Lupine (Lupinus angustifolius L.) peptide prevents non-alcoholic fatty liver disease in high-fat diet-induced obese mice

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Abstract: Bioactive peptides are related to the prevention and treatment of many diseases. GPETAFLR is an octapeptide which was isolated from lupine (Lupinus angustifolius L.) and showed anti-inflammatory properties. The aim of this study was to evaluate the potential activity of GPETAFLR to prevent non-alcoholic fatty liver disease (NAFLD) in high-fat diet (HFD)-induced obese mice. C57BL/6J mice were fed a standard diet or an HFD. Two of the groups fed the HFD diet were treated with GPETAFLR in their drinking water at 0.5 mg/kg/d or 1 mg/kg/d. To determine the ability of GPETAFLR to improve the onset and progression of NAFLD, histological studies, hepatic enzyme profile, inflammatory cytokine and lipid metabolism-related genes and proteins were analyzed. Our results suggest that HFD-induced inflammatory metabolic disorders were alleviated by treatment with GPETAFLR. In conclusion, dietary lupine consumption could repair HFD-induced hepatic damage, possibly via modifications in the liver’s lipid signalling pathways.

Keywords: GPETAFLR peptide; Protein hydrolysate; Liver; Hepatic steatosis; High-fat diet.

1. Introduction

Nowadays, population is growing exponentially, meanwhile natural sources are decreasing due to their overexploitation and changes in the climate. For these reasons, it is necessary to discover new sources where find compounds having nutritional value and that could be easy to obtain and process. This opens a new area where take advantage of the agroindustry residues to further propose adapted supply regarding specific demand of nutrients and bioactive compounds [1, 2].

Vegetables are an important source of nutrients and obtaining and purifying peptides is relatively easy; moreover, their growth is cheap and simple. Therefore, they are an ideal target for obtaining bioactive compounds. The main sources of peptides for their isolation are soy, eggs, milk, and fish [3]. The main ways bioactive peptides act on human health are: decreased inflammation, lower blood pressure, anti-obesity, and the prevention and improvement of symptoms related to diabetes [4]. In addition, the diseases that are on the rise in developed countries such as obesity, dyslipidemias, type
II diabetes, the syndrome of the metabolic, or non-alcoholic fatty liver disease (NAFLD) have pharmacological treatment that is not fully effective and also adverse side effects [5]. This is why many scientists are looking for substances of plant origin that can alleviate or prevent these diseases [6].

On the basis of these considerations, bioactive peptides are a short sequence of amino acids with different biological activities. Initially, they are found in a protein precursor in the organism of origin and they are generally not active [7,8]. The peptide is obtained from plant proteins as a result of enzymatic hydrolysis during digestion, by fermentation in micro-organisms or by in-vitro chemical or enzymatic hydrolysis processes [7].

*Lupinus angustifolius* L. is one of the targets for the search of this type of peptides because it has a high amount of proteins and low lipids [9,10]. GPETAFLR is an octapeptide isolated from *Lupinus angustifolius* L. with sequence Glycine-Proline-Glutamic Acid-Threonine-Phenylalanine-Leucine-Arginine. This peptide features a three-dimensional secondary structure of α-helix (*Figure 1*). Its immunomodulatory and anti-inflammatory capacity have been demonstrated [11]. Its effects have been previously investigated in macrophages THP-1-line derivatives [11], in osteoclasts derived from human primary monocytes [12], and in primary human monocytes [13]. Hence, it is an interesting compound to treat or prevent diseases characterized by inflammation.

*Figure 1*. Chemical (a) and secondary three-dimensional (b) structure of GPETAFLR peptide, an octapeptide isolated from *Lupinus angustifolius* L., which amino acid sequence is identified as: Glycine (G), Proline (P), Glutamate (E), Threonine (T), Alanine (A), Phenylalanine (F), Leucine (L), and Arginine (R). Yellow colour was used to side chain, green colour was used to amino group, and blue colour was used to carboxyl group.

