The Effect of High Fat High Fructose Diet (Modification of AIN-93M) on Nuclear Factor Kappa Beta Expression in the Liver Tissue of Male Sprague Dawley Rats

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Abstract. Metabolic inflammation (low-grade inflammation) remains an etiopathogenic key factor in the development of metabolic syndrome. Nuclear Factor Kappa Beta (NF-κβ) is a transcription regulator of genes having a role in immunity, the inflammatory response which can be associated with obesity-related pathological conditions like nonalcoholic fatty liver (NAFLD). Various stimuli, such as metabolic stress (hyperglycemia, ROS, fat metabolism) and proinflammatory cytokines (TNF-α, IL-6, IL-1β) could activate NF-κβ. This study was aimed to investigate the underlying molecular mechanisms of NAFLD in rats fed a modified AIN-93M HFHF (High Fat High Fructose) diet. The design of this study was experimental post-test only controlled group design. Thirty male Sprague Dawley rats were distributed into 2 treatment groups by a completely randomized design (CRD) technique. The sacrifice was performed after 17 weeks of treatment. NF-κβ expression was assessed by an immunohistochemical method (IRS score). The results showed there were significant differences in feed intake and energy intake between groups P1 and P2 (p = 0.000, p = 0.000). The average NF-κβ expression in the P2 group was significantly higher (p = 0.000) compared to the control group (P1). The correlation test between dietary intake and NF-κβ expression proved that there was a positive correlation between energy, carbohydrate and fat intake on NF-κβ expression (p = 0.001, 0.000, 0.046). However, there was a negative relationship between protein intake and NF-κβ expression (p = 0.000). This study concluded the modified AIN-93M HFHF diet increased NF-κβ expression in the liver tissue of male Sprague Dawley rats.

1. Introduction

Obesity is a complex and chronic condition, originating from several physiological interactions, specific biological, individual, and environmental factors affecting appetite and the process of energy metabolism [1, 2]. Obesity occurs due to the enlargement and increases fat/adipose tissue that accumulates excessively, that could lead into health problems and lower life expectancy [1, 3]. The prevalence of obesity has increased over the past three decades. Globally, there are 650 million people affected by obesity, while Indonesia is among the 10 highest prevalence of obesity in the world. National prevalence of obesity in adults (> 18 years) is 15.4% [3-5]. The accumulation of adipose tissue would
induce metabolic inflammation that triggers insulin resistance, type 2 Diabetes Mellitus, dyslipidemia, atherosclerosis, and nonalcoholic fatty liver (NAFLD) [6, 7]. Therefore, obesity becomes one of the risk factors of NAFLD [8].

Obesity can be induced to experimental animals with high fat diets with various percentages, ranging from 20% to 60% [8-13]. In some studies, high-fat and fructose (HFHF) diets consisting of high-fat diets (HFD) and fructose, can also trigger oxidative stress in tissue and plasma, metabolic syndrome (obesity), and obesity-related-disease, as non-alcoholic fatty liver [14-16]. The key factors in the development of metabolic diseases including obesity are chronic low-grade inflammation triggered by nutrients and metabolic signals, also known as metabolic inflammation [7].

Nuclear Factor Kappa Beta (NF-κβ) is a transcription factor that regulates inflammation, specific and nonspecific immune system, wound healing response, and cell homeostasis, activated through 2 signal cascade: classical (canonical) and alternative (non-canonical) pathways [17]. Activation of NF-κβ could be triggered by various stimuli, such as metabolic stress (hyperglycemia, ROS, fat metabolism), radiation (ultraviolet) and proinflammatory cytokines (TNF-α, IL-1β, IL-6). Active NF-κβ will translocate into the nucleus and transcribe various specific genes that produce cytokines such as TNF-α, IL-6, IL-1β, IL-10. This cytokines lead to insulin resistance and liver damage [14, 18-21]. Consequently, NF-κB is the key that can connect metabolism, inflammation & insulin work to each other [21].

Previous studies related to the expression of NF-κβ in experimental animals with metabolic syndrome (obesity and type 2 Diabetes Mellitus) used HFD as an induction feeding [22-24]. According to Cai, identification of NF-κβ expression related to insulin resistance could be carried out in the central nervous system (hypothalamus) and peripheral metabolic tissues such as the adipocytes and liver, due to its function in nutrient metabolism, where NF-κβ has an important role [22, 25, 26]. Globally, the effect of AIN-93M modification as high fat high fructose (HFHF) diet on NF-κβ expression in liver tissue of experimental animals have not been widely studied. Therefore, this research was aimed to broaden knowledge and improve science in the field of molecular nutrition.

