# IT IS NOT ENDOCANNABINOIDS BUT THE TYPE AND AMOUNT OF FOOD ARE THE MAIN CAUSE OF METABOLIC DISTURBANCES IN RATS

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**Abstract:** Obesity has been identified as a major global health problem. A major cause of the obesity is the changes in feeding behaviour. Many controversy data concerning the role of endocannabinoid system in regulation /or disturbing of the metabolic parameters. The aim of this research is to identify the effect of methanandamide (as a one of CB1 selective agonist) on some metabolic parameters in rats fed by different types of food to clarify which is the cause of metabolic abnormality in obese ?.Design: A total number of 56 healthy adult male albino rats were used to study the effect of different types of diet and daily i.p injection methanandamide (CB1 agonist) in a dose of 0.5 mg/kg BW for 6 weeks on some metabolic parameters using pair feeding paradigm.Results: a significant increase in final body weights and a significant dyslipidemia and hyperglycemia with insulin resistance was in both HFD and HFrD fed groups when compared with that of standard chow diet fed group. Moreover, a significant dyslipidemia and hyperglycemia with insulin resistance was observed in methanandamide treated ad libitum group. In addition, our study revealed an insignificant change in all parameters measured between HFD and HFrD fed groups except for TG and VLDL parameters which are significantly higher in HFrD-fed group in comparison with that of HFD fed group. Interestingly, an insignificant change in serum levels of all previously mention parameters in the three different methanandamide treated pair fed groups in comparison with that of the three different fed control groups respectivelyConclusion: we can conclude that endocannabinoid system is not the main responsible for metabolic disturbance in obese rats.

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**Key Words:** high fat, high fructose, endocannabinoid, insulin resistance, dyslipidemia.

## 1. Introduction:

The history of marijuana and its medicinal use go back thousands of years, but the endogenous counterparts of cannabis, the endocannabinoid (ECs) have only been known about 18 years ago, their discovery have been triggered by the identification of specific cannabinoid receptors in the brain[1].

Two subtypes of cannabinoid (CB) receptors have been identified to date. Both $CB_1$ and $CB_2$ receptors couple to the Gi/o subtypes of G proteins, but can also activate additional, G protein-independent pathways[2].

It is common knowledge that marijuana use improves appetite, presaging the role of ECs as endogenous orexigenic factors. However, findings as early as the 1970's suggested that $^{9}$-tetrahydrocannabinol ( $^{9}$THC), the psychoactive ingredient of marijuana, has additional metabolic effects unrelated to appetite[3].

$CB_1$ receptors are present in skeletal myocytes and are upregulated in obesity[4] and may be one of the targets for cannabinoid-induced insulin resistance. ECs may also influence insulin secretion in the endocrine pancreas, although there are conflicting reports on $CB_1$ receptors mediating a decrease[5] or an increase in insulin release[6], so ECs may share in the regulation of glucose homeostasis and insulin sensitivity.

Metabolic studies have confirmed that calories obtained from fat have a greater role in obesity and have been considered as dietary risk factors for metabolic syndrome (MS)[7,8], moreover, fructose as a common supplement of the packed food has been implicated as a possible cause of the MS[9].

$CB_1$ receptor inhibition by AM251 is capable of countering insulin resistance in adipose tissue, muscle and liver[10,11]. However, similar beneficial effects on insulin sensitivity were also observed in pair fed animals[12]. The lack of significant improvement in metabolic status in AM251 treated, in comparison to pair fed animals does not mirror this finding. Thus, further studies are necessary to confirm these initial observations. Interestingly, AM251 also increased locomotor activity in agouti mice[13].

Recently, **Sink et al.** [14] reported that all clinically available cannabinoid receptor antagonists (e.g. AM 251 and rimonabant " SR141716A") are inverse agonists that can target $CB_1$ receptors located in both central circuits regulating appetite and motivation and in peripheral organs regulating metabolism and energy expenditure. This profile (inverse agonism)

complicates understanding of cannabinoid $CB_1$ receptor blockade as a therapeutic strategy in obesity and metabolic disorders.

Unlike rimonabant, chronic adminstration of LH-21 (selective $CB_1$ antagonist only) reduces feeding but does not improve hypertriglyceridaemia or hypercholesterolaemia; nor does it reduce liver fat deposits in Zucker rats. These data explain why AM251 increased locomotor activity in agouti mice at low doses[13].

It is still uncertain whether an overactive ECS is an early cause[15] or just one of the several consequences, of HFD and the subsequent development of overweight and obesity.

The present study was done to identify the effect of methanandamide (as a one of CB1 selective agonist) on some energy metabolic parameters concerning glucose homeostasis and lipid in rats fed by different types of food (commercial, high fat and high fructose diet) using pair feeding paradigm in a trial to clarify which is the cause of metabolic abnormality in obese, it is ECS alone, type of food, amount of food intake or crosstalk between them?.

## Materials And Methods

### Animals

This study was carried out on a total number of 56 adult (4 months; body weight, 180-200 gm) healthy male albino rats. Under hygienic conditions, in the animal house of the faculty of medicine Zagazig University, all rats had free access to water and chow, supplied in separate clean containers. Rats were kept at comfortable temperature (20 to 24 ºC) and were maintained on a 12 hr light/dark cycle[16].

### Diet

**Normal (standard) diet:** consists of commercial rat standard chow [it was consisted of 25.8 % protein, 62.8 % carbohydrate and 11.4 % fat[17].

**High fat diet (HFD):** was consisted of 16.4% protein, 25.6% carbohydrate, and 58.0% fat (a total 23.4 kJ/g) in the form of cotton seed oil added to the laboratory chow diet[17.18].

**High fructose diet (HFrD)**: commercial rat laboratory chow containing 60% fructose[19].

### Methods

The rats were accommodated to the new laboratory conditions for three weeks before the beginning of the experimental regimen[20].

### Grouping of the animals

**Group I "normal fed group":** to study the effect of 6 weeks normal diet. It is consists of 24 rats which further subdivided into 3 equal subgroups (n= 8):

**Group IA**: vehicle (saline) treated group with access to ad libitum standard chow.

**Group IB:** Methanandamide (dissolved in sterile saline), treated (0.5 mg/kg BW i.p, daily)[21,22], pair fed group. Rats were given a weighed amount of standard chow each day corresponding to the amount consumed by vehicle treated rats on the previous day[12].

