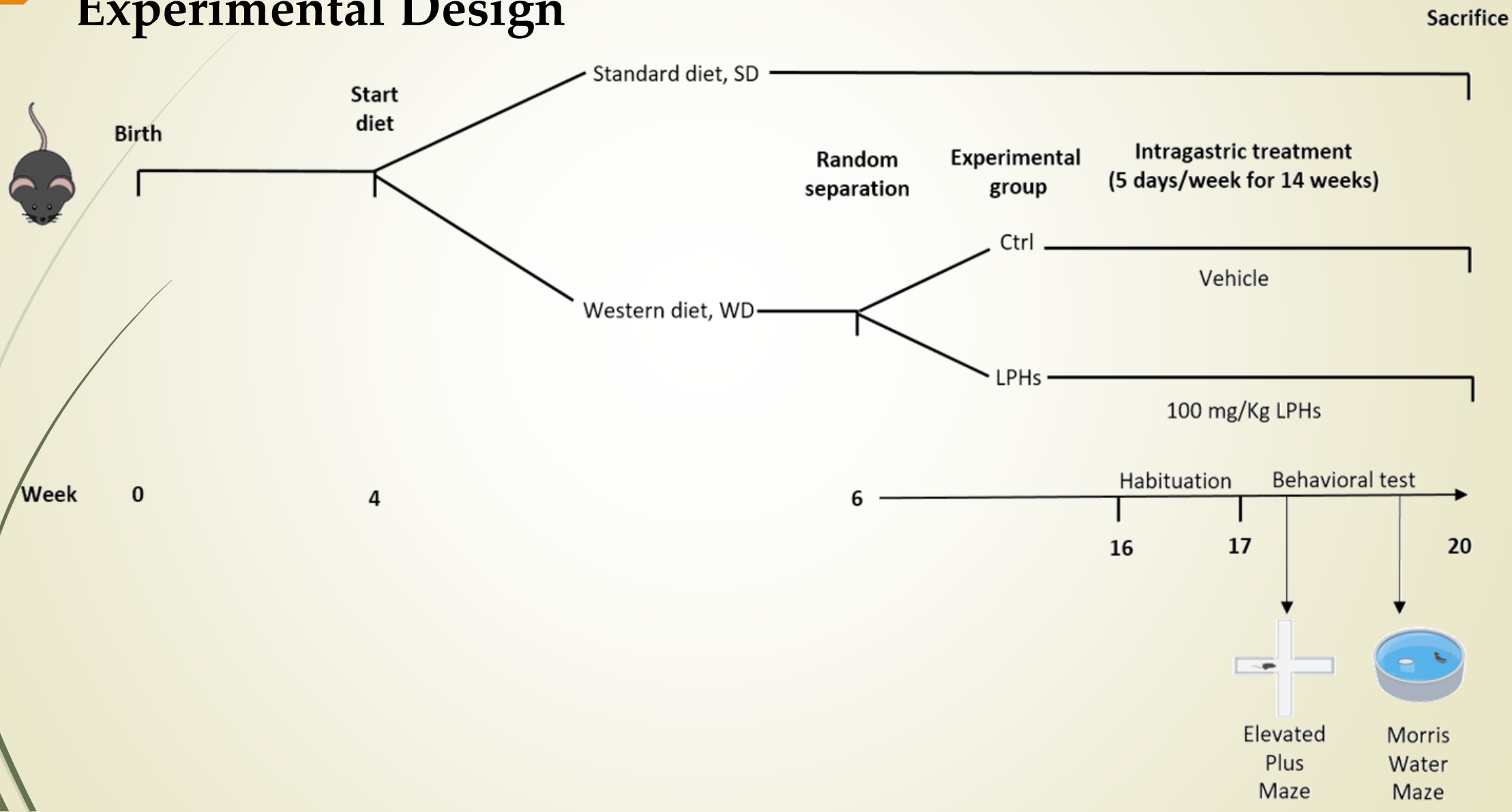
Cruz-Chamorro *et al*, Clin. Nutr. 2020 (in revision)

Aim

Evaluate the potential effects of Lupine protein hydrolysates on spatial memory and anxiety of a preclinical model of Alzheimer´ disease, the ApoE$^{-/-}$ mice.



Materials and Methods
Experimental Design



Materials and Methods
Experimental Design

- Animals were treated **intragastrically** with LPHs (100 mg/kg) or vehicle for 14 weeks, respectively.
- Individual body weight was weekly measured and recorded.

