Zagazig Veterinary Journal, ©Faculty of Veterinary Medicine,
Zagazig University, 44511, Egypt.
Volume 49, Number 1, p. 1-12, March 2021
DOI: 10.21608/zvjz.2021.54386.1127

RESEARCH ARTICLE

Effects of trans-Fatty Acid on Lipoproteins-a, Oxidative Status and Expression of Leptin and Leptin Receptor Genes in Albino Rats

Khalifa El-Dawy Ahmed[1], Doaa M. Abdel-Fattah [1], Ahmed H. Arisha[2], Mohamed E. Badawi[3], and Yasmin A. Abd El-Aal[4]*

[1]Biochemistry Department, Faculty of Veterinary Medicine, Zagazig University44511, Zagazig, Sharkia, Egypt

[2]Animal physiology Department, Faculty of Veterinary Medicine, Zagazig University 44511, Zagazig, Sharkia, Egypt

[3]Nutrition and Clinical Nutrition Department, Faculty of Veterinary Medicine, Zagazig University, 44511,Zagazig, Sharkia, Egypt

[4]Medical laboratory Department, Faculty of Applied Medical Science, 6thOctober University, 12511, Giza, Egypt

Article History: Received: 23/12/2020 Received in revised form: 15/01/2021 Accepted: 17/01 /2021

Abstract

High consumption of *trans*-fatty acids (TFAs) linked with obesity, disorders of lipoproteins, type 2 Diabetes Mellitus (T2DM), risk factors of coronary heart disease, and development of atherosclerosis. The intent of our trials was to assess the possible bad impact of TFAs on the lipid status and focusing on characterizing the physiological alters in an animal model comparing with *cis* fatty acids. Sixty male albino rats, with nearly similar average weight 170±5wereassignedinto 4 main groups (15 rats per group) and kept on a standard diet set for 12 weeks. All groups received different diet treatment for successive 12 weeks. Group A: control group received a conventional diet, group B received a diet contained 360 gm TFAs inform of partially hydrogenated vegetable oil (PHVO) l kg of diet, group C was fed a High Fat Diet (HFD) contained 360 gm beef tallow l kg of diet for, and group D received a diet containing 360 gm linseed oil l kg of diet. Both feeding of TFAs and HFD resulted in a marked increase in the body weight in reach to the obesity grade with a significant increase ($P \leq 0.05$) in leptin and leptin receptor gene expression. Also, a significant increase in Malondialdehyde (MDA) and Lipoprotein-a (Lp-a) and a marked decrease in total antioxidant capacity (TAC). It could be concluded that TFAs induce combined increased body weight, with imbalance in oxidative stress, dyslipidemia, and elevated atherogenic Lipoprotein-a may be leading to development of atherosclerosis and coronary heart disease.

Keywords: *trans*-fatty acids, lipoprotein (a), obesity, Coronary Heart Disease, leptin resistance, insulin resistance.

Introduction

Trans-fatty acids (TFAs) are unsaturated fatty acids that contain 1or more unconjugated double bond, present in the *E* or *trans* configuration [1]. It can be produced naturally in little amount in the ruminant stomach through the bacterial enzymes so, it found in cows meat (beef) and milk. Whereas the majority of TFAs are yield by artificial processing called partial hydrogenation of vegetable oils, which are found in numerous products like baked goods, deep-fried foods, spreads, margarines, and frying fats [2, 3]. Data related to TFAs have been involved as being especially harmful to human health[4].Industrial and natural fats contain similar species of TFAs but in various proportions; elaidic acid (C18:1; Δ 9tr) is more prevailing in artificial (industrial) sources whereas vaccenic acid (C18:1; Δ 11tr) is dominant in ruminant (natural) sources[5]. Brouwer *et al*. [6] reported that there is consistent evidence of contrary health impacts from TFAs industrially intake, especially on blood lipoprotein and development of Coronary Heart Disease (CHD), whereas there is no

***Corresponding author e-mail: (yasmin-ali.ams@o6u.edu.eg). Medical laboratory Department, Faculty of Applied Medical Science, 6thOctober University, 12511, Giza, Egypt.**

evidence available of any beneficial impact on the human well-being.