2. Material and Methods

2.1. Study Design

The design of this study was true experimental in vivo Post Test Only Controlled Group Design. The subjects of this study were male Sprague Dawley rats randomized into two groups, namely P1 receiving AIN-93M standard diet (control) and P2 receiving HFHF (High Fat High Fructose) AIN-93M modification diet. This research has been approved by ethical research committee from the Medical Faculty, Universitas Brawijaya Malang, Indonesia, Number 368/EC/KEPK/10/2017.

2.1.1. Criteria and Sample Size of Research Subject. The inclusion criteria of samples were male Sprague Dawley rat, age ±3 months old, complete body organs and clear eyes, body weight of 200-250 grams. Meanwhile the exclusion criteria were rats with disabilities and experience anatomic abnormalities. Formula for number of samples [Federrer Formula: (t-n) (n-1) ≥ 15, t: number of treatment groups, n: number of samples in each group]. The total sample was 36 rats.

2.2. Treatment

Twelve week old male Sprague Dawley rats were randomized into 2 groups, namely the AIN-93M standard diet group or P1, (nutritional value of 42.87%, fat 25.81%, protein 31.32% with energy density (DE) of 4.21 kcal/gram) and the HFHF with AIN-93M modification diet group or P2 (with nutritional value of carbohydrates, fat, and protein are 29.9 %, 51.64%, and 21.81%, respectively, with DE 5.08 kcal / gram and Liquid fructose with 30% concentration was added for P2 group for rat drinking). All groups were given food and drink ad libitum for 17 weeks of treatment. At the end of the study period, surgeries were performed and Nuclear Factor Kappa Beta (NF-κβ) expression in liver of subjects were assessed. The study was conducted in the Nutrition and Anafarma Laboratory, Malang Health Politeknik, Health Ministry, Malang, BioSains Laboratory, Biochemistry-Biomolecular Laboratory,
Pathology-Anatomy Laboratory, Faculty of Medicine, Brawijaya University Malang and Pathology-Veterinary Laboratory, Faculty of Veterinary Medicine, Airlangga University Surabaya.

2.3. Collecting Data
1) Data on food intake was obtained by weighing the remaining feed given, done every 2 days. The average would act as daily intake data.
2) Data on fluid intake was obtained by measuring the remaining beverage to the initial volume of daily supply, which is 250 ml.
3) Body weight data was obtained by weighing each subject every week.
4) The NF-κβ expression data was obtained by semiquantitative IRS (Immuno-reactive Score) method. NF-κβ expression was identified by brownish color in rats liver cells (cytoplasm and nucleus), using a light microscope / slide scanner in 5 different fields of view (LP) with 100x and 400x magnifications [27, 28]
5) Histopathological examination and identification of NF-κβ expression were carried out by pathologist from the Department of Veterinary Pathology, Faculty of Veterinary Medicine, Airlangga University.

2.4. Data Analysis
Normal distributed and homogeneous data was analyzed by Independent T-test and Pearson statistical test while non-parametric tests would be used if the terms were not fulfilled. The analysis was processed by IBM SPSS Statistics 16 for Windows with a confidence level of 95%. The p value was significant if it was less than 5% (p < 0.05).

3. Results
3.1. Subjects Characteristic
The characteristic of the rats in this study was presented in Table 1.

| Type             | P1                | P2                | p-value* |
|------------------|-------------------|-------------------|----------|
| Number (n)       | 14                | 16                | -        |
| Sex              | Male              | Male              | -        |
| Age              | ± 3 months        | ± 3 months        | -        |
| Initial Body Weight** | 246.11 ± 19.10   | 245.58 ± 22.05    | 0.945    |

P1 = AIN-93M standard diet, P2 = High Fat High Fructose Diet (HFHF) AIN-93M modification, *p-value = independent T-test results (p < 0.05), **Mean ± SD

The number of samples survived until the end of the study were 30 rats. Data on the initial body weight of the treatment group were normally distributed and homogeneous. Based on the results of the comparative independent T-test, it was found that there was no significant difference between the initial body weight of group P1 (AIN-93M standard diet) and P2 (AIN-93M modified HFHF diet) (p = 0.945).