LPHs: 100 mg/Kg
5 days/weeks
14 weeks
16-18h

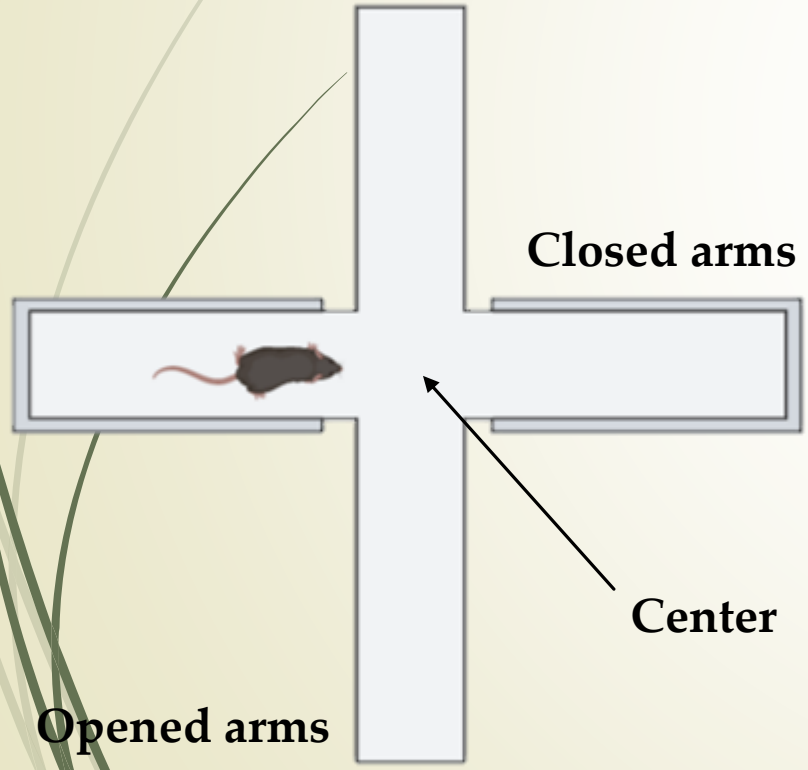
Diets

	Macronutrients		
	Fat (% of energy)	**Carbohydrates (% of energy)**	**Protein (% of energy)**
SD	13	67	20
WD	46.1	35.8	18.1

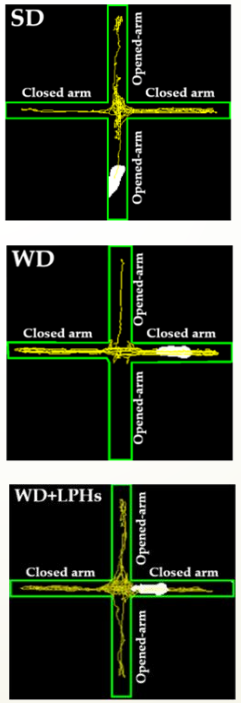
Materials and Methods
Elevated Plus Maze (EPM)

↑ *Time in closed arms* = ↑ *Anxiety*

Anxiety-like behavior was evaluated using the Elevated Plus Maze (EPM) test at 17 weeks of age, after twelve weeks of consumption of SD, WD, or WD+LPHs.



5 minutes



Analysis

- Time in opened arms
- Time in closed arms
- Time in center
- Anxiety behavior (Head dips, rears)

