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Study of the Hormones Controlling Food Intake (Leptin and Agouti Related Protein "AgRP") in Cannabis Smokers

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Authors' contributions

This work was carried out in collaboration between bath authors. Author SMA designed the study, wrote the protocol, managed the literature searches, analyses of the study and wrote the first draft of the manuscript. Author WMF designed the study, wrote the protocol, performed the laboratory analyses and also the statistical analysis. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Background: Leptin is an adipocyte derived hormone which is thought to be a key regulator of food intake and body weight. Agouti-related protein also called Agouti-related peptide (AgRP) is a neuropeptide produced in the brain by the AgRP/NPY neuron. It is one of the most potent and long-lasting of appetite stimulators. The appetite stimulating effects of AgRP are inhibited by the hormone leptin. There is a public perception that exposure to cannabis produces an increase of appetite (a phenomenon referred to as the 'munchies'). This phenomenon needs an exploration of the role of the endocannabinoid system in the regulation of obesity and associated metabolic syndrome
Aim: This study aimed to find out the food intake style, of subjects smoking cannabis by studing the relationship between the most two popular hormones controlling food intake (leptin hormone, and agouti related protein hormone) compared with healthy subjects.
Methods: The study compared two groups of personells; the test group (Group II) included ten males aged 37.40±2.67 years (mean age ±SD) with positive 9-carboxy-Tetrahydrocanabinol (THC) detected in their urine by qualitative analysis using Gas Chromatography-Mass spectrometry. And control group (GroupI) included ten healthy males aged 38.67±2.35 years (mean age ± SD) as volunteers with negative 9-carboxy-

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Tetrahydrocanabinol (THC). In both groups, age and body mass index (BMI) were matched, diabetic subjects were excluded. Plasma leptin concentration was measured by quantitative radio immunoassay (RIA). Human plasma AgRP hormone was quantitatively measured by using a kit supplied from Quantikine R&D systems INC.

Results: In group I (GI), there was an increase in mean plasma leptin concentrations ±SD (6.39±1.93) ng/ml, than that found, in group II (GII) (4.49±1.40) ng/ml, but it's statistically insignificant (P>0.05). There was an elevation, in mean plasma AgRP concentrations (27.77±2.17) pg/ml in GII, comparing with mean plasma AgRP concentrations (25.84±1.93) pg/ml, in GI but it's statistically insignificant (P>0.05). There was a statistically significant inversely correlation between plasma leptin concentrations and plasma AgRP concentrations in GI (P<0.05). There was a proportionally correlation found between plasma leptin concentrations and plasma AgRP concentrations in (GII), but it's statistically insignificant (P>0.05).

Conclusion: The relationship between plasma leptin concentrations and plasma AgRP concentrations affected by smoking cannabis, and that may change the mechanism of food intake in cannabis smokers.

Keywords: Leptin; Agouti Related Protein (AgRP); 9-carboxy-Tetrahydrocanabinol (THC); cannabis; RIA; Immunoassay.

1. INTRODUCTION

Appetite is regulated by a number of hypothalamic neuropeptides including neuropeptide Y (NPY), a powerful feeding stimulator that responds to feeding status, and drugs such as nicotine and cannabis [1].

Leptin is an adipocyte derived hormone which is thought to be a key regulator of food intake and body weight [2]. During times when leptin stores in adipocytes are decreasing (e.g., with fasting induced lipolysis), plasma leptin levels would be expected to be suppressed, stimulaing food intake [3]. Conversely, during times when lipid levels in adipocytes are increasing (following over nutrition), plasma leptin levels would be expected to rise, curbing additional food intake [4].