3.2. Diet Characteristic
Nutrient composition on feeding was presented in Table 2.

| Treatment group | P1                | P2                |
|-----------------|-------------------|-------------------|
| Energy (kcal)   | 421.00            | 508.00            |
| Carbohydrate (gram; %) | 45.12; 42.87 | 37.33; 29.39    |
| Protein (gram; %) | 32.96; 31.32 | 27.70; 21.81    |
3.3. Food and Nutrient Intake

Food and nutrient intake of P1 and P2 during the 17-week treatment period was shown in Figure 1.

![Figure 1. Average Food Intake per Week (gram).](image)

The trend of total energy intake in each week were shown in Figure 2 below.

![Figure 2. Average Total Energy Intake per Week (mL).](image)

The analysis of intake data, namely the average weight of feed intake (AIN-93M standard diet and AIN-93M modified HFHF diet), total energy, and nutrients (from food and beverages) during the 17-week treatment period are presented in Table 3.

### Table 3. The Average of Food Intake, Total Energy, and Nutrient per Day.

| Parameter          | Treatment Group | p-value |
|--------------------|-----------------|---------|
|                    | P1***           | P2***   |
| Food (g)           | 12.02 ± 1.62    | 6.31 ± 1.12 | 0.000** |
| Fructose 30% (mL)  | 50.60 ± 6.85    | 67.75 ± 6.38 | 0.000* |
| Energy (kcal)      | 5.42 ± 0.73; 42.84% | 11.27 ± 1.13; 66.53% | 0.000** |
| Carbohydrate (g); %| 3.96 ± 0.53; 31.30% | 1.74 ± 0.31; 10.27% | 0.000** |
| Protein (g); %     | 1.45 ± 0.19; 25.79% | 1.84 ± 0.32; 24.44% | 0.001* |
P1 = AIN-93M standard diet, P2 = High Fat High Fructose Diet (HFHF) AIN-93M modification, *p-value = value of independent T-test (p < 0.05), **p-value = value of Mann Whitney test (p < 0.05), ***Mean ± SD

3.4. Body Weight of Experimental Rats
The description on body weight changes of the rats during the study period was shown in Table 4.

| Parameter              | Treatment Group | p-value* | p-value** | p-value*** |
|------------------------|-----------------|----------|-----------|-----------|
| Initial Body Weight    | P1****          | 246.11 ± 19.10 | 245.58 ± 22.05 | 0.945     |
| Final Body Weight      | P2****          | 261.93 ± 29.29 | 276.92 ± 35.65 | 0.223     |
| Body Weight Changes    |                 | 15.82 ± 2.37  | 31.33 ± 2.63  | 0.103     |

Based on Table 4, the independent T-test revealed no significant differences between initial and final body weight in both treatment groups (p-value initial weight = 0.945, final p-value BB = 0.223). If final body weight and initial body weight were compared, there was a weight increase during the 17 weeks of the study. The weight changes in rats in P2 group (31.33 ± 2.63) were greater than those in group P1 rats (15.82 ± 2.37).

![Figure 3. Average Body Weight per Week (grams).](image)

The paired T-test revealed a significant difference between initial body weight and final weight at the end of the study period in each treatment group (p-value P1 = 0.027, meanwhile p-value P2 = 0.000). However, independent t test found no significant difference in body weight changes between P1 and P2 (p-value = 0.103). The fluctuation of the body weight from the first week to the end of the study (17th week) was shown in Figure 3.

3.5. NF-κβ Expression in the Liver Tissue of Experimental Rats
The images of liver tissue scans of P1 and P2 can be seen in Figure 4 below.
Figure 4. Expression of Nuclear Factor-κβ (NF-κβ) in the Liver, with Immunohistochemical Staining, Magnification of 400x.
Description: P1 = a group of rats given a standard AIN-93M diet, P2 = a group of rats given a modified AIN-93M diet. The arrow showed the NF-κβ expression identified in the field of view. In P1, NF-κβ expression shows that the total IRS value is 1 (negative category), while P2 group shows NF-κβ expression with IRS 4 value (medium category). Arrows: NF-κβ expression on hepatocytes (nucleus and cytoplasm).

The Shapiro-Wilk test showed normally distributed NF-κβ expression (p = 0.069). The average of NF-κβ expression in liver in P1 and P2 groups is presented in Figure 5 below.

Figure 5. The Average of NF-κβ Expression (IRS) in Rat Liver.
Description: P1 = AIN-93M standard diet, P2 = High Fat High Fructose Diet (HFHF) AIN-93M modification, *p-value = value of independent T-test (p <0.05), **Mean ± SD

Data showing the correlation between average dietary intake and NF-κβ expression (IRS) in liver tissue of rats are listed in Table 5.

