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RESEARCH ARTICLE

Putative role of N-Arachidonoyl glycine (NAGly) Acute hyperphagia in BALB/c mice

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ABSTRACT

Introduction: Obesity is a pandemic problem associated to development of chronic degenerative diseases. N-arachidonylglycine (NAGly) is speculated to participate in pain regulation, immunity and insulin secretion; some N-acyl amino acids induce hyperphagia; in light of this, NAGly metabolic functions related to energy homeostasis has not been clarified yet. We aim to elucidate the effect of NAGly administration on weight gain, food intake, diet preferences and inflammatory profile changes in a murine model. **Methods:** 8-week-old BALB/c mice (n = 78) were allocated into 4 groups for either sex: control, vector, NAGly-LD (1nM) and NAGly-HD (10nM). NAGly was subcutaneous administered 5 consecutive days for 3 weeks. Mice were exposed to standard diet (SD), high-fat diet (HFD) and high-sugar diet (HSD) simultaneously; weight gain, food intake and diet preferences were evaluated for 21-days. Finally, cytokines were analyzed by enzyme-linked immunosorbent assay (ELISA). **Results:** final weight gain was found higher in those following NAGly [LD: 21.6 ± 1.1 g and HD: 21.4 ± 2.1 g, (p = 0.001)]. NAGly groups consumed more food in the first days; NAGly groups consumed more HFD [5.06 ± 1.60; (p = 0.001)] and HSD [1.10 ± 0.69; (p = 0.001)]. **Conclusion:** Our data propose that subcutaneous NAGly promotes acute hyperphagia events. We propose that NAGly might play a role related to the hunger-satiety circuit.

Keywords: NAGly; Diet preference; Weight gain; High-fat diet

INTRODUCTION

Endocannabinoids are ubiquitous signaling lipidic molecules that contain long chains of polyunsaturated fatty acids, amides, esters, and ethers. There are three types of cannabinoids: botanical (marijuana and hashish), endogenous [anandamide, 2-arachidonylglycerol (2-AG), and palmitoylethanolamide (PEA)], and synthetic cannabinoids (nabilone and dronabinol). The endocannabinoid system modulates selective pathways in the brain (De Vries et al., 2001; Fattore et al., 1999; Fernández-Ruiz et al., 2002; Guindon & Beaulieu, 2010), immune and cardiovascular system (Hillard, 2000; Parolaro, 1999) and also, has been hypothesized to be implicated in energy metabolism as a "fat sensor" through a potential GPR119 activation (Hansen et al., 2012).

N-acyl amino acids (NAAAs) possess a similar structure to endocannabinoids. NAAAs biological functions are mediated by receptors and a transient receptor potential cation (TRPV); these receptors could modulate appetite and participate in other functions such as anxiety-like behavior or transmitter release suppression, which possibly implied during the food intake process (Wu et al., 2017).

NAGly is a lipoamino acid formed by enzymatic conjugation of arachidonic acid and glycine or is either synthesized as an oxidative metabolite of N-arachidonoyl etanolamide (Anandamide: AEA) (Bradshaw et al., 2009; D. McHugh et al., 2010). NAGly was first synthesized as an anandamide analog and years later was detected in its natural form in the brain, spinal cord, and gut (Burstein et al., 2002; Sheskin et al., 1997).

NAGly it is proposed to bind to GPR18 and GPR55 at the central nervous system, but this remains inconclusive. Nowadays, different functions of NAGly are reported,

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concerning pain suppression of nociceptive afferents signals (Huang et al., 2001; Lu et al., 2013), intraocular pressure (IOP) regulation (Miller et al., 2016), analgesia (Bradshaw et al., 2009; Burstein, 2014), macrophages apoptosis (Takenouchi et al., 2012), anti-inflammatory properties like cytokines signaling regulation (Jeong et al., 2010; D. McHugh et al., 2014), intervention in microglial cell migration and plasticity (D. McHugh et al., 2014; M. P. McHugh & Cosgrave, 2010) and as a novel insulin secretagogue (Ikeda et al., 2005). A possible modulation role in low-grade chronic inflammation linked to obesity has not been clarified yet (D. McHugh et al., 2014).

Food composition and the content of nutrients in diet intake are factors involved in the process of biosynthesis of these molecules, interacting and changing NAAAs concentration levels by "natural combinatory chemistry" (Di Marzo et al., 2007). Rich-lipids foods, especially in polyunsaturated fatty acids (PUFAs) like Omega-3, Omega-6, and Omega-9 having implications for health and disease. Based on this fact, dietary changes may modulate the synthesis of these PUFAs derived-endocannabinoids, being decreased or increased depending on the lipids profile contained in food (Meijerink et al., 2015).

Research has been carried out on different cell lines such as 3T3-L1 adipocytes and breast and prostate cancer cell lines, finding changes in the concentrations of amides derived from Omega-3 fatty acids (Balvers et al., 2010) and formation of N-docosahexaenoylethanolamine (DHEA) or N-eicosapentaenoylethanolamine (EPEA) (Brown et al., 2011). These events have been corroborated as well in animal models presenting variations in endocannabinoid-like molecules concentrations as a result of the content and type of lipids in diet, for instance, the class of oil consumed may contribute to altering AEA concentrations, as stated by Artmann et al., (2008), besides this, Berge et al. evaluated these events by adding to the diet, high-intakes of fish oil or supplementing specific oils such as krill oil for 24 weeks, observing a decrease in levels of AEA, OEA, and PEA (Berge et al., 2013). This influence may depend on the amount ingested and exposure time of this kind of food; concentration changes are found in multiple tissues such as the brain, jejunum, plasma, adipose tissue, liver, muscle, and others (Meijerink et al., 2015).