**Group IC:** Methanandamide treated (0.5 mg/kg BW i.p, daily), ad libitum group, rats had access to ad libitum standard chow.

**Group II "HFD fed group:** to study the effect of 6 weeks HFD diet. It consists of 16 rats which further subdivided into 2 equal subgroups (n= 8):

**Group IIA**: vehicle treated group with Access to ad libitum HFD.

**Group IIB**: Methanandamide treated (0.5 mg/kg BW i.p daily), HFD pair fed group.

**Group III " HFrD fed group:** to study the effect of 6 weeks HFrD diet. It consists of 16 rats which further subdivided into 2 equal subgroups (n= 8):

**Group IIIA**: vehicle treated group with Access to ad libitum High fructose diet.

**GroupIII B**: Methanandamide treated (0.5 mg/kg BW i.p, daily), HFrD pair fed group.

For all groups, body weight was recorded at the beginning and the end of the study period (6 weeks).

### Sampling of blood

At the end of the experimental period (at the end of $6^{th}$ week) after overnight fasting, at 8:00 a.m, blood samples were obtained from sinus orbitus vein of each rat after ether inhalation[23]. The blood samples were allowed to clot at room temperature before centrifuging at approximately 3000 rpm for 15 minutes. The serum was stored at -20° C.

### Serum analysis

**Determination of serum glucose level:** According to **Trinder**[24] using glucose enzymatic **(GOD-PAP)-** liquizyme Kits (Biotechnology, Egypt).

**Determination of serum insulin level:** By a solid phase enzyme amplified sensitivity immunoassay according to **Starr et al.** [25] using KAP1251-INS-EASIA (Enzyme Amplified Sensitivity Immunoassay) Kits (BioSource Europe S.A., Belgium).

**Determination of the Serum total cholesterol (TC):** by enzymatic colorimetric method according to **Allain**[26] using Cholesterol RTU 61218 kits: (bioMerieux S.A., Lyon, France).

**Determination of the Serum high density lipoprotein cholesterol (HDL)**: by enzymatic colorimetric method according to **Warnick et al.**[27], using Stanbio HDL-cholesterol procedure No. 0599

kits (Stanbio laboratory Inc., San Antonio, Texas, USA).

**Determination of the Serum Triglyceride levels:** It was carried out according to **Naito[28]** using triglycerides ESPAS SL kits (Elttech S.A., Sees, France.).

**Calculation of very low density lipoprotein cholesterol (VLDL) and Low density lipoprotein cholesterol:** According to **Friedewald et al. [29].**

**HOMA-IR** was assessed by homeostasis model assessment (where HOMA= fasting serum insulin (μIU/mL) x [fasting serum glucose (mmol/L)/22.5][30]**.**

The data obtained in the present study were expressed as mean ± SE for quantitative variables and statistically analyzed by using SPSS program (version 18 for windows) (SPSS Inc. Chicago, IL, USA). P value <0.05 was considered statistically significant.

**3. Results:**

**Table 1&2 and histograms 1, 2, 3, 4, 5, 6, 7, 8 & 9** show the final body weight (gm), serum glucose (mg/dL), insulin (μIU/mL), the HOMA index of insulin resistance (HOMA-IR), total cholesterol (CHO) (mg/dl), HDL-C(mg/dl), triglyceride (TG) (mg/dl), VLDL (mg/dl) and LDL-C(mg/dl) levels in all studied groups. In group IB "Methanandamide treated normal diet pair fed group" the mean values were found to be non significant (P > 0.05) when compared with that of group 1A"normal diet control group", however group IC "Methanandamide treated normal diet ad libitum" were found to be significantly higher than that of both group 1A and group IB (P < 0.01, P < 0.001, P < 0.001, P < 0.001, P < 0.001, P <0.001, P < 0.001, P < 0.001 & P < 0.001 respectively) except HDL-C levels was significantly lower. In addition, the mean values of group IIB "Methanandamide treated HFD pair fed group" and III B "Methanandamide treated HFrD pair fed group" were found to be non significant (P > 0.05) when compared with that of group IIA "HFD control group" and IIIA "HFrD control group".

It was found that both of group IIA "HFD" and group IIIA "HFrD" showed a significant increase in final body weights, serum glucose, insulin, the HOMA-IR, CHO, TG, VLDL-C LDL-C levels (P <0.001) and HDL-CHO (P < 0.01 & P < 0.05)when compared with that of group IA normal diet group". While, group IIIA "HFrD" showed an insignificant change (P > 0.05) in final body weights, serum glucose, insulin, the HOMA-IR, CHO, and LDL-C levels, however, it had significant increase in TG and VLDL (mg/dl) (P < 0.001) when compared with that of group IIA"HFD".

In addition, there were significant positive correlations between final body weights and serum glucose, serum CHO, serum TG, serum VLDL-C, serum VLDL- C, LDL-C levels and calculated HOMA-IR, accompanied by significant negative correlation between serum HDL-C levels and final body weights in all studied groups.