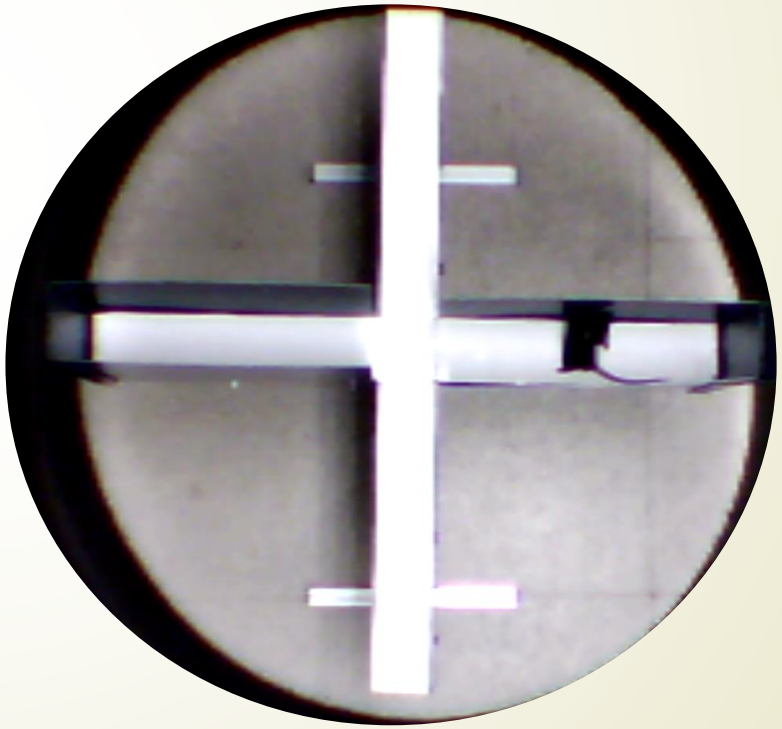
AnimalTracker for ImageJ software

Materials and Methods
Elevated Plus Maze (EPM)

↑ *Time in closed arms* = ↑ *Anxiety*

Anxiety-related behaviors like head dips and rears were considered.

Head dips

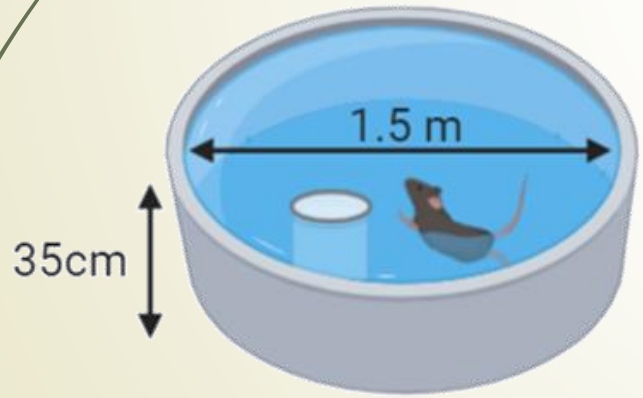


Rears

Materials and Methods
Morris Water Maze (MWM)

Morris Water Maze (MWM) was performed at 19 weeks to study the processes of **spatial memory and learning.** Experimental procedures were performed as described by Janssen et al.

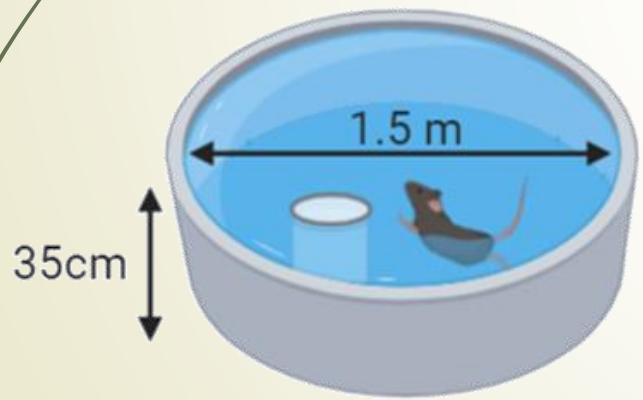
All sessions were recorded with a video tracking system facing to the pool from above.