On the other hand, obesity has become a global and worrying health problem classified as a pandemic according to the World Health Organization (WHO), several factors are considered to develop this condition mainly including overconsumption of hypercaloric food coupled to deficient vitamins and micronutrients intake, physical inactivity, and also genetic background. Obesity is a risk factor for multiple metabolic alterations and chronic-degenerative disease development; specific food and dietary intake play a key role in preventing or worsening these diseases (WHO, 2020).

Potential molecular candidates capable of modulating food choices might be helpful to revert or control overweight in humans. Of note, NAGly could be influenced by diet factors having participation in metabolic functions (Becker et al., 2015). In this context, the objective of this study was to identify if NAGly exerts a possible metabolic role on weight gain, food intake, diet preference, and modulatory effect on the inflammatory profile using a murine model. We conceive that these data might provide experimental evidence for a better understanding of NAGly and the possibility of an intervention in the hunger-satiety circuit.

MATERIALS & METHODS

Animals and ethics considerations

All the experiments were performed using 8-week-old BALB/c mice (n=78) purchased and maintained in the animal facility of the Universidad Autónoma de Aguascalientes. Mice were housed in plexiglass style cages, kept in a pathogen-free environment, at 22-24 ºC temperature, 12-hour light / 12-hour dark cycle, humidity; access to food and water was *ad libitum*. Animals were handled following the guidelines established by the Official Mexican Standard NOM-062-ZOO-1999: "Technical specifications for the production, care and use of laboratory animals". Animals were randomized in four groups of either sex in separated cages respectively as follow: control group (n=10), vector group (≥98% purity ethanol and saline solution; n=8), NAGly low-dose group (NAGly-LD: 1 nM; n=10), and NAGly high-dose group (NAGly-HD: 10 nM; n=10) see Figs. 1 and 2 for details.

N-Arachidonoyl glycine (NAGly) dosage and administration

The dose-dependent schedule of NAGly (Cayman Chemical™, item: #90051) was subcutaneously administered by single injection for five days a week (for three weeks). NAGly was dissolved in saline solution and ≥98% purity ethanol based on a 1:1 ratio, NAGly administration was given at the same time frame every day (9:00-10:00 am); the volume injection was 90 µL/mouse. Animals were allocated in three experimental groups: 1 nM of NAGly (3.25 µL/g of body weight), 10 nM of NAGly (3.25 µL/g of body weight), and vector group [NAGly vehicle: (90% saline solution / 10% ethanol)]. The dose was established based on McHugh et al. previous findings (D. McHugh et al., 2012); it has been reported NAGly effects from a minimum dose of 10 pg.

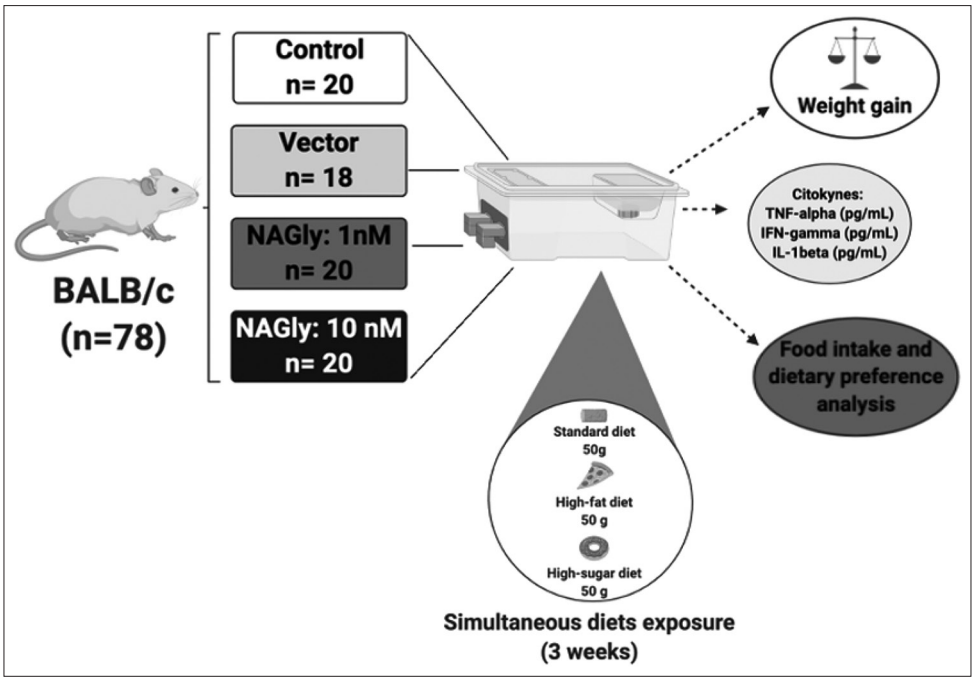

Fig 1. Material and methods.