Table 5. Correlation between average dietary intake and NF-κβ expression (IRS) in liver tissue of male Sprague Dawley rats.

|                     | p-value NF-κβ expression (IRS) | r***  |
|---------------------|-------------------------------|-------|
| Energy (kkal)       | 0.001*                        | 0.564 |
| Carbohydrate (g)    | 0.000**                       | 0.652 |
| Protein (g)         | 0.000**                       | -0.820|
other studies. However, the carbohydrate utilising a high fat high fructose and 20% fructose solution concentration. Zarghani et al. in a study related to a diet that induces NAFLD Lee a concentration of fructose solution than this study, suc fructose concentration was similar with the other studies. Castillo et al. also supported the study that in 100 grams of HFD contained 560 kcal of energy, 24% carbohydrate, 18% protein, and 58% fat. Santos et al. stated that the energy from the used High Fat High Fructose) with AIN-93M modification is a modified diet that combines High Fat Diet (HFD) and High Fructose (HF) feeding. The energy and nutrient content in 100 grams of HFD used by this study was 508 kcal of energy, 32% carbohydrate, 16% protein, 50% fat, and 2% fiber. The value of energy, protein, and fat from HFD in this study were higher than the other two studies.

However, unsimilarly, research by So et al. used 100 grams of HFD containing 516 kcal of energy, 20% carbohydrate, 20% protein, and 60% fat. Castillo et al. also supported the study that in 100 grams of HFD contained 560 kcal of energy, 24% carbohydrate, 18% protein, and 58% fat. The energy and fat content of this study was lower, compared to the latters with a difference of 8-52 kcal in energy and 6.36% - 8.36% in fat content. The High Fructose liquid of 30% concentration was given ad libitum as a drink for P2 group, with a content of 120 kcal of energy and 30 grams of carbohydrate per 100. This concentration was similar with the study conducted by Lee et al., Lambertz et al., Yoo et al. using 30% fructose solution in their research. There are also several studies with lower or higher concentrations of fructose solution than this study, such as the studies of Vilà et al. and Fariba et al. with a concentration of 10% and Vasiljević et al. with a concentration of 60%.

The HFHF diet in this study had a high proportion of fat and fructose, 51.64% and 30%, respectively. Lee et al. using the HFHF diet for one of the treatment groups, used HFD containing 60% fat percentage and 20% fructose solution concentration. Zarghani et al. in a study related to a diet that induces NAFLD utilizing a high fat high fructose (HFHFr) diet containing 53% fat and 20% fructose solution.
The fat percentage in this study was lower than fat percentage in the studied conducted by Lee et al. and Zarghani et al., but already classified as a high-fat diet (High Fat Diet). The concentration of fructose solution in this study was higher than the two.

Based on the results of the Mann Whitney statistical test, it was found that the average of daily food intake of group P1 (AIN-93M standard diet) and P2 (AIN-93M modified HFHF diet) differed significantly ($p < 0.05$). The results in Table 5.3 showed that the average feed intake in group P1 was higher than group P2, with a value of $12.02 \pm 1.62$ grams in group P1 and $6.31 \pm 1.12$ gram in group P2. This was in accordance with the results of the study of Zarghani et al. and Yoo et al. that feed intake in the control group was greater than the HFHF treatment group [36, 41]. According to Curfs et al. in the Handbook of Laboratory Animal Science, the estimated average of daily food intake in adult mice is 25-30 grams per day [42]. When compared with this number, the average of daily food intake of rats in both group was below the average.

The level of feed consumption in experimental animals was influenced by body weight, individual animals, feed types, and environmental factors. In addition there are also palatability factors for feed, taste, texture, size and consistency of feed [43]. Some factors that may cause lower intake of HFD diet (atherogenic) were rat physiological factors and feed factors. Rat physiological factors, related to eating behavior, may occur due to satiation while getting a HFHF diet. Rat feed factor was related to the form of feed, texture and state of organoleptics [44]. Moreover, there were also several environmental factors such as the state of the cage and lighting that can affect the feed intake of mice [45, 46].

Based on the texture, the modified AIN-93M HFHF feed was softer and greasy than the AIN-93M standard feed due to the addition of lard (cow fat) to the modified AIN-93M HFHF. Lard was added to increase the level of saturated fat (SFA) in the feed. Lard plays a significant role in SFA intake, because every 100 grams of lard has 39 grams of SFA [47, 48]. This soft and oily feed texture could be related to low intake because rats’ preference of solid and hard feed texture [49].