Agouti-related protein also called Agouti-related peptide (AgRP) is a neuropeptide produced in the brain by the AgRP/NPY neuron. It is only synthesised in NPY containing cell bodies located in the ventromedial part of the arcuate nucleus in the hypothalamus [5]. AgRP is primarily expressed in the adrenal gland, subthalamic nucleus and hypothalamus; with lower levels of expression in the testis, kidneys and lungs. The appetite stimulating effects of AgRP are inhibited by the hormone leptin and activated by the hormone ghrelin. Adipocytes secrete leptin in response to food intake. This hormone acts in the arcuate nucleus and inhibits the AgRP/NPY neuron from releasing orexigenic peptides [6]. It is one of the most potent and long-lasting of appetite stimulators [7]. Levels of AgRP are increased during periods of fasting. It has been shown that polymorphisms in the AgRP gene have been linked with anorexia nervosa [8]. AgRP induces obesity by chronic antagonism of the MC4-R [9]. Overexpression of AgRP causes hyperphagia and obesity [10], whilst AgRP plasma levels have been found to be elevated in obese human males [11]. Recent studies have shown that autophagy plays a key role in regulation of food intake and energy balance in maintaning neuronal AgRP levels [12].

Chronic consumption of low-fat diet leads to increased hypothalamic AgRP and reduced leptin [13].

Cannabinoids, the biologically active constituents of marijuana, have been used for millennia for their psychoactive properties. The marijuana plant contains more than 60 distinct chemical substances, of which Δ 9-tetrahydrocannabinol (THC) is the main psychoactive ingredient [14] subsequently led to the discovery and cloning of 2 specific cannabinoid receptors, CB1 and CB2 [15]. Both receptors are expressed in the CNS, as well as in the peripheral tissues. CB1 was found to be one of the most prevalent G-protein coupled receptprs in the mammalian brain, while CB2 was shown to have prominent roles in immune and haematopoietic cells, as well as osteoblasts and osteoclasts [16]. The most widespread abuse of cannabis is by smoking. It may occasionally be abuse orally. Over 80% of the excreted cannabinoid metabolite, 9-carboxy- THC is present in the urine in the form of glucuronid conjugated [17].

Obesity is one of the highest preventable causes of morbidity and mortality in the developed world. It has been well known for a long time that exposure to cannabis produces an increase of appetite (a phenomenon referred to as the 'munchies'). This phenomenon led to an exploration of the role of the endocannabinoid system in the regulation of obesity and associated metabolic syndrome [18].

2. AIM

This study aimed to find out the feeding patterns, of subjects smoking cannabis by studing the relationship between the most two popular hormones controlling appetite and food intake patterns (leptin hormone, and agouti related protein hormone) compared with healthy subjects.

3. METHODS

The study compared two groups; the test group (Group II) included ten males aged 37.40±2.67 years (mean age ±SD) with positive 9-carboxy-Tetrahydrocanabinol (THC) detected in their urine by qualitative analysis using Gas Chromatography-Mass spectrometry (Agilent 6080N- capillary coloumn 30m- 1µ diameter) collected from forensic medicin institute chemistry lab. Cairo, Egypt. And control group (GroupI) included ten healthy males aged 38.67±2.35 years (mean age ± SD) as volunteers with negative 9-carboxy-Tetrahydrocanabinol (THC). In both groups, age and body mass index (BMI) were matched, diabetic subjects were excluded. Plasma leptin concentration was measured by quantitative radio immunoassay (RIA) using a kit supplied from diagnostic systems Laboratories (DSL) Inc. (445 medical center BLVB. WEBSTEER TX 77598 USA, the method depend on a non-competitve assay in which the analyte to be measured is “sandwiched” between two antibodies. Human plasma AgRP hormone was quantitatively measured by using a kit supplied from Quantikine R&D systems INC. (614 McKinley Palace Minneapolis, MN 55413, USA.), this assay employs the quantitative sandwich enzyme immunoassay technique.

SPSS® ver 10 for Windows® (SPSS, Inc., Chicago, IL, USA) was used for data analysis. Qualitative data were presented as number and percentage; quantitative data were presented as mean±SD. The $\chi 2$ test was used to test the association between two categorical variables. Correlation between BLL and manifestations of pre-eclampsia was

measured by Pearson correlation coefficient. A p value <0.05 was considered statistically significant.