Fig 2. a) Daily weight gain; b) preparing the reagent dose for administration; c) Subcutaneous administration of NAGly-LD, NAGly-HD or vector; d) Evaluation of food preference; e) Diets formula given: Standard diet, High-fat diet and High-sugar diet. LD: Low-dose (1 nM), HD: high-dose (10 nM). (n=78).

Experimental design: diet intake, preference, and weight gain analysis

Diets were exposed simultaneously to evaluate intake and preference for standard diet (SD), high-fat diet (HFD), and high-sugar diet (HSD) for three weeks; to identify diets, these were marked according to the type of diet with food colorants. To preserve palatable food properties, 50 g of each diet were set and removed every day from the home-cages. Mice were separated according to sex and group (five mice per cage). Diet intake and preference were evaluated daily at the same schedule (8:00-9:00) by gathering and weighing food remain, subsequently the quantity obtained in the measurement were subtracted from the total of each diet (50 g/per diet), food residue was analyzed with a 0.001 g sensitivity scale (GRS 500, Torrey™); cages bedding material was changed every day. Data were registered as grams per day consumption. Weight gain was evaluated every week.

Diets: food and nutrients composition

Mice were fed with four diets formula: 1) Standard diet (SD): 23% protein, 5% lipids, and 72% carbohydrates; ingredients were: ground cereals, mixed oilseed paste, fish flour, cereal subproducts, alfalfa, and cane molasses (Agribrands Purina Mexico, Nutri-cubos™), 2) for high-fat diet (HFD): 11% protein, 53% lipids, 36% carbohydrates, and 354.46 mg of sodium; ingredients were: bacon, fried potato chips, standard diet pellets and pork pate based on a 1:3:1:2 ratio, respectively, 3) for high-sugar diet (HSD) nutrients density were: 8% protein, 2% lipids, 90% carbohydrates (63.6% complex carbohydrates / 36.4% refined carbohydrates),

and 153.4 mg of sodium; ingredients were: biscuits, liquid chocolate, marshmallow, sugar-rich cereal and standard diet based on a 1:2:2:2:1 ratio, respectively.

Cytokines determination: TNF-α, IFN-γ, and IL-1β serum quantification

Mice were sacrificed by cervical dislocation at the end of the third week. Serum fraction was obtained immediately through cardiac puncture collection and stored at -20ºC in Eppendorf™ tubes. TNF-α, IFN-γ, and IL-1β serum levels were determined by ELISA (Enzyme-Linked Immunosorbent Assay), using 100 µL serum/mouse according to manufacturer's instructions. Selective antibodies against Mouse TNF-alpha uncoated ELISA (Invitrogen™, #88-7324), Mouse IFN-gamma uncoated ELISA (Invitrogen™, #88-7314), and Mouse IL-1β uncoated ELISA (Invitrogen™, #88-7013) were used.

Statistical analysis

Distribution normality was analyzed with Kolmogorov-Smirnov Test; Two-way ANOVA Test, and One-way ANOVA test along with Bonferroni post hoc test were used to compare differences between groups, for two groups comparison Student-T test was used. Data are shown in mean and standard deviation (±SD) or percentages; analysis was done using the software SPSS version 20. $p<0.05$ were considered statistically significant; *$p<0.05$, **$p<0.01$, and *** $p<0.001$.

RESULTS

Dose-selective effect of NAGly administration on weight gain

For three weeks weight gains were evaluated in 78 BALB/c mice (n=38; female / n=40; male). Initial weight of each group was no different from each other ($p= 0.91$), at the end of the experiment, significant body weight changes were found in groups administered with NAGly compared to vector and control group ($p= 0.0001$; Table 1 & Fig. 3a). Notably, we identified a sex-dependent effect during NAGly administration, showing that male mice exposed to NAGly-HD presented differences on ponderal gains at the end of the third week compared to the control group (20.4±2.4 vs 16.9±2.5; $p= 0.01$, Table 1). NAGly does not show differences between groups in female mice.

NAGly short-term effect on food intake

Food intake was registered and later analyzed; the average intake per mouse was 4.44 g/day, treatment group intakes data is available in Table 2. NAGly-HD group had higher daily intakes than the vector group. Furthermore, it seems that NAGly may cause an acute hyperphagic effect during the first days of administration compared to the control and vector group and maintain this pattern through the first week in NAGly-HD group (Fig. 3b); during the second and third week, all groups had similar food intakes. As expected, male mice ate more of each diet compared to female group.

Food preference evaluation following NAGly administration

Food preference patterns were determined by the total amount of each diet consumed throughout the experiment. An overview in food choices patterns showed an increased preference for high-fat diet, followed by high-sugar diet and standard diet. Male mice with NAGly-HD presented an increased preference for high-fat diet compared to the rest of groups ($p= 0.001$; Table 2). On the other hand, regarding high-sugar diet we had contrasted results, while control and vector groups showed a stable intake pattern, NAGly-HD had a lower intake of this diet ($p= 0.0001$; Table 2). However, NAGly-LD was the opposite, registering the highest consumption during the experiment ($p= 0.0001$; Table 2).