Non-alcoholic fatty liver disease (NAFLD) is a health problem that affects a large percentage of the world’s population with a global prevalence of 25,24% (14). The prevalence of this disease is very common in developed countries, due to the fact that it typically occurs in people with obesity, type 2 diabetes, and metabolic syndrome, among other pathologies related to lifestyle [15-18]. NAFLD consists of the accumulation of fat in the liver tissue that it is derived from a high amount of free fatty acids (FFA) and triglycerides (TGs) in the circulating blood. These levels may be increased by a higher caloric and fat intake. For this reason, fatty liver disease is often associated with obesity and dyslipemias [15-18]. The best models for the study of this disease are animal models of induced obesity; specifically, mice and rat models present a pathogenesis more similar to humans [19]. NAFLD has 4 phases, non-alcoholic fatty liver (NAFL), accumulation of fat in the liver; non-alcoholic steatohepatitis (NASH), liver fat accumulation with inflammation; fibrosis, accumulation of fat and...
inflammation results in tissue damage that drifts into fibrosis; and cirrhosis, where most of the cells have been replaced by fibrosis and thus cellular hepatic function is seen to be very damaged. This state leads to hepatic dysfunctionality [18]. The development of NAFLD may culminate in the development of liver cancer. Specifically, cirrhosis (last stage of NAFLD) is the major risk factor for developing hepatocellular carcinoma, the most frequent liver cancer. Hepatic steatosis is also related to the appearance of hepatocarcinoma [20]. So NAFLD, even in its early stages, promotes the development of the most common type of liver cancer. Herein, the peptide GPETAFLR was used to investigate its role in the development of NAFLD in the hepatic tissue of mice with HFD-induced obesity.

2. Materials and Methods

All animal protocols received appropriate institutional approval (Animal Care and Use Committee of the University of Seville) and were performed according to the official rules formulated in the Spanish law on the care and use of experimental animals (UE Directive of 2010: 2010/63/UE; RD 53/2013).

2.1 Isolation, Purification, and Synthesis of GPETAFLR

Seeds of lupine (*Lupinus angustifolius* L.) were a gift from Koipesol Semillas, S.A. (Seville, Spain). Alcalase 2.4L was provided by Novozymes ( Bagsvaerd, Denmark). Lupine protein isolate was obtained using a previously described method [21] at pilot plant scale (Plant Protein Unit, Instituto de la Grasa, Seville, Spain). The chemical composition of lupine protein isolate in dry matter was: protein 86.83%, dietary fibre 5.97%, fat 5.08%, ash 0.78%, and carbohydrate 1.34%. Lupine protein isolate was suspended in distilled water (10% w/v), and hydrolysis with Alcalase was performed under the following conditions: pH 8, 50 °C, E/S=0.3 AU/g protein, and a hydrolysis time of 15 min. The mixture was then heated at 85 °C for 15 min to inactivate the enzyme and centrifuged at 6500 x g for 15 min, and the supernatant constituted LPH [11]. This fraction was purified by ultrafiltration and chromatographic techniques [22]. The sequence of the purified peptide was identified through sequence analysis by the nanoHPLC coupled to a Polaris Q ion-trap mass spectrometer (Mass Spectrometry Unit, Instituto de la Grasa, Seville, Spain). Finally, the peptide was synthesized by Fmoc solid-phase method (Barcelona Scientific Park Foundation, Barcelona, Spain) at 95% purity, measured by HPLC-UV at 220 nm.

2.2 Animal Diets and Experimental Design

Male mice C57BL/6J were used for the study. They were obtained from the Animal Production and Experimentation Centre at the University of Seville. The mice were divided into four groups in which 3 of them were fed with an HFD and the last group was fed with a standard chow diet and was used as a control (210 SAFE, Augy, France). Mice were fed for 8 weeks. The HFD, which contained 60% energy as fat, was prepared by Panlab Laboratories (HF 260 SAFE) and presented as pellets to the animals (*Table 1*). Two of the experimental animal groups fed on HFD were also treated with the peptide GPETAFLR, which was added to the drinking water and administered at a dose of 0.5 or 1 mg/kg/d. Mice were anesthetized by an intraperitoneal injection of ketamin (100 mg/kg) and diazepam (5 mg/kg), and then were euthanized by cervical dislocation. Hepatic tissue was immediately removed and then frozen at -80 °C until processing. Homogenization was done with TRIsure (Bioline, Memphis, TN, USA).

Table 1. Macronutrients composition of diets.
Macronutrients & CD & HFD & HFD + GPETAFLR & HFD + GPETAFLR

<table>
<thead>
<tr>
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<th>CD</th>
<th>HFD</th>
<th>HFD + GPETAFLR</th>
<th>HFD + GPETAFLR</th>
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<tr>
<td>Fat (%)</td>
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<td>58.8</td>
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<tr>
<td>Carbohydrate (%)</td>
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<td>26.7</td>
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<tr>
<td>Protein (%)</td>
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<td>1.6</td>
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<tr>
<td>GPETAFLR (mg/kg/d)</td>
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<td>0.5</td>
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Values are expressed as the percentage of energy (% Kcal) derived from fat, carbohydrate or protein. Chow diet (CD), high-fat diet rich (HFD), HFD were also treated with the peptide GPETAFLR, which was added to the drinking water and administered at a dose of 0.5 or 1 mg/kg/d.