4. RESULTS

Table (1) shows the plasma leptin and AgRP concentrations in GI and GII. Plasma leptin concentrations were lesser in GII (4.49±1.40 ng/ml) than in GI (6.39±1.9 ng/ml), while plasma AgRP concentrations were greater in GII (27.77±2.17pg/ml) than in GI (21.5-27.6 pg/ml). There was a statistically significant inversely correlation between plasma leptin concentrations and plasma AgRP concentrations in GI, with P value (P<0.05) (Fig. 1). There was a positive correlation found between plasma leptin concentrations and plasma AgRP concentrations in GII, but it's statistically insignificant with P value (P>0.05) (Fig. 2). In GI, there was a statistically significant positive correlation between plasma leptin concentrations and BMI (P<0.05) (Fig. 3) and statistically significant inverse correlation between plasma AgRP concentrations and BMI (P<0.05) (Fig. 4). In GII, there was no correlation between plasma leptin concentrations and BMI (Fig. 5) but statistically insignificant inversely correlation between plasma AgRP concentrations and BMI (P>0.05) (Fig. 6).

Table 1. Comparison between cannabis smokers (GII) and the control group (GI)

Hormone level	Control group (GI) n=10	Test group (GII) n=20	T test	p
	Mean ± SD	Mean± SD		
Plasma leptin concentrations (ng/ml)	6.39±1.9	4.49±1.40	1.90	0.094
Plasma AgRP concentrations (pg/ml)	25.84±1.93	27.77±2.17	4.20	0.003
Body mass index (BMI) kg/m2	22.78± 1.38	23.41±1.29	1.52	0.166
Serum creatinine (mg/dl)	0.72±0.27	0.87±0.27	4.21	0.003
Serum ALT (U/L)	19.6±7.7	20.2±7.78	2.26	0.054
Serum AST (U/L)	16.6±7	20.5±5.4	1.37	0.20

5. DISCUSSION

This study demonstrates that cannabis smokers have a lower plasma leptin and a higher AgRP concentrations than comparable individuals not smoking cannabis, that is associated with increased BMI in cannabis smokers. This denote that cannabis smoking can modulate the body weight through affection of appetite and feeding pattern by changing leptin and AgRP concentrations. This finding is consistent with [19] that suggest that AgRP, an endogenous negative regulator of the hypothalamic melanocortin system, constitutes a novel regulatory mechanism of leptin action within the hypothalamus. Antagonism of leptin action by AgRP should be attributable mostly to its ability to block the hypothalamic a-MSH/MC4-R signaling activated by leptin. The discussion above also supports the notion that the hypothalamic melanocortin system plays a pivotal role in leptin action.