The major biochemical problems related to consumption of TFAs including an elevated serum level of LDL-C, decreased serum level of HDL-C, and modifications to the structure and fluidity of the phospholipid bilayer that led to an increased risk of CHD [7-10]. In addition, TFAs have several adverse health impacts including increased Lp (a),increased triacylglycerol, and hence dramatically increased risk of cardiovascular diseases (CVDs), insulin resistance, disruption of glucose homeostasis, weight gain, and obesity [11]. Moreover, there was a positive relationship between the levels of Lp (a) and the risk of CVD[10]. TFAs have been reported to have a systemic or localized inflammation[12], and pro-inflammatory effects [13].

Feeding of TFAs resulted in the elevation of serum leptin and insulin levels [14].Also, the feeding of TFAs/high-fat diets induced obesity of rats, and alteration in the blood and brain physiological parameters, as well as High Fat Diet (HFD) may cause leptin resistance[15]. The positive association between the leptin level and the serum insulin is that the leptin level raises with an increase in the serum insulin which may be a good indicator of the insulin resistance syndrome, where there is a hyperinsulinemic state, it predicts that there is an increase in the leptin levels [16].The progression of undesirable insulin resistance and obesity resulted in the development of type 2 Diabetes Mellitus (T2DM)[17].

The excessive intake of TFAs might accelerate atherosclerotic lesion formation and oxidative stress induction, due to the changes of the fatty acid composition in the cell membrane [18],and contributes to worse coronary patient outcomes[19] which confirmed by the presence of reactive lipids such as malondialdehyde (MDA) [20].

Replacement of TFAs with unsaturated fatty acids diminishes CHD risk, in part, by improving the negative impacts of TFAs on the blood lipids[21]. Also, there are indications that TFAs may raise endothelial dysfunction, adiposity, and systemic inflammation [22].The detrimental impacts of TFAs feeding have been considered to be worse than Saturated Fatty Acids (SFAs) on cardiovascular health and *cis* unsaturated fatty acids [23], and TFAs do not act any vital functions [24]. Due to the observations that have connected the magnitude of the physiological impacts of dietary TFAs with biochemical values, this study was designed to estimate the potential health risk from TFAs through the investigation of the biochemical and molecular parameters.

Materials and methods

Animal treatment

Sixty adult male Albino rats were allocated to four groups with 15 rats in each group selected with a nearly similar range between 170-175 g body weights. They were purchased from the Animals House of Faculty of Veterinary Medicine; Zagazig University. They were adapted for 2 weeks under standard laboratory conditions. The rats were housed in plastic cages with water and food *ad libitum*. The experimental protocol was approved by the Institutional Animal Care and Use Committee, Zagazig University, Zagazig, Egypt (ZU-IACUC/2/F/24/2019).

Diet

The rats were fed experimental diets for 12 weeks. The experimental diets that differed in the quantity and the quality of fat (Table 1) were prepared as described previously [25]. Mineral salts and vitamins were added to the mixtures to ensure that the daily requirement of the animals were fulfilled, but with various types of dietary fat source: PHVO (rich in TFAs), beef tallow (rich in both monounsaturated fatty acids and saturated fatty acids) and linseed oil (rich in n-3 polyunsaturated fatty acids). The fatty acid compositions of PHVO was assayed by GC-Mass analysis performed at the National Research Center (Cairo, Egypt), and the fatty acid content of beef tallow and linseed oil were previously reported [26, 27], respectively. Pellets were produced daily.