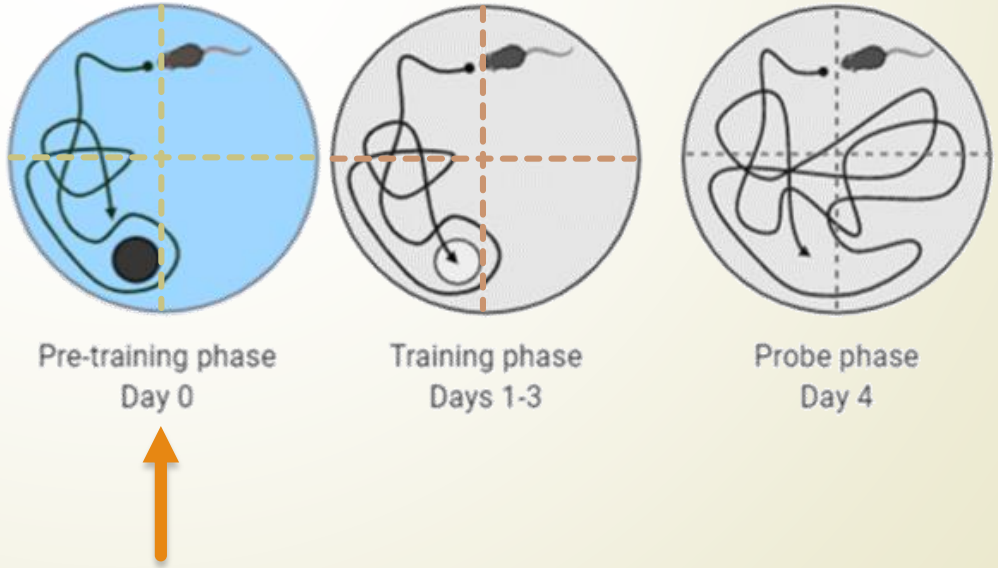


Janssen *et al*, Plos One, 2016

Materials and Methods
Morris Water Maze (MWM)

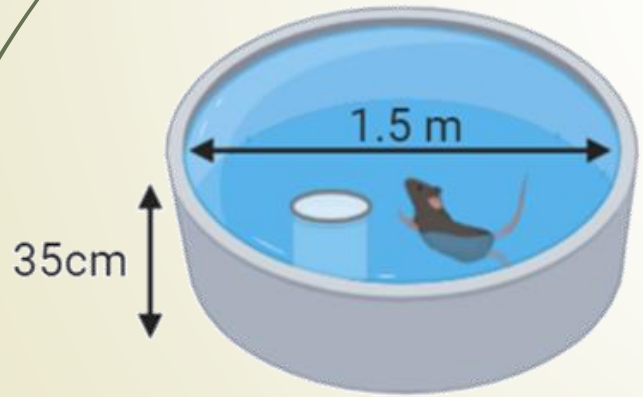
- On **day 0**, mice received two habituation trainings.
- Mice were located in two different quadrants and allowed to swim for 90 sec until reaching the **visible platform** (2 cm above the water surface).
- Once over the platform, mice stand there for 15 sec.

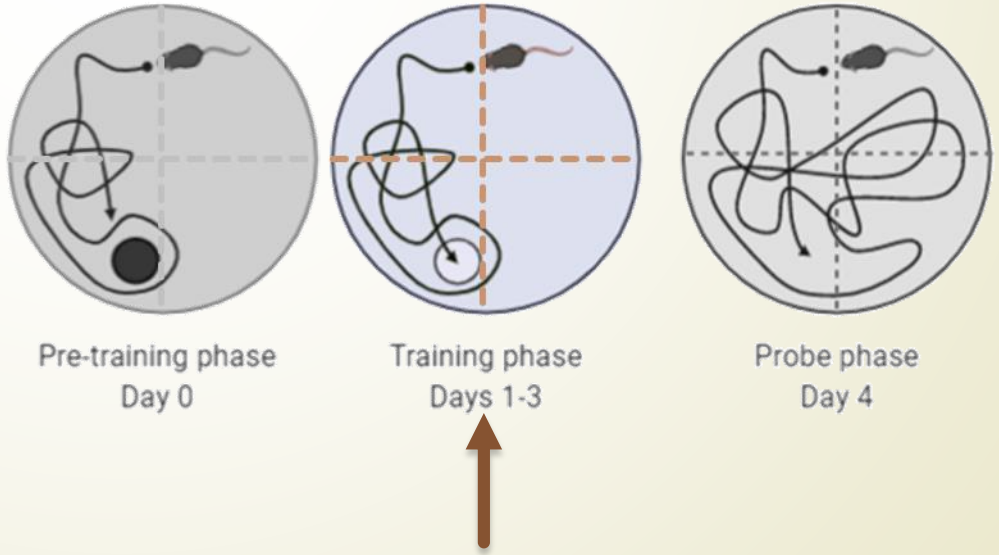




Materials and Methods
Morris Water Maze (MWM)

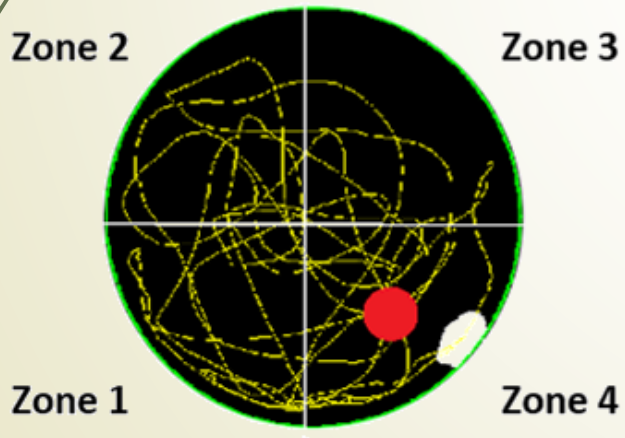
- On **days 1-3**, animals were placed in each quadrant and allowed to swim for 90 sec or until they reach the non-visible platform.
- In this phase **water was opaque** by adding white-dye (lime) and the time between trial was 45 min.
- **Latency time** was analysed