NAGly effect on food choice patterns throughout time

A dose dependent NAGly effect promoted an acute increase on food intake during the first week (Fig. 4a, b & c). All groups presented a predilection for high-fat diet during the three weeks, beside this, in the second week we registered changes in diet preference; standard diet intake was considerably lower in all groups compared to control mice (Fig. 4a); additionally, NAGly-HD group ate less high-sugar diet as opposed to the other groups and previous week (Fig. 4c). Finally, there was no differences noticed during the third week.

NAGly administration does not show changes in immune profile

Finally, TNF-α, IFN-γ and IL-1β plasma levels were under lower detection limit in the four groups, there was no Th1 reaction produced by high amounts of fat and sugar in diet, therefore there were no statistical differences.

DISCUSSION

We aimed to identify the potential effect of NAGly on weight gain, food intake, diet preferences and inflammatory profile changes. Our data confirm that after three weeks of NAGly administration at 1nM or 10 nM dosage, a difference was noticed in weight gain, being higher than control and vector group. We propose that it might be due to an acute hyperphagic effect presented during week one, where it reached a peak in food consumption during the first days in those mice with NAGly administered. Previous studies where NAAAs are tested to study their effect on food intake showed that N-Oleoylglycine (OLGly)

Table 1: General weight gains according to treatment group

Group Time	Control (n=20)	Vector (n=18)	NAGly-LD (n=20)	NAGly-HD (n=20)	F	p
First week weight (g)	16.7±3.4	16.4±2.1	17.1±2.4	17±3.5	0.17	0.91
Second week weight (g)	18.6±2.4	19.3±1.8	20.4±1.3	20.5±2.2	3.79	0.01†
Third week weight (g)	20.1±1.2	19.8±1.1	21.6±1.1	21.4±2.1	6.57	0.001‡
Weight gain according to sex and treatment group						
Female Time	**Control (n=10)**	**Vector (n=9)**	**NAGly-LD (n=10)**	**NAGly-HD (n=10)**	**F**	**p**
First week weight (g)	18.5±1.7	17.9±1.7	18.7±0.9	18.2±1.3	0.52	0.66
Second week weight (g)	20.0±1.1	19.5±1.4	21.0±1.0	20.5±2.1	1.81	0.16
Third week weight (g)	20.8±0.6	20.2±1.0	21.8±1.2	20.6±2.4	2.08	0.12
Male Time	**Control (n=10)**	**Vector (n=9)**	**NAGly-LD (n=10)**	**NAGly-HD (n=10)**	**F**	**p**
First week weight (g)	14.6±3.8	15.1±1.6	15.5±2.4	15.8±4.6	0.19	0.89
Second week weight (g)	16.9±2.5	19.2±2.2	19.7±1.3	20.4±2.4	4.33	0.01*
Third week weight (g)	19.5±1.3	19.5±1.2	21.3±0.9	22.1±1.6	9.6	0.0001‡

Data are displayed as: mean and SD; One-way ANOVA test, $p<0.05$ was considered significant; (n=78).
†: Control group vs NAGly groups.
‡: Control & Vector group different from NAGly groups.
*: Control group vs NAGly-HD.

Table 2: Diets intakes and preference according to sex & treatment group

Food intake (g/day/mouse)	Control (n=20)	Vector (n=18)	NAGly-LD (n=20)	NAGly-HD (n=20)	F	p
Male	5.11±1.30	4.30±1.27	5.48±1.54	5.87±1.61	4.56	0.005a
Female	3.76±1.25	4.07±2.14	3.45±1.38	3.46±1.38	0.73	0.53
Group	4.44±1.13	4.20±1.34	4.46±1.63	4.67±1.70	0.48	0.69
Diet type	**Control (n=20)**	**Vector (n=18)**	**NAGly-LD (n=20)**	**NAGly-HD (n=20)**	**F**	**p**
Standard diet (g/day/mouse)						
Male	0.07±0.10	0.05±0.16	0.06±0.14	0.07±0.13	0.18	0.90
Female	0.01±0.06	0.01±0.01	0.01±0.03	0.10±0.02	0.02	0.99
Group	0.04±0.07	0.03±0.09	0.04±0.09	0.04±0.09	0.10	0.95
High-fat diet (g/day/mouse)						
Male	3.95±0.92	3.64±1.01	3.77±1.12	5.06±1.60	6.18	0.001b
Female	3.01±1.00	3.12±2.12	2.86±1.31	3.02±1.25	0.11	0.95
Group	3.48±0.89	3.41±1.26	3.32±1.22	4.04±1.57	1.81	0.15
High-sugar diet (g/day/mouse)						
Male	1.08±0.54	0.61±0.54	1.64±0.72	0.73±0.44	13.65	0.005c
Female	0.73±0.64	0.93±0.62	0.57±0.45	0.42±0.42	3.36	0.02d
Group	0.90±0.38	0.75±0.39	1.10±0.69	0.57±0.45	6.98	0.0001*,e

Data are displayed as: mean and SD, ANOVA test was used; a: Vector different from NAGly groups; b: NAGly-HD different from rest; c: NAGly-LD different from rest; d: NAGly-HD different from vector; e: NAGly-LD different from vector & NAGly-HD. *: Control & Vector vs NAGly-HD group; $p<0.05$ was considered significant; (n=78.)