Table (1) Chemical components (%) of the experimental diets fed to rats along 12 weeks

Ingredients	Experimental diets (Amount, g)			
	Control	Partially hydrogenated vegetable oil	Beef tallow	Linseed oil
Casein 80%	134.00	176.00	176.00	176.00
DL- Methionine 98%	1.80	1.80	1.80	1.80
Corn starch	623.64	214.64	214.64	214.64
Sucrose	100.00	150.00	150.00	150.00
Cellulose	50.00	50.00	50.00	50.00
Soybean oil	43.00	-	-	-
PHVO		360[185.6*]		
Beef tallow	-	-	360	-
Linseed oil				360
Antioxidant, BHT	0.06	0.06	0.06	0.06
Minerals	35.00	35.00	35.00	35.00
Vitamins	10.00	10.00	10.00	10.00
Choline chloride	2.50	2.50	2.50	2.50
Total (g)	1000.00	1000.00	1000.00	1000.00
Calculated composition	**Amount (%)**	**Amount (%)**	**Amount (%)**	**Amount (%)**
Metabolizable energy Kcal/100g	3.86	5.07	5.07	5.07
Metabolizable energy KJ/100g	16.17	21.23	21.23	21.23
Protein, %	13.58	17.78	17.78	17.78
Carbohydrate %	77.36	41.46	41.46	41.46
Lipid %	4.30	36.00	36.00	36.00

[*] (C18:1 9t + C18:2 9,12t) × g/100. Value in table each 360 g PHVO contain 185.6 g TFA

Content of fatty acids in dietary fat sources

The fatty acids composition of PHVO is C17:0 =43%, C18:0=5.43%, C18:1(trans-9) =41.95, and C18:2(all-trans-9,12) = 9.62%, while beef tallow is C16:0=25.7%, C18:0=26.7% and C18:1(cis-9) = 37.9%, and linseed oil is C18:3(all-cis-9,12,15) = 62.6%, C18:1(cis-9) = 14.87% and C18:2(all-cis-9,12) = 11.4%.

Sampling

The body weight was followed up weekly. Rats were euthanized humanly after 12 h of fasting. The blood was collected for serum preparation by centrifugation for biochemical investigations. Liver, adipose tissues, and hypothalamus were separated rapidly, washed, and stored at -80 °C until analysis.

Biochemical determination

The serum levels of total cholesterol (TC), triacylglycerol(TAG), and HDL-C were measured by enzymatic colorimetric methods using spectrum reagents [28,29]. Both LDL-C and VLDL were estimated by Friedwald formula [30]. Serum levels were expressed as mg/dL.

The serum levels of lipoprotein (a) were determined according to Rifai *et al*.[31]. Liver samples were removed rapidly, homogenized, washed with cold phosphate-buffered saline (PBS) to eliminate the contaminated blood, and then centrifuged at 5000 rpm for 5 min at 4ºC.The supernatant was used for the measurement of MDA and Total Antioxidant Capacity (TAC) according to Allard *et al*. [32].

Molecular investigation

Total RNA was extracted from the adipose tissue and hypothalamus tissues

Using TRIzol™ reagent (Cat. No. 15596026 and 15596018, Invitrogen, Thermo Fisher Scientific, Waltham, MA, U.S.A.) Following the manufacturer instructions. The obtained RNAs were estimated for purity using the Nano Drop® ND-1000 Spectrophotometer (Nano Drop Technologies, Wilmington, Delaware, USA). cDNA synthesis was performed using a

HiSenScript™ RH (-) cDNA Synthesis Kit following the guidelines of the manufacturer (iNtRON Biotechnology Co., South Korea).One microliter of cDNA was used for semi-quantitative real-time PCR using TOP real™ qPCR 2X Pre MIX(SYBR Green with low ROX) (Intron Biotechnology cat. No. RT500S, South Korea).The cycling conditions were performed in Stratagenean Mx3005P Real-Time PCR System following the manufacturer's instructions. The PCR cycling conditions for all reactions included an initial denaturation for 12 min at 95°C, then 40 cycles of denaturation for 20 s at 95°C,annealing for 30 s at 60°C, and extension at 72°C for 30 s. The oligonucleotide specific primers were synthesized by Sangon Biotech (Beijing, China). A melting curve analysis was performed following PCR amplification. The primer sequences for Leptin were F: 5′CAATGACATTTCACACACGCAG-3′andR:5′AGATGGAGGAGGTCTCGCAG-3′. Leptin receptor gene primer sequences were F: 5′GTGTCCTTCCTGACTCCGTAG-3′and R: 5′GTTATTCTCTGGAAAGACTGGCT-3′[33]. The relative gene expression was expressed by $2^{-\Delta\Delta CT}$ method relative to the GAPDH gene (primer F: 5′GGCACAGTCAAGGCTGAGAATG-3′and R:5′ATGGTGGTGAAGACGCCAGTA-3) as fold change [34].