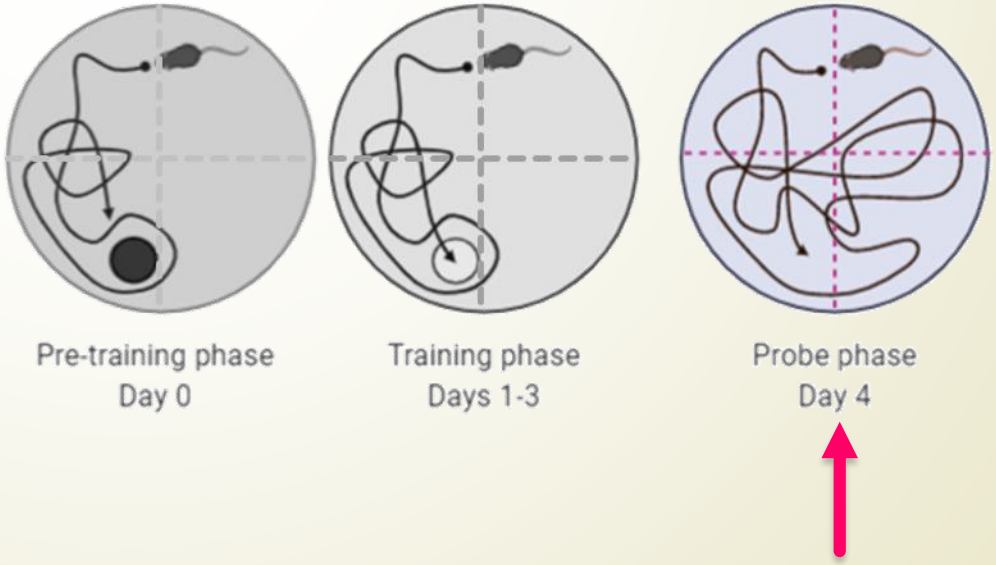




Materials and Methods
Morris Water Maze (MWM)

- At last, in the **fourth day**, **platform was removed** from the pool and mice were placed into the pool for 90 sec.
- **Traveled distance** and **the time spent in each quadrant** were analyzed using the plugin Animal Tracker for ImageJ software.





Results and discussion

LPHs treatment does not change body weight.

- **No differences** were observed in the baseline body weight (BBW), final body weight (FBW), and body weight gain (BWG) between groups.

Table 1. Body weight parameters

Parameter	SD	Ctrl	LPHs
BBW (g)	20.35 ± 0.41	20.98 ± 0.36	20.88 ± 0.49
FBW (g)	26.20 ± 0.87	26.50 ± 0.54	27.15 ± 0.69
BWG (g)	5.85 ± 1.18	5.53 ± 0.65	6.28 ± 1.09

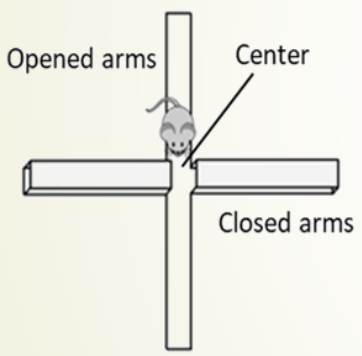
Baseline body weight (BBW), final body weight (FBW), and body weight gain (BWG) in ApoE-/- mice. Values are shown as the mean and standard error of the mean of each group (n=4). Ctrl, control group. LPHs, lupine protein hydrolysates group. SD, standard group.

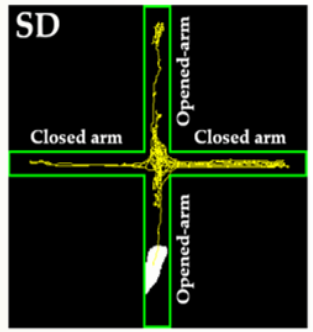
- With these results we can conclude that the effects observed in this study were not related to BWG of mice, that remained unchanged between the three experimental groups.