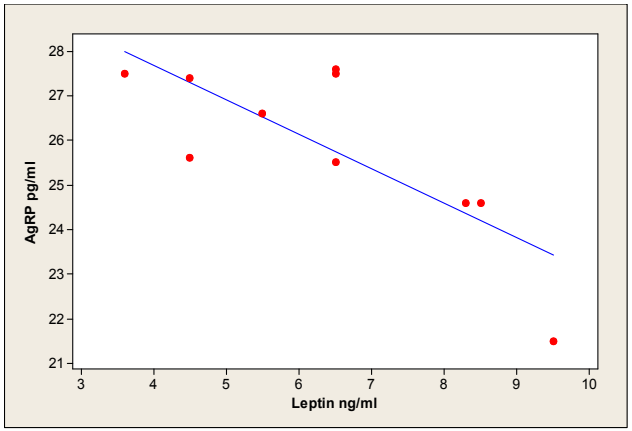

Fig. 1. Correlation between plasma leptin concentrations and AgRP in GI

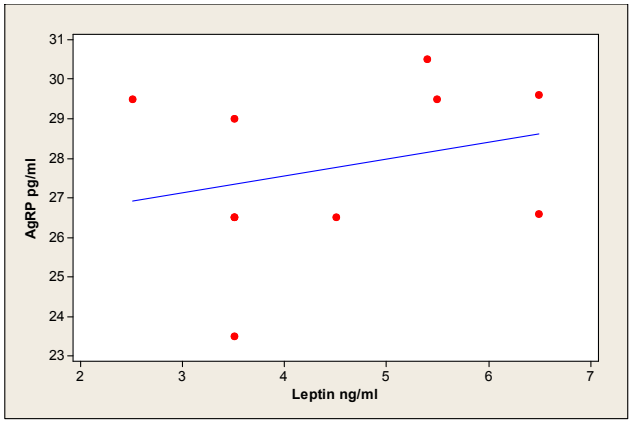

Fig. 2. Correlation between plasma leptin concentrations and AgRP in GII

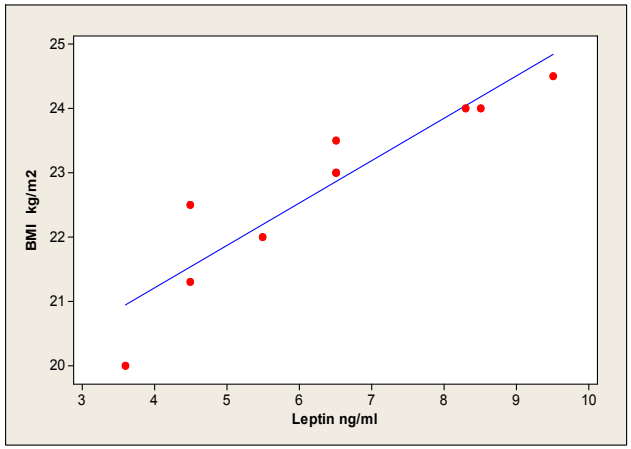

Fig. 3. Correlation between plasma leptin concentrations and BMI in GI

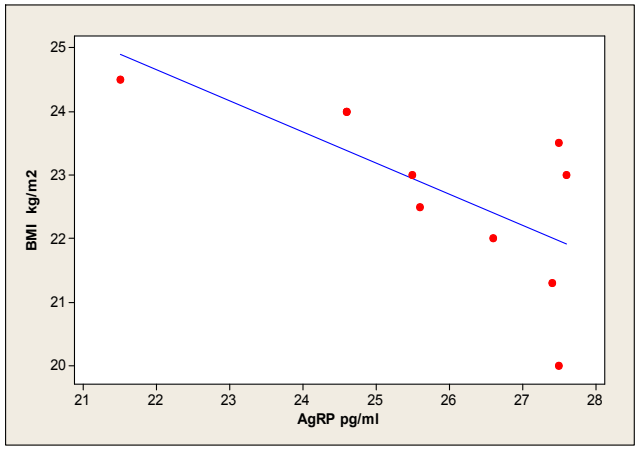

Fig. 4. Correlation between plasma AgRP concentrations and BMI in GI

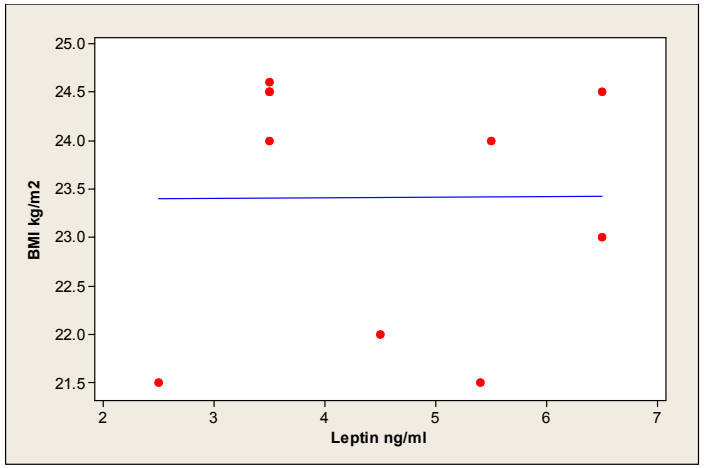

Fig. 5. Correlation between plasma leptin concentrations and BMI in GII

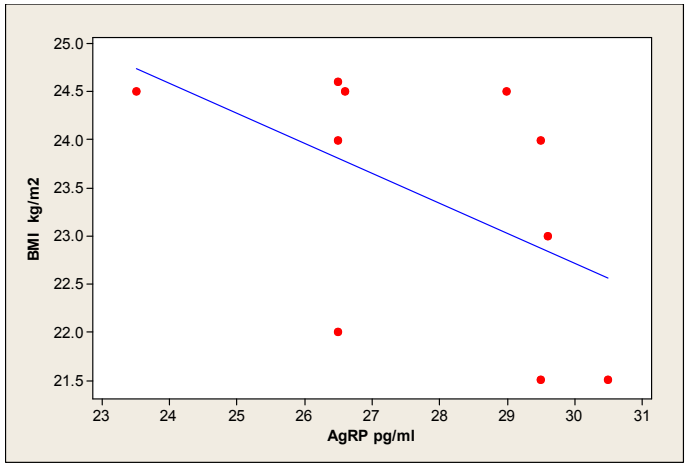

Fig. 6. Correlation between plasma AgRP concentrations and BMI in GII

Our results are in concordance with that of [20] who suggested that leptin's inhibition of fodd intake is in part mediated by downregulation of CB1R and that of leptin-deprivation conditions. These results are consistent with other findings showing increased levels of endocannabinoids of the ob rats corroborating the regulation of cannabinoid signaling by leptin.

It has been well known for a long time that exposure to cannabis produces an increase of appetite (a phenomenon referred to as the 'munchies'). This phenomenon led to an exploration of the role of the endocannabinoid system in the regulation of obesity and associated metabolic syndrome [18].

Leptin regulates hypthalamic signaling that underlies the motivation to hyperphagia but the interaction between leptin and Cannabinoid signaling is poorly understood [20].

Results of the present study showed that cannabis smoking modulate the relation between leptin and AgRP that was associated with increased BMI. There was statistically insignificant positive correlation between plasma leptin and plasma AgRP concentrations in cannabis smokers comared to statistically significant inverse correlation between them in non smokers. Both animal and human data show that the endocannabinoid system is up-regulated in obesity [21]. The plant Cannabis Sativa has been used to promote caloric intake by enhancing appetite for many years ago [22]. It seems that Cannabinoids interact with a number of hormonal systems and possibly mediate their effects as leptin, Ghrelin adiponectin and Neuropeptide Y (NPY) and others [21].