Statistical analysis

The obtained data are expressed as mean values ± S.E. The data were tested for normality by the Shapiro-Wilk test. For statistical analysis, comparisons between groups were analyzed using one-way analysis of variance (ANOVA). The differences between the groups were evaluated by the Tukey's post-hoc test. *p*-value< 0.05 was considered statistically significant.

Results

Effects of trans fatty acids-containing diet on body weight

Feeding of PHVO and beef tallow diet for 12 weeks resulted in a significant increase ($P \leq 0.05$) in body weight in *trans* and HFD groups compared to the control group, while there was a non-significant difference in body weight between rats fed linseed oil and control group (Figure1).

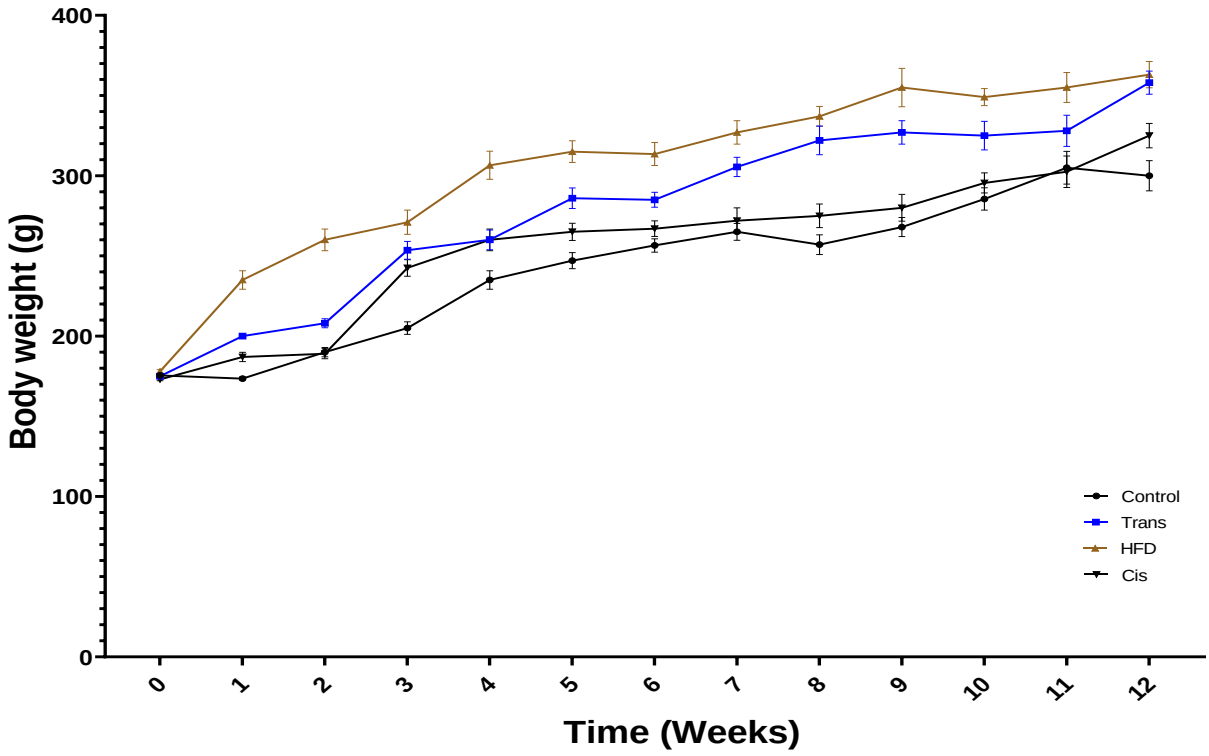

Figure 1: Weight analysis of the groups. The groups were analyzed using two-way ANOVA and Bonferroni post hoc test (n = 15 animals/group). Data are expressed as the mean ± SE of the mean and *P* < 0.05.