**Table 1: Final body weights, serum glucose, insulin, the HOMA-IR, levels in all studied groups.**

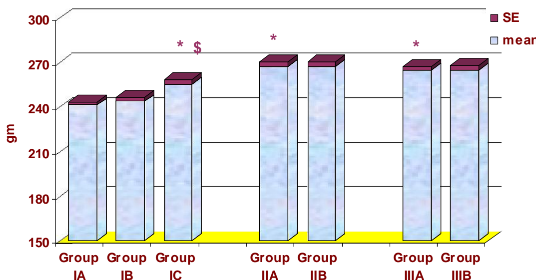
| parameter | N= 8 | Normal Diet | | | HFD | | H Fr. D | |
|---|---|---|---|---|---|---|---|---|
| | | Group IA | Group IB | Group IC | Group IIA | Group IIB | Group IIIA | Group IIIB |
| Final body weight (gm) | $\bar{X}$ ± SE | 241.25± 2.13 | 243.63± 2.93 | 255.25 ± 3.47 | 266.86 ± 3.47 | 267.25 ± 3.06 | 264.50± 2.49 | 264.87 ± 2.81 |
| | P | NS | | <0.01*$ | NS | | NS | |
| Glucose (gm/dl) | $\bar{X}$ ± SE | 89.63 ± 6.0 | 93.13 ± 7.75 | 219.63± 3.64 | 272.0± 6.67 | 273.5 ± 5.5 | 281.75± 4.41 | 280.75 ± 4.89 |
| | P | NS | | < 0.001*$ | NS | | NS | |
| | r<br>P | +.752<br>< 0.05 | +.795<br>< 0.05 | + .771<br>< 0.05 | + .890<br>< 0.01 | + .948<br>< 0.01 | + .866<br>< 0.01 | +.881<br>< 0.01 |
| Insulin ( IU/mL) | $\bar{X}$ ± SE | 21.04±0.93 | 21.05± 0.69 | 36.44± 1.78 | 40.25± 1.61 | 39.17± 1.21 | 38.68± 1.18 | 39.76± 1.22 |
| | P | NS | | < 0.001*$ | NS | | NS | |
| | r<br>P | + 0.749<br>< 0.05 | + 0.804<br>< 0.05 | + 0.920<br>< 0.01 | + 0.932<br>< 0.01 | + 0.977<br>< 0.01 | + 0.775<br>< 0.05 | + 0.932<br>< 0.01 |
| HOMA index | $\bar{X}$ ± SE | 4.69 ± 0.44 | 4.88± 0.49 | 20.12±1.26 | 27.57± 1.71 | 26.93± 1.34 | 27.35± 1.22 | 28.00± 1.19 |
| | P | NS | | < 0.001*$ | NS | | NS | |
| | r<br>P | + 0.901<br>< 0.01 | + 0.921<br>< 0.01 | + 0.920<br>< 0.01 | + 0.937<br>< 0.01 | + 0.981<br>< 0.01 | + 0.832<br>< 0.05 | + 0.848<br>< 0.01 |

* VS group IA $ VS group IB

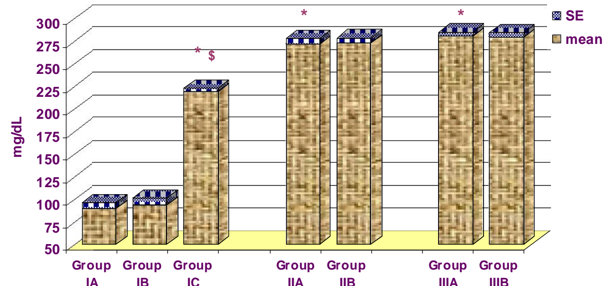
**Table 2: Serum CHO, TG, VLDL-C LDL-C levels in all studied groups.**

| Parameter | N= 8 | Normal Diet | | | HFD | | H Fr. D | |
|---|---|---|---|---|---|---|---|---|
| | | Group IA | Group IB | Group IC | Group IIA | Group IIB | Group IIIA | Group IIIB |
| CHO | X̄ ± SE | 106.0± 2.66 | 101.50± 2.24 | 133.88± 5.30 | 186.75± 3.87 | 190.63± 3.87 | 189.38± 4.17 | 191.88± 2.68 |
| | P | NS | | < 0.001 *$ | NS | | NS | |
| | r<br>P | + 0.839<br>< 0.01 | + 0.828<br>< 0.05 | + 902<br>< 0.01 | + 0.724<br>< 0.05 | + 0.793<br>P < 0.05 | + 935<br>< 0.01 | + 0.925<br>< 0.01 |
| HDL-C (mg/dl) | X̄ ± SE | 51.0± 1.65 | 47.75± 3.08 | 38.63± 1.55 | 38.25±1.75 | 37.75± 1.33 | 39.63± 1.05 | 38.13± 1.74 |
| | P | NS | | < 0.001 *$ | NS | | NS | |
| | r<br>P | - 0.800<br>< 0.05 | - 0.734<br>< 0.05 | - 0.955<br>< 0.01 | - 0.958<br>P < 0.01 | - 0.886<br>< 0.01 | - 0.865<br>< 0.01 | - 0.877<br>< 0.01 |
| TG (mg/dl) | X̄ ± SE | 56.86± 1.8 | 57.75±2.31 | 91.37± 4.85 | 125.63± 2.90 | 130.13± 2.49 | 150.75± 4.19 | 161.5±3.91 |
| | P | NS | | < 0.001 *$ | NS | | NS | |
| | r<br>P | + 0.716<br>< 0.05 | + 0.834<br>< 0.05 | + 0.968<br>< 0.01 | + 0.737<br>< 0.05 | + 779<br>< 0.05 | + 0.831<br>< 0.05 | + 0.970<br>< 0.01 |
| VLDL-C (mg/dl) | X̄ ± SE | 11.38±0.36 | 11.55±0.46 | 18.28±0.97 | 25.13±0.58 | 26.0±0.50 | 30.15±0.84 | 32.30±0.78 |
| | P | NS | | < 0.001 *$ | NS | | NS | |
| | r<br>P | + 0.716<br>< 0.05 | + 0.834<br>< 0.05 | + 0. 968<br>< 0.01 | + 0.737<br>< 0.05 | + 779<br>< 0.05 | + 0.831<br>< 0.05 | + 0.970<br>< 0.01 |
| LDL-CHO (mg/dl) | X̄ ± SE | 43.63±4.04 | 42.20±4.11 | 76.98±5.75 | 122.25± 5.94 | 126.85± 4.54 | 119.60±4.4 1 | 121.45±3.5 3 |
| | P | NS | | < 0.001 *$ | NS | | NS | |
| | r<br>P | + 0.813<br>< 0.05 | + 0.908<br>< 0.01 | + 0.925<br>< 0.01 | + 0.822<br>< 0.05 | + 0.853<br>< 0.01 | + 0.933<br>< 0.01 | + 0.919<br>< 0.01 |