It appears likely that some of the obesity-producing effects of AgRP expression in the brain may be due to its interference with signal generation by MSH at MC4-R, a signal which normally acts to suppress food intake and we support this explanation which agreed with [23,24,25] who showed that melanocortinergic neurons inhibit feeding behavior. Chronic disruption of this inhibitory signal causes the agouti obesity syndrome, and [26] who reported that agouti signal protein gene expression is negatively associated with the degree of obesity in men whereas an opposite relationship was observed in women.

Another explanation [25] reported that recombinant agouti protein directly increased [Ca2+] in a variety of cells and stimulated both the expression and activity of adipocyte fatty acid synthase and increased triglyceride accumulation in a Ca2+ dependent manner. These effects can be mimicked by stimulation of Ca2+ influx and blocked by Ca2+ channel inhibition, while treatment of mice with a Ca2+ antagonist decreases agouti-induced obesity.

Regulation of food intake by the neuropeptide Y (NPY)/Agouti-related protein (AgRP) neurons of the hypothalamic arcuate nucleus is an area of active investigation [27,28,29]. Recently [18], it was discovered that the prevalence of obesity is paradoxically much lower in cannabis users as compared to non-users and that this difference is not accounted for by tobacco smoking status and is still present after adjusting for variables such as sex and age. Here, we propose that this effect is directly related to exposure to the Δ^9-tetrahydrocannabinol (THC) present in cannabis smoke. We therefore propose the seemingly paradoxical hypothesis that THC or a THC/cannabidiol combination drug may produce weight loss and may be a useful therapeutic for the treatment of obesity and its complications.