Serum lipid profiles

Results in Table 2 revealed a significant increase ($P \leq 0.05$) in Total cholesterol, Triacylglycerol, LDL-C and VLDL levels; 107.67±2.03, 95.0±4.16, 51.67±1.67 and 19.0±0.83, respectively in the *trans* group which consumed PHVO. While the rats fed with beef tallow and linseed oil in the HFD and *cis* groups showed a non-significant difference when compared with the control group (Table 2).

Table 2: Effect of feeding different diets on the biochemical parameters serum of adult male rats

Groups	Control	*trans*	HFD	*cis*
T. C (mg/dl)	99.3 ± 2.60^{b}	107.67 ± 2.03^{a}	102.3 ± 3.93^{ab}	98.0 ± 4.04^{b}
TAG (mg/dl)	77.0 ± 3.21^{b}	95.0 ± 4.16^{a}	85.3 ± 4.33^{ab}	78 ± 5.13^{b}
HDL-C (mg/dl)	45.0 ± 3.06^{a}	37.0 ± 1.15^{b}	38.6 ± 1.2^{b}	46.0 ± 2.0^{a}
LDL-C (mg/dl)	38.9 ± 1.57^{b}	51.67 ± 1.67^{a}	46.64 ± 3.56^{b}	36.4 ± 3.71^{b}
VLDL (mg/dl)	15.4 ± 0.64^{b}	19.0 ± 0.83^{a}	17.06 ± 0.87^{ab}	15.6 ± 1.03^{b}
Lp a (mg/dl)	$0.18^{d}\pm7$	$1.17^{a}\pm38.2$	$1.07^{b}\pm16.5$	$1.2^{c}\pm8.3$
MDA (nmol/g tissue)	48 ± 1.7^{c}	90 ± 2.8^{a}	76 ± 3.5^{ab}	61 ± 2.3^{c}
TAC (ng/g tissue)	$1.15^{a}\pm15$	$1.09^{c}\pm5.7$	9.8 ± 0.44^{bc}	$0.28^{b}\pm11.5$

T. C: Total Cholesterol, TAG: Triacylglycerol, HDL-C: High Density Lipoprotein-Cholesterol, LDL-C: Low Density Lipoprotein-Cholesterol, Lp a: Lipoprotein-a, MDA: Malondialdehyde, TAC: total antioxidant capacity. Means ± SE in the same row and carrying different superscripts are significantly different at $P < 0.05$.TFAs*: trans*-fatty acids,, HFD: high fat diet and *cis* :cis fatty acids.

The HDL-C was significantly decreased in the *trans* and HFD groups which consumed PHVO and beef tallow (37.0±1.15 and 38.6±1.2) (Table 2), respectively. Meanwhile, there was a non-significant difference between both groups. While the rats fed with linseed oil in the *cis* group showed a non-significant increase when compared with the control group (46.0±2.0) (Table 2).

Serum lipoprotein (a)

Results in Table 2 revealed a significant increase ($P \leq 0.05$) in the serum lipoprotein (a) level in *trans*, HFD, and *cis* groups compared to the control group, while there was a significant decrease ($P \leq 0.05$) in serum lipoprotein (a) in HFD and *cis* groups compared to *trans* group.

Hepatic malonaldehyde (MDA)

There was a significant increase ($P \leq 0.05$) in the hepatic MDA level in *trans* and HFD groups and compared to control group, while there was a non-significant difference in hepatic MDA level in the *trans* group when compared to the HFD group. In addition, there was a non-significant difference in hepatic MDA level in the *cis* group when compared to the control group.