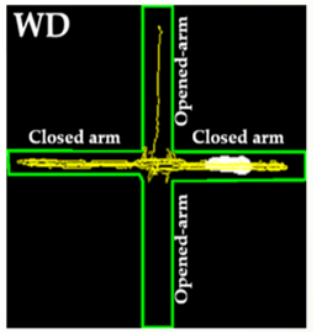
Results and discussion

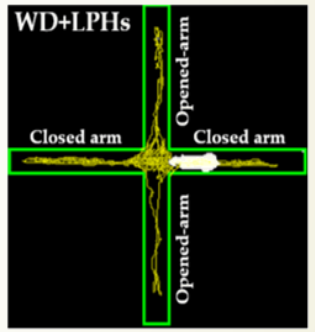
LPHs exert an anxiolytic-like effect

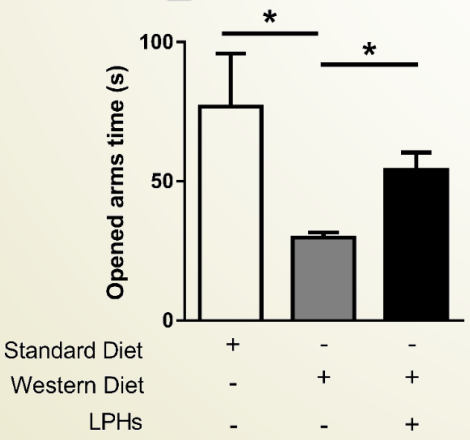
- WD mice spent significantly less time in opened arms and more in closed arms compared to SD group. This effect was reversed after LPHs supplementation
- Moreover, the time spent in the center was significantly lower in WD compared to SD and WD+LPHs.