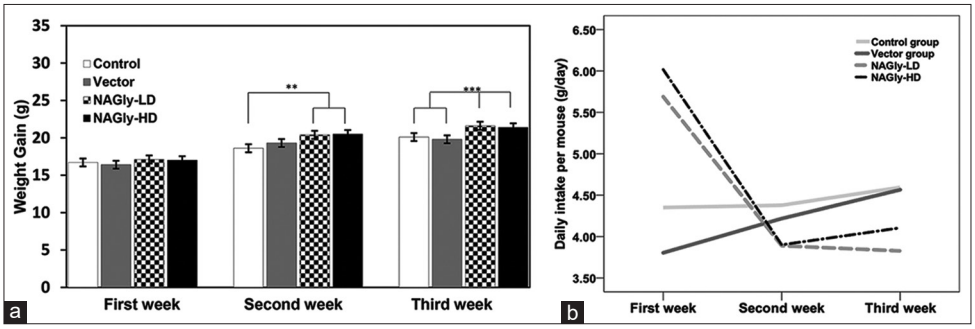

Fig 3. a) Weight gain across time; b) Daily food intake comparison between experimental groups across time. NAGly-LD (Low dose:1 nm); NAGly-HD (High dose: 10 nM). $p<0.05$ was considered significant. *: $p<0.05$; ***: $p \leq 0.001$. (n=78).

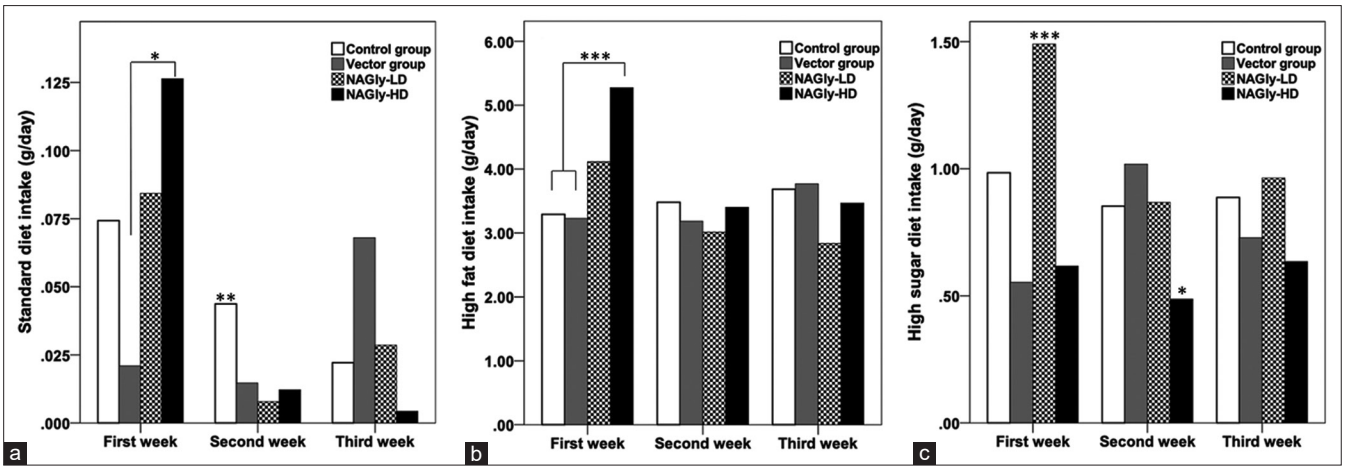

Fig 4. a) Standard diet intake comparison between experimental groups across time; b) High-fat diet intake comparison between experimental groups across time; c) High-sugar diet intake comparison between experimental groups across time. NAGly-LD (Low dose:1 nm); NAGly-HD (High dose: 10 nM). $p<0.05$ was considered significant. *: $p<0.05$; **: $p<0.01$ ***: $p \leq 0.001$. (n=78).

induced hyperphagia by increasing c-Fos protein expression in Agouti-Related protein (AgRP) neuron, nonetheless, such results were not found in NAGly (Wu et al., 2017), unlike our results, it seems NAGly may participate in these hyperphagic episodes; neuropeptide Y (NPY), AgRP and orexigenic molecules actions and expression levels should be considered for further studies. This might allow to determine if hypothalamic neuronal activity during NAGly administration may play a role as intermediary in CB1 and GPR's activation on feeding behavior and metabolic regulation.

Sexual dimorphism on food intake and weight gain has been identified to present differences (Morselli et al., 2014), our results agreed with this, by showing a sex-dependent effect where a considerable substantial food intake and weight gain were observed in male mice rather than female group. Increased food intake in males is a common behavior in mammals feeding determined by genetic and hormonal factors that will conditionate sex-dependent energy intake and caloric expenditure (Shi et al., 2009; Voigt et al., 2014). It has been studied that males might change feeding patterns and females their energy expenditure, these events may be the result of changes in NPY and proopiomelanocortin (POMC) neurons profile expression (Ikeda et al., 2005; Palmer & Clegg, 2015; Shi et al., 2009).