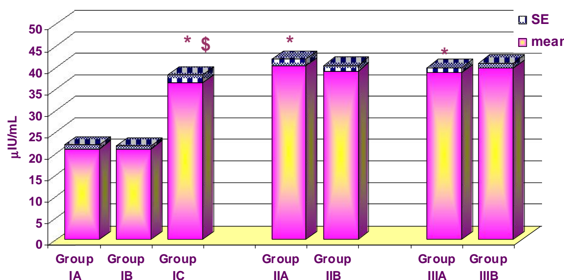
* VS group IA $ VS group IB



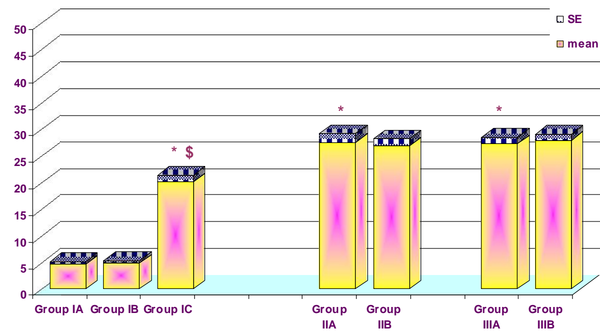
Histogram 1 : The final body weights (gm ) in all studied groups.
* VS group IA $ VS group IB



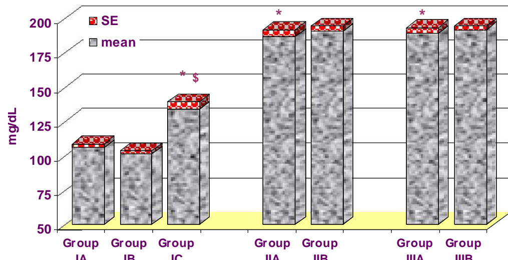
Histogram 2: illustrates serum glucose levels ( mg/dl) in all studied groups.
* VS group IA $ VS group IB



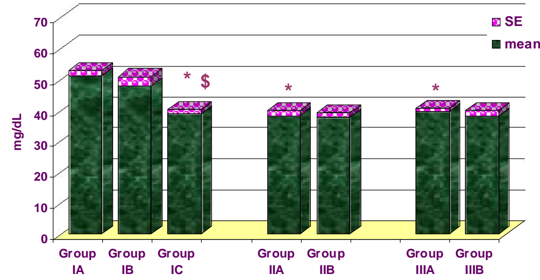
Histogram 3: illustrates serum insulin levels µIU/ml in all studied groups.
* VS group IA $ VS group IB



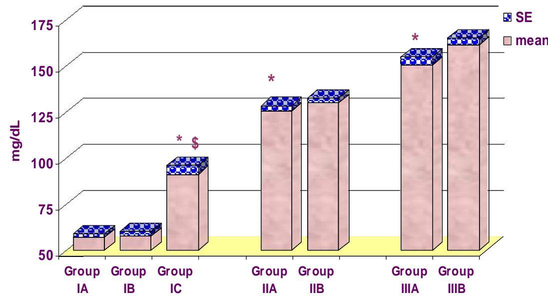
Histogram 4: illustrates HOMA index of insulin resistence in all studied groups.
* VS group IA $ VS group IB



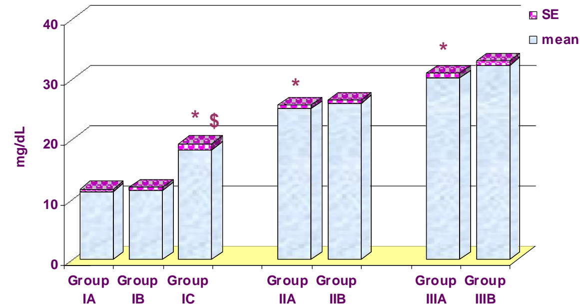

Histogram 5: illustrates total serum cholesterol levels mg/dl in all studied groups.
* VS group IA $ VS group IB



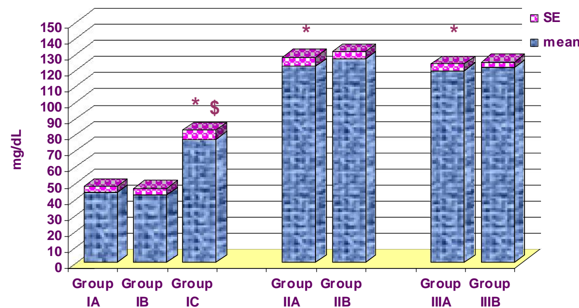

Histogram 6: illustrates serum HDL-C levels (mg/dl) in all studied groups.
* VS group IA $ VS group IB




Histogram 7: illustrates serum TG levels (mg/dl) in all studied groups.
* VS group IA $ VS group IB




Histogram 8: illustrates serum VLDL levels (mg/dl) in all studied groups.
* VS group IA $ VS group IB




Histogram 9: illustrates serum LDL-C levels (mg/dl) in all studied groups.
* VS group IA $ VS group IB

## 4. Discussion:

In mammals, body weight and composition are maintained within a narrow range by the integrated control of energy intake, storage and expenditure. The endocannabinoid system (ECs) which consists of cannabinoid receptors, the endogenous lipid ligands (endocannabinoids), and the machinery for their biosynthesis and metabolism share in this control[31].

To explore the exact role of diet and ECs in metabolic disturbance, the present study used the 6 weeks pair-feeding paradigm to ascertain if the metabolic effect of ECs were related to type and/or amount of food intake or not.

As regard HFD ad libitum group, It showed a significant increase in final body weight with preservation of its correlation with all metabolic parameters, and a significant disturbance in glucose metabolism, distinguished by significant increase in fasting serum glucose levels, fasting serum insulin levels, and HOMA index of insulin resistance. Ad libitum HFD fed group also showed dyslipidemia proved by significant increase in serum total cholesterol, TG, VLDL-cholesterol levels, and LDL-cholesterol levels, accompanied by a significant decrease in HDL-cholesterol levels when compared with that of ad libitum normal diet group.

These data are in consistent with **Alsaif and Duwaihy**[32] who found that in general, HFD significantly increased weight gain, impaired glucose tolerance and increased insulin resistance.

**Buettner et al.**[33] stated that long term HFD feeding often impairs lipid metabolism by reducing insulin sensitivity of peripheral tissue.

HFD decrease insulin-stimulated glucose disposal in skeletal muscle and increase TG, long-chain acyl-CoA and diacylglycerol contents[34]**.** In addition, Glut 4 mRNA in WAT is down-regulated by HFD[35]**.**

The HFD induced glucose intolerance and dyslipidemia which proved in the present study can be explained by disturbance of some metabolic hormones such as insulin resistance which has been proved in our results. In addition, **Naitoh et al.**[36] confirmed that mRNA expression level of adiponectin in WAT and plasma adiponectin levels were found to be decreased in HFD-fed mice compared with commercial diet-fed mice. This hypoadiponectinemia was closely linked to insulin resistance and low HDL cholesterol[37], as adiponectin enhance lipid clearance from plasma and increasing fatty acid beta oxidation in muscle[38].