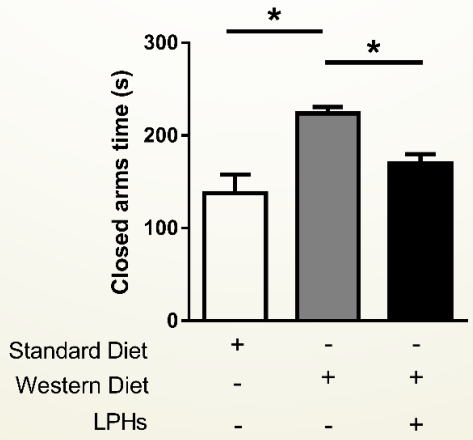


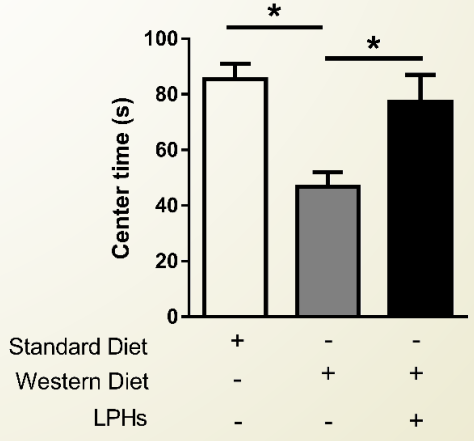








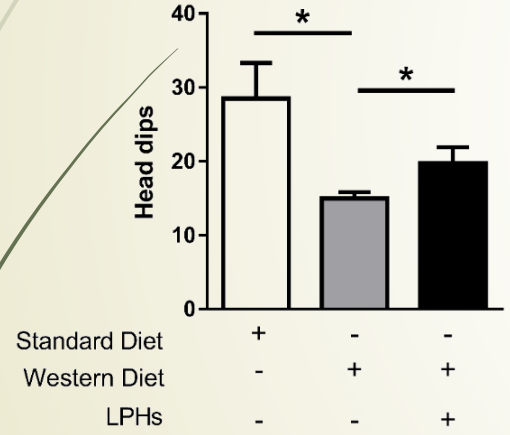


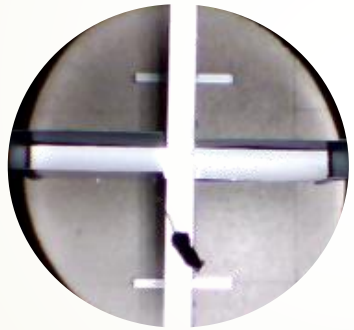


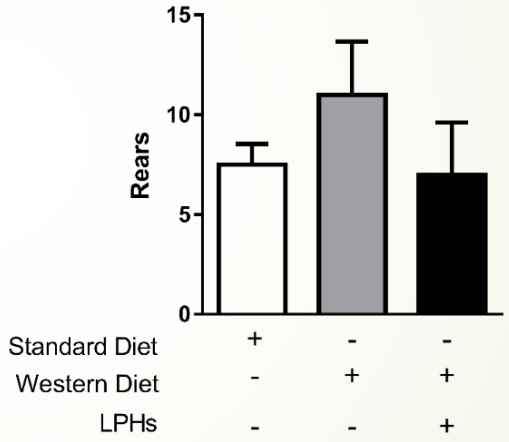
Results and discussion

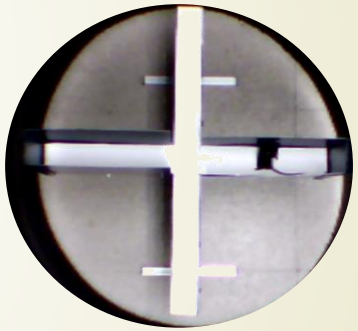
LPHs exert an anxiolytic-like effect

- The number of head dips was significantly lower after WD intake compared to SD, but not in WD+LPHs group. There were no differences in the rears between groups.









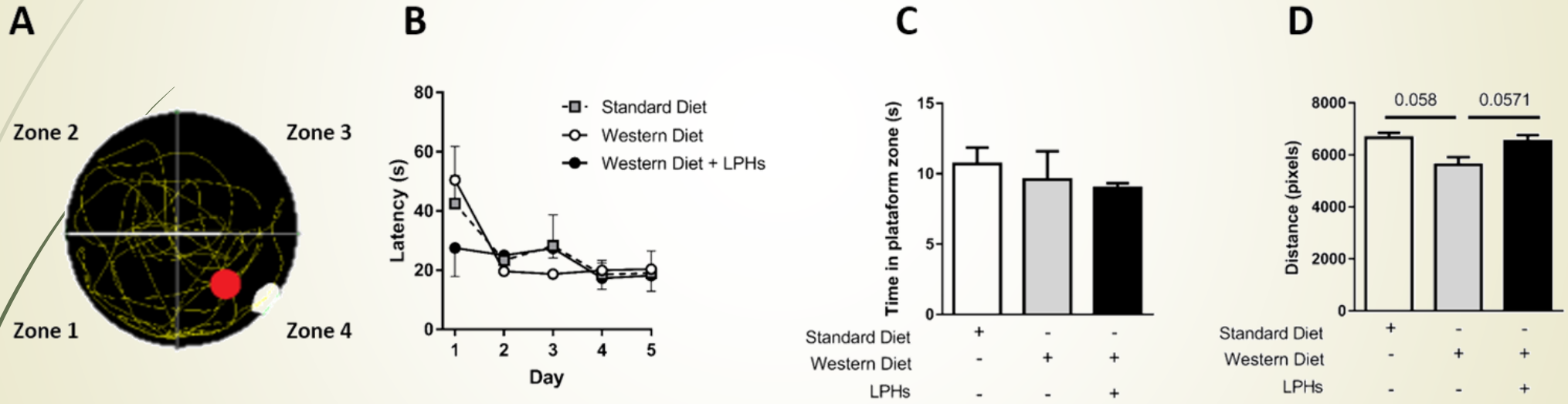
In the present study, we observed that <u>WD increases significantly anxiety levels.</u>

It has been already shown that bioactive peptides from soy may exert an anti-anxiety activity, but to the best of our knowledge, **<u>it is the first time that a Lupine hydrolysate show anxiolytic-like properties.</u>**

Results and discussion

LPHs do not alter spatial memory.

- We did not observe any significant differences between groups in latency and time spent in the platform zone among groups.



Effects of LPHs on WD-induced cognitive deficits. Representative image of the MWM (A); learning effects (B), time in platform zone (C), and distance traveled of each experimental group. Values are shown as the mean and standard error of the mean of each group (n=4).

Our results reveal **no impairment of memory or spatial learning after WD eating**.

Unlike other peptides, LPHs did not improve neither the spatial memory nor learning after a treatment of 14 weeks.

Conclusion

This is the first study showing that a LPHs treatment causes anxiolytic effects, **pointing out LPHs as an effective component of future nutritional therapies in AD patients with anxiety**, serious side effect of this disease.