The average intake of 30% fructose in P2 group rats was $29.73 \pm 3.88$ mL daily ~ energy $31.02$-$40.33$ kcal per day. The intake in the P2 group had met the standards (rat daily water intake was about 15-30 mL) [43]. Another study showed that drinking intake in the treatment group given a standard chow diet and fructose solution was 30% higher than the control group, with a value of $38.6 \pm 7.6$ mL per day [50]. This shows that giving fructose can affect the average volume of drink intake in rats.

The total daily energy intake was obtained by summing the energy from feed and drinks. The results of the Independent T-test stated that the energy intake of the P1 and P2 groups differed significantly ($p < 0.05$). Based on the results, the average daily energy intake of P2 group ($67.75 \pm 6.38$ kcal) was higher than that in group P1 ($50.60 \pm 6.85$ kcal), although the feed intake of the P2 group was actually lower than group P1. This occurs because the energy density of the P2 group is greater ($5.08$ kcal / gram) and was also added by the energy intake from 30% fructose liquid, while the energy density in group P1 feed was 4.21 kcal / gram without any addition from the drink. The results of this study were similar with Yoo’s study which is stated that calorie intake of the treatment group (30Frc + 45Fat) was greater than the control group (RD) [36]. Decreasing feed intake could originate from subjects learning to compensate for high energy density diets by consuming less amounts of feed [51].

### 4.2. Changes in Experimental Rat Weight

The development of body weight of experimental animals was observed during the study period (17 weeks) and body weight was measured every 7 days. The average body weight description in experimental animals can be seen in Figure 3 where the increase in body weight in group P1 continued to occur from week 1 to 7, then fluctuates along the rest of study period. While in P2 group, body weight tended to increase starting from week 1 to week 13, then began to experience weight loss in the 14th week. The average initial body weight of group P1 rats was $246.11 \pm 19.10$ grams while the P2 group was $245.58 \pm 22.05$ grams. Based on the data in Table 4, there was an increase in body weight in both groups, $15.82 \pm 2.37$ grams in the P1 (6.42%) and $31.33 \pm 2.63$ grams in the P2 (12.76%). Although there were differences in weight gain between groups P1 and P2, the values did not differ significantly.
On the contrary, Zarghani’s study in Wistar rats with a composition of dietary HFHF concentration of 20% fructose and 53% fat content of total energy and the composition of a standard diet containing 14.5% fat from thermal energy showed that body weight in the HFHF diet group was significantly higher than other groups [41]. Another study conducted by Yoo also showed the results of the group given the HFHF diet significantly had higher body weight than the control group [36]. Although the increase in body weight in groups P1 and P2 in this study did not differ significantly, the pattern of differences between treatment groups had been seen. Meanwhile, the paired T-test stated that there was a significant difference in body weight between the initial and final body weight at the end of the study period in each treatment group. So that it can be said that the weight of both group had increased significantly. The results of this study were in line with of Lee et al. stating that weight in the group given the HFHF diet increased significantly [35]. Obesity in experimental animals can be assessed based on several criteria, such as (1) increase in body weight or Lee Index and/or (2) increase in body fat levels [52]. According to some studies, experimental animals were said to be moderately obese if there was an 10-25% increase in body weight, and severe obese if the increase in body weight exceeded 40% of the initial body weight [53-55]. Based on the cut-off, the P2 group was not classified as obese. Lee's index is an approach to measure BMI (Body Mass Index) of the rats using the body weight and body length of mice [56]. The previous part of this study stated that the average Lee Index calculation in P2 group (285.06 ± 10.15 g / cm) was greater than group P1 (285.06 ± 10.15 g / cm) but not significantly different [57]. It was also written that the P2 group rats were more obese compared to group P1 rats.

Hariri & Thibault wrote that obesity occurs when the stored energy is greater than the energy released, so that the reserved energy in body fat increases, especially in adipose tissue. The state of obesity can involve one or both of the increase in the number of adipocytes (hypertrophy) or their size (hypertrophy) [52]. The previous part of this study, discussed the effect of the modified AIN-93M HFHF diet on body fat index [BFI] of male Sprague Dawley rats [58]. The results of this study found that BFI in P2 group (3.69 ± 0.77%) was smaller than group P1 (3.93 ± 1.18%) and did not differ significantly. Rats in P2 group cannot be categorized as obese because they have a low BFI value. Based on these parameters, it can be seen that the P2 rats could not be classified as obese. It can be concluded that the modified AIN-93M HFHF diet in this study had not been proven to cause obesity in male Sprague Dawley rats.