It was found that, diet induced obesity in humans and rodents have very high amounts of circulating leptin, this hyperleptinemia is associated with leptin resistance which neither reduces appetite nor increases energy expenditure[39].

Apelin has been reported as a beneficial adipokine up-regulated in obesity as an attempt to overcome either insulin resistance or obesity-related cardiovascular diseases[40], elevated plasma apelin has been estemated in moderately[41] and in severe obese[42]. It has also been shown that plasma apelin levels were increased in diabetic subjects and positively correlated with BMI, HOMA-IR and fasting plasma insulin[43], suggesting a role of apelin in the pathogenesis of type II diabetes induces by overweight[44].

**Watanabe et al.**[45] stated that disturbed ECs levels that accompanied HFD are related to onset, duration and its fatty acid composition.

Cannabinoids promote lipogenesis and the storage of adipose tissue via $CB_1$, and the expression of $CB_1$ in adipose tissue is up-regulated in rodent models of obesity[15,46].

On the other hand, antagonists of $CB_1$ promote lipolysis and fatty acid oxidation and increase in insulin sensitivity[47,48].

As regard the comparison between the HFrD and the normal diet fed groups, HFrD fed group showed a significant increase in final body weight with preservation of its correlation with all metabolic parameters and a significant increase in fasting serum glucose levels, fasting serum insulin levels, and HOMA-IR, HFrD fed group also showed dyslipidemia proved by significant increase serum total cholesterol, serum triglyceride, VLDL-cholesterol levels, and LDL-cholesterol, accompanied by a significant decrease in HDL-cholesterol.

These data are in line with studies on rodents that stated that HFrD increases intra hepatocellular lipid and stimulates hepatic de novo lipogenesis within a few days and induce hyperlactatemia and hypertriglyceridemia[49].

Studies of pure fructose fed to laboratory animals show increased plasma free fatty acids, and abdominal adipose tissue, as well as impaired insulin sensitivity[50].

Fructose consumption by adult rats has been shown to produce diminished glucose tolerance and insulin sensitivity as well as elevated TG, cholesterol, and body fat[51]. The insulin resistance in this animal model probably results from the impairment of insulin-stimulated glucose uptake in insulin-responsive tissues as well as changes in hepatic glucose metabolism[52] Insulin resistance may also contributed to this hypertriglyceridemia by reducing the inhibitory effect of insulin on TG secretion rate in the liver of fructose fed rats[53].

The increase in TG level may be due to stimulation of hepatic VLDL-triacylglycerol synthesis and secretion and decreased VLDL-triacylglycerol clearance[54]. **Stanhope et al.**[55] indicate an increase in weight gain on diets rich in fructose, and a correlation between body fat and circulating TG has been established. In addition, HFrD induce leptin and insulin resistance. Taken together, leptin or insulin resistance and elevated TG serum levels may cause food over-consumption and contribute to the corresponding obesity, moreover, ingesting fructose-sweetened food raises calorie intake resulting in an over-consumption of energy which is not balanced by energy output, leading to weight gain[55].

As regard the comparison between HFD and HFrD fed groups, both groups showed dyslipidemia and glucose intolerance, however there were no significant differences between both groups in final body weight, fasting serum levels of glucose, insulin, total cholesterol, HDL-cholesterol, and LDL-cholesterol and HOMA-IR. While a significant increase in serum triglyceride, VLDL-cholesterol in HFrD fed group when compared with HFD fed group. These data are in consistent with that of **Liu and Manson**[57] who proved that diet high in carbohydrates is associated with glucose intolerance and obesity.

One of the main differences between glucose and fructose metabolism is that glucose must advance through a negatively regulated step using phosphofructokinase which regulates glycolysis in the liver. Fructose can bypasses this regulatory step and continue to be metabolized in the liver into glycerol-3-phosphate and acetyl coenzyme, these latter metabolites serve as substrates for glyceride synthesis leading to increased formation of VLDL and TG in the liver[58]. In contrast to glucose, when large amounts of fructose are ingested, the glycolytic pathway becomes saturated, and TG production is facilitated[59].

The present results showed an insignificant disturbance in all metabolic parameters measured in methanandamide treated normal diet pair fed group and methanandamide treated HFD pair fed group and methanandamide treated HFrD pair fed group when

compared with that of normal diet fed, HFD fed, and HFrD fed control groups respectively. However, methanandamide treated normal diet; ad libitum showed a significant disregulation of glucose metabolism "significant hyperglycemia, hyperinsulinemia, and increase HOMA-IR and significant dyslipdemia when compared with that of both ad libitum normal diet and methanandamide treated normal diet pair fed groups.

Taken together, this results proved that the ECS affects the metabolism by increasing the substrates that are needed for the lipogenesis, through increasing food intake and this concept is in line with **Williams and Kirkham**[21], as they proved that AEA injections in rats activated CB1 and promoted overeating, similar results were reported in rats injected with 2-AG[60]**.** ECS is considered to be primarily involved in the regulation of food intake via effects in the hypothalamus and nucleus accumbens[4]**.** $CB_1$ is selectively expressed in ventro-medial hypothalamus neurons. Absence of these neurons leads to weight gain, and their excitability is decreased in the presence of $CB_1$ agonists and increased by leptin[61].

ECs appear to interact with several other anorexigenic and orexigenic factors, clearly implicating the ECS in appetite regulation in a central control mechanism[2]**.** Cannabinoid and leptin signals are integrated in lateral hypothalamic neurons[62]**. Di Marzo et al.**[63] demonstrated that injecting mice with leptin, an anorexigenic adipokine that acts on the hypothalamus, resulted in a significant decrease in both AEA and 2-AG in the hypothalamus. They also showed that defective leptin signaling in the hypothalamus of obese db/db, ob/ob mice and Zucker rats was associated with an increase in endocannabinoid content.

Some studies stated that $CB_1$ antagonism has shown an improvement in insulin resistance and plasma glucose parameters, and a decrease in insulin and free fatty acid levels[12,64].