On the other hand, the main food preference intake was for HFD in all groups. Although, HFD intake was decreasing at the end of the third week in NAGly administered mice; a lower fat intake was observed in males. NAGly has been identified as a novel insulin secretagogue; as is known, insulin has a role as an anorexigenic hormone, at the same time, stimulates leptin synthesis and secretion, these two hormones work synergically to stimulate satiety and extending time for the next food intake; NAGly insulinotropic action has been hypothesized through Ca^{2+} stimulating VR1 (vanilloid receptor 1) that will be modulated insulin secretion in beta cells from pancreas (Akiba et al., 2004; Goldbach-Mansky, 2012; Kleiner et al., 2013; Ramírez-Orozco et al., 2019). In addition, leptin circulant levels and AgRP expression in NAGly administered groups could elucidate better these events. Furthermore, dietary factors could modify NAGly synthesis and expression (Inam et al., 2016), given the results it is necessary to study NAGly thoroughly; analyze probable mediated expression through GPR18, GPR119 or GPR55 to evaluate influence and association over hunger and satiety circuitry that could solve part of the possible metabolic effect of NAGly.

Contrasted observations were the preference for high-sugar diet in NAGly exposed groups, i.e., NAGly-LD consumed more than any group, nevertheless, NAGly-HD was the opposite to this result. Firstly, high intake of sugar might affect serotonin metabolism; for instance, Inam et al. demonstrated a down-regulation of serotonin pathway in rats of both genders which were fed freely for five weeks on a sugar-rich diet (Inam et al., 2016). Serotonin has a major role in mood state, perception, reward system, memory, rage, appetite control and other important functions; a diminished function of serotonin has been associated with hyperphagia in both sexes and other collateral effects like anxiety and depression, being more severe these alterations in females (Zhang et al., 2018). In contrast, our results showed different food preference pattern of HSD in males vs females, which it is preserved during the three weeks. Also, we observed an increase in food intake in NAGly treated groups in the first week. Sweet taste flavor is potentially powerful to induce a strong flavor in humans, rodents and flies, probably explained by a greater activity in dopamine release through brain reward

circuitry and the presence of macronutrients infusions in gut causing an enduring flavor-choice (Zhang et al., 2018). These results could be conceptualized perhaps by NAGly role on intracellular GPR55-dependent calcium increase, promoting serotonin receptors excitability along to the effect of sugary food in this system, which might enhance this feeding pattern by eating more sugar, remembering that a poor serotonin activity will lead to an unbalanced appetite regulation (Console-Bram et al., 2017).

Cytokines (TNF-α, IFN-γ and IL-1β) are known to be proinflammatory markers under selective pathological scenarios. We tested whether chronic excesses of fat and sugar intake modulate inflammatory profiles in mice. We found not significant TNF-α, IFN-γ and IL-1β plasma cytokines identification in our experimental models. According to Kleiner et al. previous results, it will be very low or non-detectable cytokine levels in healthy subjects (Kleiner et al., 2013); as it is known, food has different effects (pro or anti-inflammatory) in the organism, these will depend and vary on the food type, such as: carbohydrates and fat source (e.g., simple vs complex or unsaturated vs saturated), other factors that have been demonstrated that could modulate inflammation response is glycemic index, calories intake or eating patterns and habits (Inam et al., 2016). Our results could be explained by the fact that mice were young and healthy, and a short time-dependent diet exposure to promote metabolic alterations. It will be ideal a diet-induced obesity model to test and clarify possible NAGly role.

CONCLUSIONS

To summarize, our results have shown that NAGly actively promotes acute hyperphagia at low and higher doses administration and forward increases weight gain, although this effect disappears with time. On the other hand, as time goes on, NAGly increases fat and sugar intake, probably modulated by hunger mechanisms or other orexins regulation processes; no acute changes were observed in the pro-inflammatory cytokine production profile due to an excess in the intake of fats and sugars, however, it should be considered in a model of chronic exposure to this type of food or with the presence of pathologies that may condition this response to study the effect of NAGly on this. NAAAs participation in biological processes is still unclear; more research is needed, the study of lipids profile contained in food and their function in the synthesis modulation of NAGly in the body. Physiological concentrations of NAGly and the type of diet consumed should be evaluated in detail since these conditions may play a relevant role in the activity of this molecule, causing variation in NAGly functions. These findings lead to future studies related to food composition and NAGly possible activity in energy homeostasis metabolism that contribute to a better understanding of NAAAs.

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AUTHOR CONTRIBUTIONS

Álvarez-Miramontes SA and Martínez-Miranda CA: Mice maintenance and NAGly administration; Masuoka D and Camacho-Morales A: partial manuscript drafting and statistical analysis; Pedroza-García KA: partial manuscript drafting and experimental procedures and Ramírez-Orozco RE: Project coordinator, main manuscript draft, diet formula designer and graphical art designer.

REFERENCES

Akiba, Y., S. Kato, K. I. Katsube, M. Nakamura, K. Takeuchi, H. Ishii and T. Hibi. 2004. Transient receptor potential vanilloid subfamily 1 expressed in pancreatic islet β cells modulates insulin secretion in rats. Biochem. Biophys. Res. Commun. 321: 219-225.

Artmann, A., G. Petersen, L. I. Hellgren, J. Boberg, C. Skonberg, C. Nellemann, S. H. Hansen and H. S. Hansen. 2008. Influence of dietary fatty acids on endocannabinoid and N-acylethanolamine levels in rat brain, liver and small intestine. Biochim. Biophys. Acta Mol. Cell Biol. Lipids. 1781: 200-212.