4.3. NF-κβ Expression in Experimental Rat Liver
Obesity is one of the risk factors for nonalcoholic fatty liver disease (NAFLD). NAFLD can be identified by the presence of fat accumulation (triglycerides) in hepatocytes [15]. The NAFLD pathogenesis is known as the 'two-hit' hypothesis, (1) fat metabolism and insulin resistance could induce simple fat accumulation and fatty liver and (2) associated with oxidative stress, inflammation and certain other factors. Oxidative stress in NAFLD can cause accumulation of triglycerides and free fatty acids in the liver, and mitochondrial dysfunction. Fatty acid oxidation can trigger oxidative stress in the liver, decreasing of antioxidants, and mitochondrial dysfunction, as a result, proinflammatory cytokines such as TNF-α, IL-6, IL-1β were released [21, 23].

Nuclear beta kappa factor (NF-κβ) is a transcription factor that becomes a central mediator of inflammation and can be associated with pathological conditions related to obesity [9]. NF-κβ is also one of the key regulators in the inflammatory process that can stimulate the synthesis of TNF-α, IL-6, and adipokine, which in turn, increase the level of inflammation [59]. Based on immunohistochemical examination, it was found that the average NF-κβ (IRS) expression in the P2 group (5.18 ± 1.12) was greater than group P1 (1.66 ± 1.03). The difference in IRS of NF-κβ expression in both groups was significantly high, ± 3.53. The results of the independent T-test showed a significant difference between the NF-κβ expression values (IRS) between P1 group rats and P2 group rats (p-value = 0.000).

The results of this study were in line with the research which was aimed to determine the role of various types of diets on hepatic inflammation and expression of lipogenic factors mRNA. The study also included male Sprague Dawley rats as subjects and had 4 treatment groups, the control group (chow
diet standard), HF (high fructose 20%), HFAT (high fat) and HFHF (high fructose high fat). After a 16-week treatment period, it was found that NF-κβ expression in the HF, HFHF and HFAT groups were significantly higher than the control group (p <0.05), with the highest expression in the HFHF group [60]. Another study showed that the liver tissue from the HF (high fructose 30%) group induced for 8 weeks was significantly having higher NF-κβ levels than the control group [61].

The previous part of this study, examined the effect of the AIN-93M modified HFHF diet on fatty liver in male Sprague Dawley mice. The study found significant difference in the normal group of AIN-93M modification compared with P2 group, p value = 0.000 (α = 0.05). In the group of rats given a normal diet of 93M AIN modification, the average percentage of fatty liver cells was 19.64%, whereas in the group of rats fed a modified AIN-93M HFHF diet, the average number of fatty liver cells was higher (62.19%) [62]. Research by He stated that HFAT and HFHF treatment groups showed higher NAFLD scores than the control group and HF, and the percentage of NASH was 80% in the HFAT group and 90% in the HFHF group [60]. Another study by Jarukamjorn also stated that the group given HFFD (high fat fructose diet) had more steatosis than the control group [15].

5. Limitations of Research
The high energy density and texture of HFHF feed which was soft and oily due to the high fat proportion can reduce the appetite of mice, leading to lower feed intake in HFHF group. Consequently, the final weight was not significantly different between the two groups. In addition, the bottle drinking containing fructose solution was leaked several times, and certainly affects the volume of drinking intake of mice and causing the rat's hair wetness and moist condition of the husk.

6. Conclusion
1) The average daily feed intake of the P1 group was higher than the P2 group. But the average energy intake in P2 group was higher than in group P1. The results of statistical tests found significant differences between daily feed intake and energy intake of group P1 mice (AIN-93M standard diet) with mice P2 (AIN-93M modified HFHF diet), p = 0.000 in both.
2) The average body weight of rats in P2 group was higher than in group P1. But the results of the statistical test showed that there was no significant difference between the average final weight of the end of the group P1 and the P2 group with the p value = 0.223.
3) The average NF-κβ expression in P2 group was higher than in group P1. The results showed a significant difference between the average NF-κβ expression (IRS) between P1 group rats and P2 group rats (p = 0.000). The results of the correlation test between dietary intake and NF-κβ expression in rat liver tissue showed that there was a positive correlation between energy, carbohydrate and fat intake with NF-κβ expression (p = 0.001, 0.000, 0.046). On the other hand, there was a negative correlation between protein intake and NF-κβ expression in rat liver tissue (p = 0.000).

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