2.3 Dosage Information

Doses of 0.5 and 1 mg/kg/d for GPETAFLR were established according to previous studies [23-28] and to expected water consumption of the C57BL/6 strain [29]. These selected doses for animals were comparable to human equivalent doses (HED) of 40 and 80 µg/kg, respectively [30].

2.4 RNA Isolation and Real-Time quantitative PCR Analysis

RNA from hepatic tissue was isolated to quantify gene expression by RT-qPCR. Total RNA was extracted by using TRIsure Reagent (Bioline). RNA quality was assessed by A260/A280 ratio in a NanoDrop ND-1000 Spectrophotometer (ThermoFisher Scientific, Madrid, Spain). Briefly, RNA (250 ng) was subjected to reverse transcription (iScript, Bio-Rad). An amount of 40 ng of the resulting cDNA was used as template for RT-qPCR amplifications. The mRNA levels for specific genes were determined in a CFX96 system (Bio-Rad). For each PCR reaction, cDNA template was added to Brilliant SYBR green QPCR Supermix (Bio-Rad) containing the primer pairs for either gene or for hypoxanthine phosphoribosyltransferase (HPRT) as a housekeeping gene. All amplification reactions were performed in triplicate and average threshold cycle (Ct) numbers of the triplicates were used to calculate the relative mRNA expression of candidate genes. The magnitude of change of mRNA expression for candidate genes was calculated by using the standard 2^(-ΔΔCt) method. All data were normalized to endogenous reference (HPRT) gene content and expressed as relative fold-change of control. The sequences of the designed oligonucleotides are shown in Table supplementary 1.

2.5 Histological analysis

Dissected liver sections were fixed overnight with 4% paraformaldehyde, cryoprotected with 15%-30% sucrose and embedded in Tissue-Tek OCT embedding medium (Sakura Finetek Europe, The Netherlands). Cryostat sections (8-10 microns) were rinsed with PBS and stained with a 0.3% solution of Oil Red O for 10 minutes at room temperature. After washing in PBS, sections were counterstained with hematoxylin and eosin for 1 minute and washed with water [31]. The percentage of stained area was determined using Image J software.

2.6 Biochemical analysis

The levels of leptin in supernatants were measured by ELISA, following the indications of the manufacturer (ThermoFisher). The adipokine concentrations were expressed in ng per mL, as calculated from the calibration curves from serial dilution of mice recombinant standards. In addition, the TG content in serum and liver tissue was determined by colorimetric enzyme assays (Bio-Science-Medical). The enzyme activity of liver enzymes, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH), were measured by a commercial kit (Bio-Science-Medical), following the indications. Absorbance measurements were taken on a plate reader.
2.7 Statistical Analysis

All values in the figures and text are expressed as the arithmetic mean ± SD. Experiments were carried out in triplicate. Data were evaluated with GraphPad Prism Version 7.0 software (San Diego, CA, USA). The statistical significance of any difference in each parameter among the groups was evaluated by one-way analysis of variance (ANOVA), using Tukey’s test for multiple comparison analysis. P values of < 0.05 were considered statistically significant.

3. Results

3.1. GPETAFLR decreases body and liver weight gain in HFD-induced obese mice

After 8 weeks of diet, the animals showed a gain in body weight (Figure 2A), and the increase in body weight of the groups fed with HFD was higher than the control group (Figure 2B). The increase in weight during the 8 weeks with HFD and treatment with GPETAFLR at 1 mg/kg/d was significantly lower than those animals fed with HFD but not the peptide. Liver weight was also significantly higher in the obese control (animals fed only with HFD) with respect to those animals that were treated with GPETAFLR in their drinking water (Figure 2C). In line with the results described above, the level of TGs in the blood was significantly higher in the obese control group with respect to those animals that, in addition to HFD, were treated with GPETAFLR in their drinking water (Figure 2D). In this case, as in liver weight, the effect of GPETAFLR was dose-dependent.