Hepatic total antioxidant capacity (TAC)

There was a significant decrease ($P \leq 0.05$) in the hepatic TAC in *trans*, HFD, and *cis* groups compared to the control group. While there was a significant increase ($P \leq 0.05$) in hepatic TAC in the *cis* group compared to the *trans* group.

Molecular investigation

Effects of trans fatty acids-containing diet on leptin gene expression

Feeding of PHVO and beef tallow diet resulted in a significant increase ($P \leq 0.05$) in the expression levels of leptin mRNA, and there was a non-significant difference between them (Figure2a). While there was a non-significant difference in leptin mRNA expression levels in the *cis* group compared to the control group.

Effects of trans fatty acids-containing diet on leptin receptor gene expression

The *trans* group showed a significantly increased ($P \leq 0.05$) gene expression of leptin receptor when compared with the control group. However, the rats in the HFD and *cis* groups showed a significant decrease ($P \leq 0.05$) in gene expression of leptin receptor when compared with the *trans* group (Figure 2b).

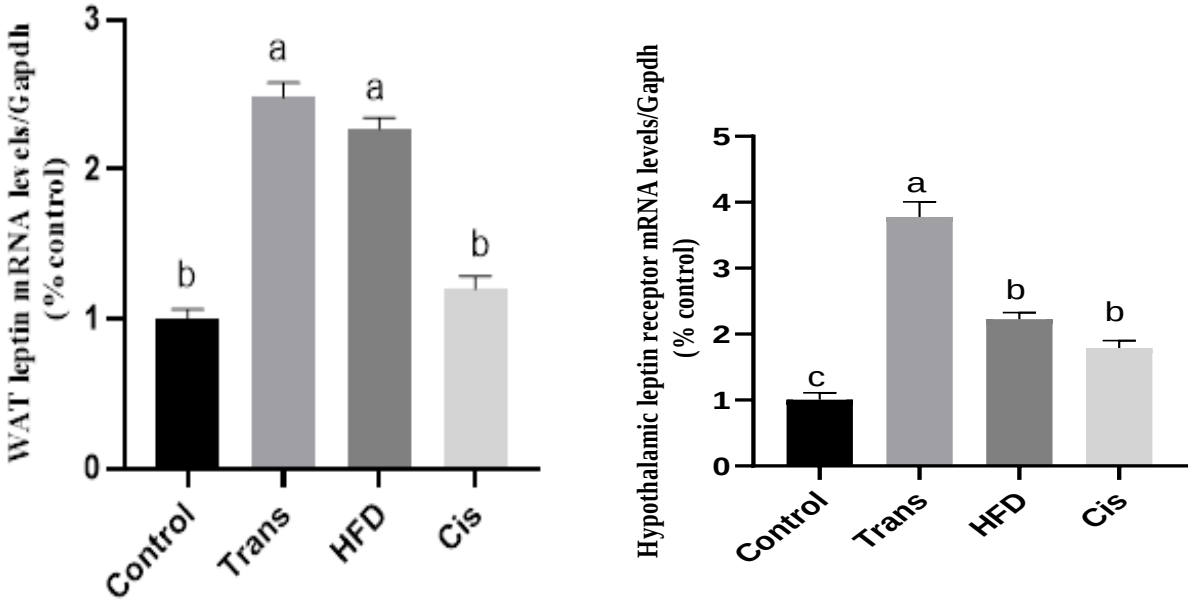

Figure 2: Changes of relative gene expression of leptin (ob) in adipose tissue (A) and leptin receptor (B) in the hypothalamus of control, *trans*-fatty acids, and high fat diet and cis fatty acids.

Discussion

The current study revealed a weighty increase in the *trans* and HFD groups that consumed food with PHVO (51.57% of the energy of TFAs) and beef tallow in the body weight comparing with the control group and this result was noticed previously by Kubant *et al*.[35]that may reflect the induction of obesity by feeding PHVO and beef tallow for 12 weeks and causes enhancement of the leptin gene. But there was no significant difference between *trans* and HFD groups. This result agreed with those reported by Longhi *et al*.[25]who did not find a significant increase in the body weight of adult male Wistar rats fed partially hydrogenated soybean oil(57.3 % of the energy of TFAs) and high-fat lard (57.3 % of energy) for 12 weeks.