On studing the relation between BMI and appetite regulating hormones, it was found that in cannabis smokers there is no correlation between plasma leptin concentrations and BMI in

comparison to significant positive correlation between them in non cannabis smokers. It was found also that cannabis smoking had modulated the statistically significant inverse correlation between plasma AgRP concentrations and BMI in non cannabis smokers to be insignificant inversely correlation between them. These findings are consistent with modulation of appetite hormones mediated through endogenous cannabinoid receptors as previously concluded by [30], whose study results showed that cannabis administration was associated with significant increases in plasma levels of ghrelin and leptin, and decreases in PYY, but did not significantly influence insulin levels and these findings are consistent with modulation of appetite hormones mediated through endogenous cannabinoid receptors, independent of glucose metabolism.

It is demonstrates that [31] agouti directly up regulates adipose tissue leptin as well as plasma leptin and ob mRNA levels. Since agouti is normally expressed in human adipose tissue. In addition, they indicate, “Agouti may function as an autocrine regulator of leptin in human adipocytes.” This is the first report of regulation of an obesity gene product by another obesity gene product, suggesting that interaction between obesity genes may play a key role in obesity syndromes.

AgRP could antagonize leptin action by antagonism of MC4-R. It is also, reported that AgRP is unregulated in the Arc from leptin-deficient o b/o b mice and leptin receptor–deficient db/db mice, suggesting that leptin may, in turn, regulate hypothalamic AgRP production. All the findings suggest resence of complex interactions between leptin and AgRP to elucidate the role of AgRP in leptin action [7,24,32].

So we can confirm that AgRP may play an important role in affecting plasma leptin concentration this regard may give us a clue to understand the mechanism of leptin resistance, the MCR signaling system is a key regulator of body weight. Profound obesity develops leptin level that leads to activated in MC4R, when the gene encoding the MCR ligand α-MSH is activated, or when AgRP can’t antagonist the MC3/4R, that leads to decrease in AgRP expression in the hypothalamus,"

Leptin signaling can influence 2AG (2-arachidonoyl glycerol) biosynthesis in the hypothalamus and anandamide hydrolysis in T-lymphocytes [33]. CB1 is located mostly presynaptically allowing for retrograde actionn of endocannabinoids. CB1 signaling affects the expression of oreigenic and anorectic mediators in the hypothalamus. In the hypothalamus, changes in endocannabinoids levels seemed to be inversely correlated with the changes that are known to occur in blood vessels of the neurohormone leptin,which is pivotal in regulating the hypothalamic orexigenic and anorectic signals. Indeed, leptin decreases endocannabinoid levels in the hypothalamus, much as it does for other orexigenic mediators, and obese rodents with defective leptin signaling show significantly higher hypothalamic endocannabinoid concentrations [34].

Endocannabinoids may play an integral role in the leptin pathway, which may be the key to understanding their role in appetite stimulation. Leptin is the main signal in which the hypothalamus senses nutritional state and modulates food intake. In one study, a defective leptin signaling pathway resulted in increased levels of hypothalamic endocannabinoids which points to a strong association between the leptin signaling pathway and the endocannabinoid system and this suggestion may agreed with [34].

Cannabinoid type 1 receptor-mediated appetite stimulation by Δ9tetrahydrocannabinol (Δ9THC) is well understood. Recently, it has become apparent that at least one of non-

Δ9THC phytocannabinoids could definitively stimulate feeding, although further trials using individual phytocannabinoids are required to fully understand the observed effects [35].

6. CONCLUSION

It can be concluded that there is a possible link between endocannabinoid mechanisms and the appetite-regulating hormones and that smoking cannabis, increases food intake by by affecting the relation between plasma leptin concentration, and plasma Agouti related protein concentrations.

Leptin and AgPR hormones may be consider as smoking cannabis markers when there was not any other effects on these hormones.

CONSENT

Both authors declare that 'written informed consent was obtained from the participants for sample collection for research and publication.

ETHICAL APPROVAL

Both authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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