Balvers, M. G. J., K. C. M. Verhoeckx, P. Plastina, H. M. Wortelboer, J. Meijerink and R. F. Witkamp. 2010. Docosahexaenoic acid and eicosapentaenoic acid are converted by 3T3-L1 adipocytes to N-acyl ethanolamines with anti-inflammatory properties. Biochim. Biophys. Acta. Mol. Cell Biol. Lipids. 1801: 1107-1114.

Becker, A. M., D. J. Callahan, J. M. Richner, J. Choi, J. F. DiPersio, M. S. Diamond and D. Bhattacharya. 2015. GPR18 controls reconstitution of mouse small intestine intraepithelial lymphocytes following bone marrow transplantation. PLoS One. 10: e0133854.

Berge, K., F. Piscitelli, N. Hoem, C. Silvestri, I. Meyer, S. Banni and V. di Marzo. 2013. Chronic treatment with krill powder reduces plasma triglyceride and anandamide levels in mildly obese men. Lipids Health Dis. 12: 78.

Bradshaw, H. B., N. Rimmerman, S. S. J. Hu, S. Burstein and J. M. Walker. 2009. Chapter 8 novel endogenous N-acyl glycines. Identification and characterization. Vitam Horm. 81: 191-205.

Brown, I., K. W. J. Wahle, M. G. Cascio, R. Smoum-Jaouni, R. Mechoulam, R. G. Pertwee and S. D. Heys. 2011. Omega-3 N-acylethanolamines are endogenously synthesised from omega-3 fatty acids in different human prostate and breast

cancer cell lines. Prostaglandins Leukot. Essent. Fatty Acids. 85: 305-310.

Burstein, S. H. 2014. The cannabinoid acids, analogs and endogenous counterparts. Bioorg. Med. Chem. 22: 2830-2843.

Burstein, S. H., S. M. Huang, T. J. Petros, R. G. Rossetti, J. M. Walker and R. B. Zurier. 2002. Regulation of anandamide tissue levels by N-arachidonylglycine. Biochem. Pharmacol. 64: 1147-1150.

Console-Bram, L., S. M. Ciuciu, P. Zhao, R. E. Zipkin, E. Brailoiu and M. E. Abood. 2017. N-arachidonoyl glycine, another endogenous agonist of GPR55. Biochem. Biophys. Res. Commun. 490: 1389-1393.

De Vries, T. J., Y. Shaham, J. R. Homberg, H. Crombag, K. Schuurman, J. Dieben, L. J. M. Vanderschuren and A. N. M. Schoffelmeer. 2001. A cannabinoid mechanism in relapse to cocaine seeking. Nat. Med. 7: 1151-1154.

Di Marzo, V., T. Bisogno and L. de Petrocellis. 2007. Endocannabinoids and related compounds: Walking back and forth between plant natural products and animal physiology. Chem Biol. 14: 741-756.

Fattore, L., M. C. Martellotta, G. Cossu, M. S. Mascia and W. Fratta. 1999. CB1 cannabinoid receptor agonist WIN 55, 212-2 decreases intravenous cocaine self-administration in rats. Behav. Brain Res. 104: 141-146.

Fernández-Ruiz, J., I. Lastres-Becker, A. Cabranes, S. González and J. A. Ramos. 2002. Endocannabinoids and basal ganglia functionality. Prostaglandins Leukot. Essent. Fatty Acids. 66: 257-267.

Goldbach-Mansky, R. 2012. Immunology in clinic review series; focus on autoinflammatory diseases: Update on monogenic autoinflammatory diseases: The role of interleukin (IL)-1 and an emerging role for cytokines beyond IL-1. Clin. Exp. Immunol. 167: 391-404.

Guindon, J. and P. Beaulieu. 2010. The role of the endogenous cannabinoid system in peripheral analgesia. Curr. Mol. Pharmacol. 2: 134-139.

Hansen, H. S., M. M. Rosenkilde, J. J. Holst and T. W. Schwartz. 2012. GPR119 as a fat sensor. Trends Pharmacol. Sci. 33: 374-381.

Hillard, C. J. 2000. Endocannabinoids and vascular function. J Pharmacol. Exp. Ther. 294: 27-32.

Huang, S. M., T. Bisogno, T. J. Petros, S. Y. Chang, P. A. Zavitsanos, R. E. Zipkin, R. Sivakumar, A. Coop, D. Y. Maeda, L. de Petrocellis, S. Burstein, V. Di Marzo and J. M. Walker. 2001. Identification of a new class of molecules, the arachidonyl amino acids, and characterization of one member that inhibits pain. J. Biol. Chem. 276: 42639-42644.

Ikeda, Y., H. Iguchi, M. Nakata, R. X. Ioka, T. Tanaka, S. Iwasaki, K. Magoori, S. Takayasu, T. T. Yamamoto, T. Kodama, T. Yada, T. Sakurai, M. Yanagisawa and J. Sakai. 2005. Identification of N-arachidonylglycine, U18666A, and 4-androstene-3, 17-dione as novel insulin secretagogues. Biochem. Biophys. Res. Commun. 333: 778-786.

Inam, Q. A., H. Ikram, E. Shireen and D. J. Haleem. 2016. Effects of sugar rich diet on brain serotonin, hyperphagia and anxiety in animal model of both genders. Pak. J. Pharm. Sci. 29: 757-763.