More studies have reported the impact of TFAs diet feeding on obesity, and glucose homeostasis but till now there is no exact data for the detailed impact on the molecular mechanism of the leptin-resistant state and metabolic disorder state generate by TFAs diet feeding [6]. Therefore, this study dealt with that problem and the obtained results revealed that TFAs diet feeding resulted in a significant increase in the expression level of leptin and leptin receptor genes.

In obesity and leptin dysfunction, the hyperleptinemia in executing its biological impact of suppressing the appetite has brought us to understand the development of leptin resistance. Such resistance can result from various mechanisms in the kinetic and dynamic process of leptin; the first cause of leptin resistance is reduced leptin transport across the blood-brain barrier. The second cause is impaired leptin signal transduction in neurons [36].

Leptin resistance is linked with the development of insulin resistance in individuals with T2DM [37].The development

of T2DM is associated with obesity, hyperinsulinemia, and insulin resistance has been observed and obesity is related with a marked elevation in the circulating leptin concentration [38]. Our results demonstrated an elevation in the insulin level in TFAs and HFD groups. These results agreed with Hua *et al*.[39]who mentioned that insulin resistance syndrome has been related with an increased risk of developing obesity.

The obtained results came in agreement with Aronis *et al*.[40]who suggested that an improvement of lipid metabolism disorders and also worst in insulin resistance in TFAs and HFD groups suffered from obesity. Lp (a) level was markedly increased in the *trans* group and this result was in agreement with Dhibi *et al*.[41]. They reported the inflammatory impacts and endothelial dysfunction after PHVO consumption which has been shown to induce accumulation of inflammatory cells, linked with increased activity of Lp (a), inducing cardiovascular risk and atherosclerotic.

The lipid metabolism disorder induced by feeding of PHVO and beef tallow to rats was indicated by an elevated level of plasma lipoproteins like TC, TAG, LDL-C, VLDL, and Lp (a). The raise of Lp (a) in serum suggested a result of lipotoxicity induced through the consumption of PHVO and beef tallow[42].According to Nestel *et al*.[10] the feeding of TFAs and HFD to rats caused diminished HDL-C and so, presumably, the risk of CVDs. Diminishing this parameter is a negative factor because the HDL-C concentration is directly related to the protective impact on CVDs. However, the difference among the groups was not significant. More studies will be required. In addition, one of the important risk markers for CVDs is LDL concentration [43]. Our results showed an increase in the LDL in TFAs and HFD groups which in the same line with the findings of Markey *et al*.[44]. Even though, the difference was not significant between the groups. The differences demonstrated in this study are proportionate with the other studies that the feeding of sources of *trans*-fatty acid raises levels of LDL-C versus MUFA or PUFA feeding [45]. This probably due to the content of TFAs in PHVOs that alters various metabolic processes as the fluidity of cell membrane, causing a difficult coupling of the LDL receptor with its ligand[46].

Epidemiological studies demonstrated that n-6 PUFA, especially a linoleic acid dietary feeding, significantly decreases levels of blood LDL-C[47]. Linoleic acid acts as a ligand of Peroxisome proliferator activated receptor gamma (PPAR) transcription factors and prevents transcription of certain gene-encoding enzymes concerned inside the synthesis of hepatic lipogenesis, and as an alternative provide the synthesis of enzymes involved in β-oxidation [48]. So, linseed oil rich in linoleic fatty acid may do a hypocholesterolemic impact that coincides with our findings. Although, Czernichow, *et al.* [47]estimated that more consumption of n-6 PUFA can cause adverse outcomes on health and hence recommend achieving a balance between fatty acids of n-6 and n-3 feeding and setting an upper limit.