The absence of significant disturbance in methanandamide treated pair fed groups in comparison to other ad libitum control groups is in line with **Irwin et al.**[12] who suggested that the effect of ECS on disturbing the metabolism is due to increase food intake, depending in their study on the anorectic effect of the $CB_1$ blocker (AM 251) they observed that subchronic AM251 treatment in ob/ob mice caused weight reduction, improved the impaired metabolism and decrease insulin resistance, but these observations were found also in pair-fed control animals suggesting that the beneficial actions of $CB_1$ receptor antagonism is due to reduced food intake.

Specific central $CB_1$ blockade decreased body weight and food intake in diet induced obese rats, but had no beneficial effects on glucose metabolism; on the other hand, peripheral $CB_1$ blockade also reduced food intake and body weight but, in addition, enhanced insulin sensitivity. However, this improvement in insulin sensitivity was also detected in vehicle pair-fed rats, which suggest that decreased energy intake was the major factor responsible for these effects[65]**.**

Finally, collecting data from previous researches concerned with ECS and ECs benefits revealed that ECs have been used therapeutically for alleviating pain[66], And induces neuroprotection in ischemic brain areas[67]. Moreover, endocannabinoid signaling was proposed to protect against the consequences of stress in a certain dose range as low doses of methanandamide was proved to induce anxiolytic effects which are $CB_1$ receptor-mediated. In addition, $CB_1$ receptor activation at amygdala promotes fear extinction[68]. Cannabinoids are also effective in treatment of models of nausea and vomiting[69]**,** gastric ulcers, irritable bowel syndrome, ulcerative colitis, Crohn's disease, secretory diarrhea, paralytic ileus and gastro-esophageal reflux disease[70]**.**

In fact, many side effects related to the use of $CB_1$ receptor antagonists were proved as impairs fear extinction, increase anxiety-related behaviours in the elevated plus maze. In addition, both pharmacological antagonism and genetic inactivation of $CB_1$ receptors impair extinction of conditioned fear memories[71]. Moreover, acute injections of rimonabant or AM251 (CB1 antagonists) also increase both basal and stress-induced serum corticosterone levels[72]. Furthermore, mice lacking $CB_1$ receptors are impaired in actively coping with stress in a model predictive for antidepressant-like activity[73]. Thus, $CB_1$ receptor antagonism may induce psychiatric side-effects, in humans, mainly anxiety- and depression-like states, being in accordance with the notion that the endocannabinoid system acts to keep a set point counteracting aversive emotions[71]. Obese patients already suffer from anxiety or depression more frequently than non-obese subjects[74].

Taken together with the results of the present study, it can be concluded that ECS should not be palmed for metabolic disturbances but the type and amount of diet is the main cause and its receptors antagonists must not be the main target for treatment obesity.

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**5. References:**

**1- Devane W.A., Hanus L., Breuer A., Pertwee R.G., Stevenson L.A., Griffin G., and Mechoulam R.**

**(1992):** Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science*, 258:1946–1949.

**2- Pacher P., Batkai S. and Kunos G. (2006):** The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol Rev* , 58: 389-462.

**3- Greenberg I., Kuehnle J., Mendelson J. H., and Bernstein J.G. (1976):** Effects of marihuana use on body weight and caloric intake in humans. *Psychopharmacology*, 49:79-84.

**4- Pagotto U., Marsicano G. and Cota D. (2006):** The emerging role of the endocannabinoid system in endocrine regulation andenergy balance. *Endocrine Reviews*, 27(1): 73–100.

**5- Nakata M and Yada T. (2008):** Cannabinoids inhibit insulin secretion and cytosolic Ca2+ oscillation in islet beta-cells via CB1 receptors. *Regul Pept*, 145:49-53.

**6- Bermudez-Silva F.J., Suarez J., Baixeras E., and Cuesta-Munoz AL. (2008):** Presence of functional cannabinoid receptors in human endocrine pancreas. *Diabetologia* , 51:476–487.

**7- Thomas CD., Peters JC., Reed GW. (1992):** Nutrient balance and energy expenditure during ad libitum feeding of high-fat and high-carbohydrate diets in humans. *Am J Clin Nutr*., 55:934-942.

**8- Giugliano D., Ceriello A. andosito K. (2006):** The effects of diet on inflammation: emphasis on the metabolic syndrome. *J. Am. Coll. Cardiol.*, 48:677–685.

**9- Torres I., El Hafidi M., Gonzdlez J., Infante O., and Baos G. (2007:** Modulation of aortic vascular reactivity by sex hormones in a male rat model of metabolic syndrome. *Life Sciences* 80 2170–2180.

**10- Osei-Hyiaman D., Harvey-White J., Batkai S., Kunos G. (2006):** The role of the endocannabinoid system in the control of energy homeostasis. *Int. J. Obes.*, 30:S33–S38.

**11- Liu YL., Connoley IP., Wilson CA. and Stock MJ. (2005):** Effects of the cannabinoid CB1 receptor antagonist SR141716 on oxygen consumption and soleus muscle glucose uptake in Lep(ob)/Lep(ob) mice. *Int J Obes Relat Metab Disord*, 29:183–187.

**12- Irwin N., Hunter K., Frizzell N. and Flatt PR. (2008):** Antidiabetic effects of sub-chronic administration of the cannabinoid receptor (cb1) antagonist, am251, in obese diabetic (ob/ob) mice. *Eur.. J. of. Pharmacol.*, 581:226-233.

**13- Zhou D. and Shearman LP. (2004):** Voluntary exercise augments acute effects of CB1-receptor inverse agonist on body weight loss in obese and lean mice. *Pharmacology*, *Biochemistry*, *and Behavior*, 77(1): 117–125.

**14- Sink KS, Segovia KN, Collins LE, Markus EJ, Vemuri VK and Salamone JD. (2010)**: The CB1 inverse agonist AM251, but not the CB1 antagonist AM4113, enhances retention of contextual fear conditioning in rats. *Pharmacol Biochem Behav.* 95(4):479-84.

**15- Starowicz K.M., Cristino L. and Matias I. (2008):** Endocannabinoid dysregulation in the pancreas and adipose tissue of mice fed with a high-fat diet. *Obesity*, 16(3): 553–565.

**16- Lesourd B and Mazari L: (1999):** Nutrition and immunity in the elderly. *Proceeding of Nutrition Society*, 58 (3):94-200.