Figure 2. Effect of GPETAFLR on the evolution of body weight (a), percentage of body weight gain (b), liver weight (c) and blood triglyceride levels (d) in mice with HFD-induced obesity. CD, chow diet, was used as a control group. Values are presented as means ± SD (n=10) and those marked with different letters are significantly different (P < 0.05).
3.2. GPETAFLR decreases hepatic leptin resistance in HFD-induced obese mice

In obesity, blood levels of leptin are increased due to a lack of sensitivity between leptin and its receptor (OB-R), in a similar way to what happens in insulin resistance. After 8 weeks of HFD, the animals presented higher serum levels of leptin to those animals fed with the standard diet (Figure 3A). The treatment with GPETAFLR significantly decreased those levels. Similarly, OB-R mRNA levels were significantly higher in animals fed with HFD in contrast to those animals that did not receive GPETAFLR in their drinking water (Figure 3B).

Figure 3. Effect of GPETAFLR on leptin resistance. Serum leptin levels (a) and gene expression in leptin receptor hepatic tissue (OB-R) (b) in mice with HFD-induced obesity. CD, chow diet, was used as a control group. Values are presented as means ± SD (n=10) and those marked with different letters are significantly different (P < 0.05).

3.3. GPETAFLR decreases hepatic steatosis in HFD-induced obese mice

Figure 4 shows the results of the histological study carried out with the ORO and H&E staining for the determination of lipid content in hepatocytes. Figure 4A shows representative staining images of histological slices of liver from each experimental group. After 8 weeks of HFD, mice presented higher levels of hepatic lipids to those animals fed with a standard diet (Figure 4B). Mice treated with GPETAFLR significantly decreased the hepatic steatosis induced by HFD.

3.4. GPETAFLR decreases hepatic steatosis in HFD-induced obese mice

After 8 weeks of HFD, levels of TGs in liver tissue were higher with respect to the CD (Figure 5A). However, TG levels were lower in those animals that were fed HFD and received GPETAFLR as treatment. Significantly, GPETAFLR at 1 mg/kg/day restored the values of the CD. In line with these results, the expression of the fatty acid synthase enzyme (FAS) (Figure 5B) and the peroxisome proliferator-activated receptor α (PPARα) were determined (Figure 5C). Animals fed HFD and those that were not treated had FAS mRNA levels significantly superior to those animals fed a CD or treated with GPETAFLR. In addition, the transcriptional activity of PPARα was increased in those groups that received the octapeptide. Finally, mRNA levels of mitochondrial decoupling protein 1 (UCP1) were determined (Figure 5D). The treatment with GPETAFLR in the drinking water significantly increased the hepatic transcriptional activity of UCP1 regarding those groups which did not receive the peptide.
Figure 4. Effect of GPETAFLR on hepatic steatosis. Representative images (10x) of stained histological slices with Oil Red O (ORO) and hematoxylin-eosin (H&E) (a) and quantification of the stained area with ORO from each experimental group (b) in mice with HFD-induced obesity. CD, chow diet, was used as a thin control group. Values are presented as means ± SD (n=10) and those marked with different letters are significantly different (P < 0.05).

3.5. GPETAFLR decreases hepatic inflammation in HFD-induced obese mice

After 8 weeks of HFD, the gene expression of proinflammatory cytokines on hepatic tissue such as tumor necrosis factor α (TNF-α) (Figure 6A), interleukin 1β (IL-1β) (Figure 6B) and interleukin 6 (IL-6) (Figure 6C) were significantly higher in the animals fed exclusively with HFD. In both doses, GPETAFLR significantly decreased the mRNA levels of the pro-inflammatory cytokines. The gene expression of the anti-inflammatory cytokine interleukin 10 (IL-10) significantly increased in those mice that received GPETAFLR in their drinking water compared to the groups that did not receive the peptide (Figure 6D).
Figure 5. Effect of GPETAFLR on the TG levels in the liver tissue (a) and the expression of genes related to lipid and energy metabolism, such as the fatty acid synthase (FAS) (b), peroxisome proliferator-activated receptor α (PPARα) (c) and uncoupling protein 1 (UCP1) (d) in mice with HFD-induced obesity. CD, chow diet, was used as a control group. Values are presented as means ± SD (n=10) and those marked with different letters are significantly different (P < 0.05).