Jeong, H. J., R. J. Vandenberg and C. W. Vaughan. 2010. N-arachidonyl-glycine modulates synaptic transmission in superficial dorsal horn. Br. J. Pharmacol. 161: 925-935.

Kleiner, G., A. Marcuzzi, V. Zanin, L. Monasta and G. Zauli. 2013. Cytokine levels in the serum of healthy subjects. Mediators Inflamm. 2013: 434010.

Lu, V. B., H. L. Puhl and S. R. Ikeda. 2013. N-arachidonyl glycine does not activate G protein-coupled receptor 18 signaling via canonical pathways. Mol. Pharmacol. 83: 267-282.

McHugh, D., S. S. J. Hu, N. Rimmerman, A. Juknat, Z. Vogel, J. M. Walker and H. B. Bradshaw. 2010. N-arachidonoyl glycine, an abundant endogenous lipid, potently drives directed cellular migration through GPR18, the putative abnormal cannabidiol receptor. BMC Neurosci. 11: 44.

McHugh, D., J. Page, E. Dunn and H. B. Bradshaw. 2012. Δ 9-tetrahydrocannabinol and N-arachidonyl glycine are full agonists at GPR18 receptors and induce migration in human endometrial HEC-1B cells. Br. J. Pharmacol. 165: 2414-2424.

McHugh, D., D. Roskowski, S. Xie and H. B. Bradshaw. 2014. Δ9-THC and N-arachidonoyl glycine regulate BV-2 microglial morphology and cytokine release plasticity: Implications for signaling at GPR18. Front. Pharmacol. 4: 162.

McHugh, M. P. and C. H. Cosgrave. 2010. To stretch or not to stretch: The role of stretching in injury prevention and performance. Scand. J. Med. Sci. Sports. 20: 169-181.

Meijerink, J., M. Balvers, P. Plastina and R. Witkamp. 2015. Omega-3 polyunsaturated N-acylethanolamines: A link between diet and cellular biology. Endocannabinoidome. 15-32.

Miller, S., E. Leishman, O. Oehler, L. Daily, N. Murataeva, J. Wager-Miller, H. Bradshaw and A. Straiker. 2016. Evidence for a GPR18 role in diurnal regulation of intraocular pressure. Invest. Ophthalmol. Vis. Sci. 57: 6419-6426.

Morselli, E., E. Fuente-Martin, B. Finan, M. Kim, A. Frank, C. Garcia-Caceres, C. R. Navas, R. Gordillo, M. Neinast, S. P. Kalainayakan, D. L. Li, Y. Gao, C. X. Yi, L. Hahner, B. F. Palmer, M. H. Tschöp and D. J. Clegg. 2014. Hypothalamic PGC-1α protects against high-fat diet exposure by regulating ERα. Cell Rep. 9: 633-645.

Palmer, B. F. and D. J. Clegg. 2015. The sexual dimorphism of obesity. Mol. Cell. Endocrinol. 402: 113-119.

Parolaro, D. 1999. Presence and functional regulation of cannabinoid receptors in immune cells. Life Sci. 65: 637-644.

Ramírez-Orozco, R. E., R. García-Ruiz, P. Morales, C. M. Villalón, J. R. Villafán-Bernal and B. A. Marichal-Cancino. 2019. Potential metabolic and behavioural roles of the putative endocannabinoid receptors GPR18, GPR55 and GPR119 in feeding. Curr. Neuropharmacol. 17: 947-960.

Sheskin, T., L. Hanuš, J. Slager, Z. Vogel and R. Mechoulam. 1997. Structural requirements for binding of anandamide-type compounds to the brain cannabinoid receptor. J. Med. Chem. 40: 659-667.

Shi, H., R. J. Seeley and D. J. Clegg. 2009. Sexual differences in the control of energy homeostasis. Front. Neuroendocrinol. 30: 396-404.

Takenouchi, R., K. Inoue, Y. Kambe and A. Miyata. 2012. N-arachidonoyl glycine induces macrophage apoptosis via GPR18. Biochem. Biophys. Res. Commun. 418: 366-371.

Voigt, R. G., M. W. Mellon, S. K. Katusic, A. L. Weaver, D. Matern, B. Mellon, C. L. Jensen and W. J. Barbaresi. 2014. Dietary docosahexaenoic acid supplementation in children with autism. J. Pediatr. Gastroenterol. Nutr. 58: 715-722.

WHO. 2020. Obesidad y Sobrepeso. Available from: https://www.who.int/es/news-room/fact-sheets/detail/obesity-and-overweight.

Wu, J., C. Zhu, L. Yang, Z. Wang, L. Wang, S. Wang, P. Gao, Y. Zhang, Q. Jiang, X. Zhu and G. Shu. 2017. N-oleoylglycine-induced hyperphagia is associated with the activation of agouti-related protein (AgRP) neuron by cannabinoid receptor type 1 (CB1R). J. Agric. Food Chem. 65: 1051-1057.

Zhang, L., W. Han, C. Lin, F. Li and I. E. de Araujo. 2018. Sugar metabolism regulates flavor preferences and portal glucose sensing. Front. Integrat. Neurosci. 12: 57.