**17- Ahren B. and Scheurink A.J.W. (1998):** Marked hyperleptinemia after high fat diet associated with severe glucose intolerance in mice. *Eur. J.Endocrinol.,* 139:461-467.

**18- Cha M.C., Chou J., Boozer C.N (2000):** High-fat diet feeding reduces the diurinal variation of plasma leptin concentration in rats. *Metab.,* 48:503-507.

**19- Shapiro A, *Mu W, Roncal CA, Cheng K-Y, Johnson RJ, Scarpace PJ* (2008):** Fructose-induced leptin resistance exacerbates weight gain in response to subsequent high fat feeding. *Am J Physiol Regul Integr Comp Physiol*., 295(5):R1370-1375.

**20- Gui Y., Silha J.V., Murphy L.J. (2004):** Sexual dimorphism and regulation of resistin, adiponectin, and leptin expression in the mouse. *Obes. Res.,* 12:1481–1491.

**21- Williams C.M. and Kirkham T.C. (1999):** Anandamide induces overeating: mediation by central cannabinoid (CB1) receptors. *Psychopharmacology*, 143:315-317.

**22- Costa B, Conti S, Giagnoni G, Colleoni M (2002):** Therapeutic effect of the endogenous fatty acid amide, palmitoylethanolamide, in rat acute inflammation: inhibition of nitric oxide and cyclo-oxygenase systems. *Br J Pharmacol;*137(4):413-420.

**23- Yang H., Lauv S., Sinclair D.A. (2006):** Nampt/PBEF/Visfatin: a regulator of mammalian health and longevity. *Exper. Geront.,* 41:718-726.

**24- Trinder P (1969):** Enzymatic determination of glucose. *An. Clin. Bioch.*, , 6:24-27.

**25- Starr J.I., Mako M.E., Juhn D., Rubenstein A.H. (1978):** Measurement of serum pro-insulin–like material: cross reactivity of porcine and human proinsulin. *J. Lab. Clin. Med.,* 91:691-692.

**26- Allain C (1974):** Enzymatic determination of total serum cholesterol. *Clin. Chem*., 20:470-475.

**27- Warnick GR, Benderson V, Albrs N (1983):** Selected methods. *Clin. Chem.,* 10:91-99.

**28- Naito HK (1989):** Triglycerides in clinical chemistry: theory, analysis and correlation. (2nd ed) Eds, Kaplan LA and Pesce AJ.U.S.A., p: 997.

**29- Friedewald WT, Levy RI, Fredrickson DS (1972):** Estimation of the concentratin of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem*., 18:499-502.

30- **Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. (1985):** Homeostasis model assessment: insulin resistance and beta-cell function from fasting serum glucose and insulin concentrations in man. Diabetologia, 28:412:419.

31- **De Petrocellis L., Cascio MG. and Di Marzo V. (2004):** The endocannabinoid system: a general view and latest additions. British Journal of Pharmacology, 141:765–774.

32- **Alsaif M.A. and Duwaihy M.M.S. (2004):** Influence of dietary fat quantity and composition on glucose tolerance and insulin sensitivity in rats. *Nutrition Research*, 24:417–425.

33- **Buettner R., Scholmerich J., Bollheimer L.C. (2007):** High-fat diets modeling the metabolic disorders of human obesity in rodents *Obesity (Silver Spring),* 15: 408–409

34- **Kraegen EW, Cooney GJ.(2008):** Free fatty acids and skeletal muscle insulin resistance. *Curr Opin Lipidol. 19*(3): 235-241.

35- **Pedersen O., Kahn C.R. and Flier J.S. (1991**): High fat feeding causes insulin resistance and a marked decrease in the expression of glucose transporters (GLUT4) in fat cells of rats. *Endocrinology*, 129: 771-777.

36- **Naitoh R, Miyawaki K, Harada N, Mizunoya W, Yamada Y, Seino Y, Inagaki N. (2008):** Inhibition of GIP signaling modulates adiponectin levels under high-fat diet in mice. *Biochem Biophys Res Commun.* 2008 376(1):21-25.

37- **Zietz B., Herfarth H., Paul G., Ehling A., Muller-Ladner U., Scholmerich J. (2003):** Adiponectin represents an independent cardiovascular risk factor predicting serum HDL-cholesterol levels in type 2 diabetes. *FEBS Lett.*, 545:103–104.

38- **Yamauchi T., Kamon J., Minokoshi Y., Ito Y., Waki H., Uchida S. (2002):** Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat. Med.*, 8:1288–1295.

39- **Tschop and Heiman. (2001):** Rodent obesity models: an overview, Exp. Clin. Endocrinol. *Diabetes*, 109:307–319.

40- **Castan-Laurell I., Boucher J., Dray C., Daviaud D., Guigne C., Valet P. (2005):** Apelin, a novel adipokine overproduced in obesity: friend or foe? Molecular and Cellular Endocrinology, 245:7–9.

41- **Boucher J., Masri B., Daviaud D., Gesta S., Guigne C., Mazzucotelli A., Audigier Y., and Valet P. (2005):** Apelin, a newly identified adipokine up-regulated by insulin and obesity. *Endocrinology*, 146:1764–1771.

42- **Heinonen MV., Purhonen AK., Miettinen P., Pirinen E., Alhava E., Akerman K., Herzig KH. (2005):** Apelin, orexin-A and leptin plasma levels in morbid obesity and effect of gastric banding. *Regul Pept*, 130:7–13.

43- **Li L., Yang G., Li Q., Tang Y., Yang M., and Li K. (2006):** Changes and relations of circulating visfatin, apelin, and resistin levels in normal, impaired glucose tolerance, and type 2 diabetic subjects. *Experimental* Clinical Endocrinology and Diabetes, 114:544–548.

44- **Beltowski J. (2006):** Apelin and visfatin: unique "beneficial" adipokines upregulated in obesity? *Medical Science Monitor.,* 12:112–119.

45- **Watanabe S., Doshi M. and Hamazaki T. (2003):** n-3 Polyunsaturated fatty acid (PUFA) deficiency elevates and n-3 PUFA enrichment reduces brain 2-arachidonoylglycerol level in mice. *Prostaglandins Leukot. Essent. Fatty Acids*, 69:51–59.