Figure 6. Effect of GPETAFLR on expression of pro-inflammatory and anti-inflammatory cytokine genes in mice with HFD-induced obesity. The mRNA levels of TNF-α (a), IL-1β (b), IL-6 (c) and IL-10 (d) were measured in the liver tissue. CD, chow diet, was used as a control group. Values are presented as means ± SD (n=10) and those marked with different letters are significantly different (P < 0.05).
3.6. GPETAFLR decreases blood markers of liver damage in HFD-induced obese mice

After 8 weeks of HFD, mice presented higher serum activity values for AST (Figure 7A) and ALT (Figure 7B) than those animals that received peptide in their drinking water. GPETAFLR restored the values of transaminases to levels of those animals that were fed a CD. The activity of serum ALP was significantly higher in the obese control (Figure 7C). GPETAFLR at 1 mg/kg/d restored the values of this marker to similar levels to the animals that were fed a CD. On the other hand, values from serum activity of the enzyme LDH were higher in the obese control (Figure 7D). GPETAFLR at 1 mg/kg/d significantly decreased the values of that enzyme compared to the mice fed with HFD without the GPETAFLR treatment.

![Figure 7. Effect of GPETAFLR on serum liver damage markers such as aspartate aminotransferase (AST) (a), alanine aminotransferase (ALT) (b), alkaline phosphatase (ALP) (c) and lactate dehydrogenase (LDH) (d) in mice with HFD-induced obesity. CD, chow diet, was used as control group. Values are presented as means ± SD (n=10) and those marked with different letters are significantly different (P < 0.05).](#)

4. Discussion

The use of bioactive peptides obtained from food is increasingly gaining importance because of its ability to improve human health and prevent chronic diseases, whose incidence is increasing worldwide [4]. Many chronic diseases are characterized by a marked inflammatory process. Therefore, GPETAFLR, a novel anti-inflammatory biopeptide obtained from *Lupinus angustifolius* L., can be a potent molecule for the prevention of these type of diseases. It has demonstrated its anti-inflammatory activity. Specifically, both the peptide and the extracts of hydrolyzed lupin proteins have shown the ability to decrease the expression of pro-inflammatory cytokines and to increase the expression of anti-inflammatory cytokines [11-13].

The liver is the main metabolic organ of the body. NAFLD affects its functionality, endangering the homeostasis metabolic system. This disease is typically associated with obesity [15-18], wherefore a substance which has the effect of reducing body weight and obesity rates will greatly alleviate the...
symptoms and the development of NAFLD. The octapeptide GPETAFLR prevents the weight gain resulting from a diet rich in fat in contrast to other substances with hepatoprotective properties that do not have the capacity to prevent weight gain such as isoliquiritigenin [32]. It can be said that GPETAFLR interferes by preventing the progression of the main cause of NAFLD, obesity.

On the other hand, the data obtained corroborate that HFD-induced obese mice, presented a NAFLD pathophysiology similar to humans. The physiopathology of this disease is characterized by increased body weight and liver, high levels of TGs in the blood, fat accumulation in adipose tissue, hepatic inflammation and elevated markers of liver damage [33]. In our project, the obese control group showed a greater hepatic weight derived from a greater accumulation of hepatic fat. The accumulation may be due to high levels of TGs in the blood and the increase in FAS gene expression. In addition, this group also presented a pro-inflammatory state, provoked by high levels of expression of pro-inflammatory cytokines as IL-6, IL-1β, and TNF-α, since the Kupffer cells increase their expression in response to hepatic fat accumulation [34]. Finally, it presented elevated markers of liver damage, since the activity of transaminases, LDH, and ALP in serum were significantly higher. These values were also obtained in other similar studies on HFD-induced obese animals [19,32,35-37].

All these parameters of the pathology were palliated with the treatment with GPETAFLR, owing to the fact that liver weight and liver fat accumulation were lower in the animals treated with the octapeptide. The factors that can influence the minor accumulation of hepatic fat in these individuals are a decreased gene expression of FAS and an increased expression of enzymes that increase energy metabolism, such as UCP1 and PPARα. In addition, there is less circulation of TGs in the blood and the availability for its accumulation in different tissues, including the liver. GPETAFLR could mediate the accumulation of hepatic fat by influencing the signaling pathways involved in the regulation of gene expression or the activity of lipid metabolism proteins, such as the kinase pathway activated by AMP (AMPK).

In previous studies, GPETAFLR has been demonstrated to present anti-inflammatory activity that is also present on the hepatocytes. The octapeptide decreased pro-inflammatory cytokine levels and increased levels of the anti-inflammatory cytokine IL-10. IL-10 did not show a decrease in expression in the obese control compared to the standard control, but it greatly increased its expression in the groups treated with GPETAFLR. These data are related to previous studies on other cell lines and primary cultures [11-13]. Pro-inflammatory cytokines present several actions related to metabolism, for example, TNF-α decreases the degradation of fats and increases their accumulation [38] and IL-6 inhibits lipolysis and increases fat deposition [35]. For this reason, in addition to contributing to tissue damage from chronic inflammation, they contribute to the development of NAFLD by boosting fat accumulation in the liver. Chronic inflammation is primarily mediated by the nuclear factor κB (NF-κB) pathway, which stimulates the production of cytokines such as TNF-α [39]. For this reason, GPETAFLR may decrease inflammation by influencing the NF-κB pathway.

Another parameter used as a marker of NAFLD pathogenesis in this study was the measurement of blood markers of liver damage, specifically transaminases, ALP, and LDH. The activity of these enzymes in serum was lower in both GPETAFLR treatments compared to the obese control group. This effect is due, probably, to the fact that peptide decreases liver damage by reducing the accumulation of fat and inflammation (influenced by the lower accumulation of fat) and thus all the tissue damage associated with a chronic inflammatory process.

On the other hand, leptin is an adipokine produced by adipose tissue, and the levels of this hormone in the blood are indicative of the body’s fat reserves, because it is produced proportionally to the amount of adipose tissue. Therefore, a higher amount of leptin results in a higher amount of fat reserve [40]. Leptin resistance is a characteristic of most obesity-associated diseases. This syndrome is characterized by a rise in serum leptin due to the increase of adipose tissue, but there is a lack of it
signaling because the tissues with receptors show resistance. Proof that leptin resistance is occurring
is a high value of leptin in serum and gene expression of its receptor in the tissues that express it,
such as occurs in animals of the obese control group. The liver is one of the organs that presents
receptors for leptin and signaling by this molecule produces an increase in catabolic metabolism,
increases the degradation of fatty acids, decreases the synthesis of fatty acids and effects glucose
metabolism, specifically hypoglycemic [40,41]. Leptin resistance in NAFLD could mediate an
increase in the accumulation of hepatic fat in its direct form by a lack of signaling in the liver. In
addition, leptin resistance may enhance the progression of NAFLD for its role in intake and satiety.
Leptin can cross the blood-brain barrier, once inside the central nervous system (CNS), activate the
production of anorexigenic peptides and inhibit the production of orexigenic peptides. This role
translates into an inhibition of appetite and therefore into a decrease in caloric intake [40]. When there
is resistance to the signaling, the effect of leptin (which should be greatly enhanced in obese
individuals due to the high amount of this adipokine) is lower or does not occur in proportion to the
amount of leptin, so there is no decrease in appetite or intake. Despite the high body weight, obese
individuals do not have functional circuits of adjustment of energy expenditure and intake.
Supplementation with GPETAFLR produced a decrease in obesity-associated leptin resistance. These
effects may be due to the fact that GPETAFLR decreases body weight or GPETAFLR decreases
resistance to the leptin and therefore there is a decrease in body weight. It may also be considered
that both effects are produced. Other plant bioactive substances have been shown to have effects by
decreasing resistance to leptin without significantly affecting body weight [32].

5. Conclusions

To conclude, GPETAFLR decreased body weight gain, liver weight and fat accumulation in liver, as
well as TGs levels in the blood and leptin resistance associated with obesity. GPETAFLR showed
anti-inflammatory properties at the hepatic level, decreasing the mRNA levels of pro-inflammatory
cytokines such as TNF-α, IL-6 and IL-10, and increasing the expression of anti-inflammatory cytokine
IL-10 in liver tissue. The octapeptide also reduced blood markers related to liver damage such as AST,
ALT, ALP, and LDH. Taken together, our findings suggest that GPETAFLR is a potential treatment,
as well as an important preventive therapy for NAFLD. Thus, the dietary supplementation with
*Lupinus angustifolius* L. would also contribute to a reduction of this pathology.

**Supplementary Materials:** The following are available online at www.mdpi.com/xxx/s1, Table S1: Sequence and GenBank accession number of oligonucleotides used in RT-qPCR.

**Author Contributions:** Conceptualization, M.C.M.L. and S.M.P.; methodology, L.M.V. and C.C.; formal
analysis, R.T.; investigation, A.L.C, E.G.C., and R.T.; writing—original draft preparation, S.M.P.; supervision,
M.C.M.L. and S.M.P.; funding acquisition, J.P. and F.M.

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