46- **Yan ZC., Liu DY. And Zhang LL. (2007):** Exercise reduces adipose tissue via cannabinoid receptor type 1 which is regulated by peroxisome proliferator-activated receptor-delta. *Biochemical and Biophysical Research Communications*, 354(2): 427–433.

47- **Herling AW., Kilp S. and Elvert R. (2008):** Increased energy expenditure contributes more to the body weight-reducing effect of rimonabant than reduced food intake in candy-fed wistar rats. *Endocrinology* , 149(5): 2557–2566.

48- **Motaghedi R. and McGraw TE. (2008):** The CB1 Endocannabinoid System Modulates Adipocyte Insulin Sensitivity. Obesity (Silver Spring) , 16(8): 1727–1734.

49- **Lê KA, Faeh D, Stettler R, Ith M, Kreis R, Vermathen P and Tappy L. (2006):** A 4-wk high-fructose diet alters lipid metabolism without affecting insulin sensitivity or ectopic lipids in healthy humans. *Am J Clin Nutr.*, 84(6):1374-1379.

50- **Alzamendi A, Castrogiovanni D, Ortega HH, Gaillard RC, and Spinedi E (2010):** Parametrial adipose tissue and metabolic dysfunctions induced by fructose-rich diet in normal and neonatal-androgenized adult female rats. *Obesity,*18(3):441-448.

51- **de Moura RF, Ribeiro C, de Oliveira JA and de Mello MA (2009):** Metabolic syndrome signs in Wistar rats submitted to different high-fructose ingestion protocols. *Br J Nutr.* 101(8):1178-1184.

52- **Boivin A, Deshaies Y.(1995):** Dietary rat models in which the development of hypertriglyceridemia and that of insulin resistance are dissociated. *Metabolism*, 44(12):1540-1547.

53- **Storlien LH., Oakes ND., Pan DA., Kusunoki M. and Jenkins AB. (1993):** Syndromes of insulin resistance in the rat: inducement by diet and amelioration with benfluorex. *Diabetes*, 42:457-62.

**54- Parks EJ. and Hellerstein MK. (2000):** Carbohydrate-induced hyper triacylglycerolemia: historical perspective and review of biological mechanisms. *Am J Clin Nutr.*, 71:412–433.

**55- Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, and Hellerstein MK, (2009):** Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J Clin Invest*. 119(5):1322-1334.

**56- Bray GA., Nielsen SJ. and Popkin BM. (2004):** Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *Am J Clin Nutr.,* 79:537– 543.

**57- Liu S and Manson JE.(2001):** Dietary carbohydrates, physical inactivity, obesity, and the 'metabolic syndrome as predictors of coronary heart disease. *Curr Opin Lipidol.* 12(4):395-404.

**58- Mayes PA (1993):** Intermediary metabolism of fructose. *Am J Clin Nutr.*, 58(5):754S-765S.

**59- Elliott SS., Keim NL., Stern JS. and Havel PJ. (2002):** Fructose, weight gain, and the insulin resistance syndrome. *Am J Clin Nutr.*, 76:911–922.

**60- Kirkham T.C., Williams C.M., Fezza F.and Di Marzo V. (2002):** Endocannabinoid levels in rat limbic forebrain and hypothalamus in relation to fasting, feeding and satiation: stimulation of eating by 2-arachidonoyl glycerol. *Br J Pharmacol*, 136:550-557.

**61- Dhillon H., Zigman JM.and Ye C. (2006):** Leptin directly activates SF1 neurons in the VMH, and this action by leptin is required for normal body-weight homeostasis. *Neuron*, 49(2): 191–203.

**62- Jo YH., Chen YJ. And Chua Jr. SC. (2005):** Integration of endocannabinoid and leptin signaling in an appetite-related *neural circuit. Neuron*, 48(6): 1055–1066.

**63- Di Marzo V., Goparaju SK., Wang L. (2001):** Leptin-regulated endocannabinoids are involved in maintaining food intake. *Nature*, 410: 822–825.

**64- Ravinet Trillou C., Arnone M., Delgorge C., Keane P., Maffrand J.P., Soubrie P. (2003):** Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.,* 284:R345-R353.

**65- Nogueiras R, Rohner-Jeanrenaud F., Woods S.C., Tschöp M.H. (2008):** The endocannabinoid system and the control of glucose homeostasis. *J Neuroendocrinol.* 1:147-151.

**66- Agarwal N., Pacher P. and Tegeder I**. **(2007)**: Cannabinoids mediate analgesia largely via peripheral type 1 cannabinoid receptors in nociceptors, *Nature Neuroscience* ,**10**: 870–879.

**67- Ashton J.C., Rahman R.M., Nair S.M., Sutherland B.A., Glass M., Appleton I. (2007):** Cerebral hypoxia-ischemia and middle cerebral artery occlusion induce expression of the cannabinoid CB2 receptor in the brain. *Neurosci Lett*., 412:114-117.

**68- Marsicano G., Wotjak C.T. and Azad S.C. (2002):** The endogenous cannabinoid system controls extinction of aversive memories, *Nature* **418**: 530–534

**69- Rock E.M., Limebeer C.L., Mechoulam R. (2008)**: The effect of cannabidiol and URB597 on conditioned gaping (a model of nausea) elicited by a lithium-paired context in the rat, *Psychopharmacology,* **196:** 389–395.

**70- Di Carlo G. and Izzo A. A. (2003):** Cannabinoids for gastrointestinal diseases: potential therapeutic applications. *Expert. Opin. Investig. Drugs*., 12(1): 39-49.

**71- Moreira F.A. and Lutz B. (2008):** The endocannabinoid system: emotion, learning and addiction, *Addiction Biology*, 13: 196–212.

**72- Patel S., Roelke C.T., Rademacher D.J. (2004):** Endocannabinoid signaling negatively modulates stress-induced activation of the hypothalamic-pituitary-adrenal axis, *Endocrinology* **145**: 5431–5438.

**73- Steiner M.A., Wanisch K., Monory K. (2008):** Impaired cannabinoid receptor type 1 signaling interferes with stress-coping behavior in mice, *The Pharmacogenomics Journal* **8**: 196–208.

**74- Dixon J.B., Dixon M.E., O'Brien P.E. (2003):** Depression in association with severe obesity: changes with weight loss, *Archives of Internal Medicine,* **163**: 2058–2065

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