In addition to the impact of linseed oil to decrease the levels of cholesterol and blood glucose by decreasing and delaying their absorption from the intestine that agreed with our result even though it is not significant. It has an impact on reducing the risk of several chronic diseases such as hyperlipidemia, atherosclerosis, CVD, and cancer [49].

Linseed oil has an anti-inflammatory and antioxidant activity which may decrease the oxidative damage in several diseases such as diabetes and heart disease [50] in agree with our result that showed marked reduction in MDA and elevation in TAC of linseed oil group.

Feeding TFAs and HFD raised free radicals as MDA concentration causing destroy to the fat cell membrane fluidity and high leptin[51]. The increase in leptin levels after feeding TFAs and HFD is in agreement with the findings of Koppe *et al*.[14]. TFAs and HFD feeding cause an elevation in the

leptin receptor level that affect the leptin signaling pathway and block the leptin action to bring into the hypothesis of leptin resistance and obesity [52]. When *trans* fats are incorporated into the cell membranes, the membrane fluidity is diminished, and the cells do not act as well. The impact is then to elevate more production of reactive oxygen species that explain the increase in lipid peroxidation in the groups fed with TFAs and HFD diet and a significant decrease of TAC[20].

Zambonin *et al*.[53]declared that the free radicals that formed during stressful cases can able to induce TFAs production by isomerization of *cis*-*trans* of their *cis* precursor *in vivo*. Indeed, it has been showed that the free-radical stress joined with weakened defense systems can change the molecular shape of unsaturated fatty acid (UFA) residues *in vivo*. In this study, a clear relation was observed between oxidative stress and TFAs.

Conclusions

It can be concluded that excessive intake of *trans*-fatty acids may be linked with various disturbances including dyslipidemia, lipoprotein (a), metabolic hormones instability, and imbalance in oxidative stress.

Conflict of Interest

The authors have no conflict of interest to declare.

References

[1] Ginter, E. and Simko, V .(2016): New data on harmful effects of *trans*-fatty acids. Bratislavske lekarske listy. 117:251-253.

[2] Wanders, A.J.; Zock, P.L. and Brouwer, I.A.(2017):*Trans* fat intake and its dietary sources in general populations worldwide: a systematic review. Nutrients, 9:40.

[3] Gotoh, N.; Kagiono, S.; Yoshinaga, K.; Mizobe, H.; Nagai, T.; Yoshida, A.and Nagao, K. (2018): Study of *trans* fatty acid formation in oil by heating using model compounds. JOS, 67: 273-281.

[4] Kris-Etherton, P. M.; Lefevre, M.; Mensink, R. P.; Petersen, B.; Fleming, J.and Flickinger, B. D. (2012). *Trans* fatty acid intakes and food sources in the US population: NHANES 1999–2002. Lipids, 47, 931-940.

[5] Brouwer, I.A.; Wanders, A.J. and Katan, M.B. (2010): Effect of animal and industrial *trans*-fatty acidson HDL and LDL cholesterol levels in humans–a quantitative review. PloS one. 5:9434.

[6] Brouwer, I.; Wanders, A. and Katan, M. (2013):*Trans*-fatty acids and cardiovascular health: research completed? EJCN. 67:541-547.

[7] Tzeng, Y.-Z and Hu, C.-H. (2014): Radical-induced *cis–trans* isomerization of fatty acids: A theoretical study. JPC A. 118: 4554-4564.

[8] Wang, Y.and Proctor, S.D. (2013): Current issues surrounding the definition of *trans*-fatty acids: implications for health, industry and food labels. British journal of nutrition. 110:1369-1383.

[9] Tokede, O. A.; Petrone, A. B.; Hanson, N. Q.; Tsai, M. Y.;Weir, N. A.; Glynn, R. J. and Djoussé, L. (2013): Plasma phospholipid *trans-fatty acids* and risk of heart failure. AJCN, 97: 698-705.

[10] Nestel, P. (2014): *Trans* fatty acids: are its cardiovascular risks fully appreciated? Clinical therapeutics. 36:315-321.

[11] Teegala, S.M.; Willett, W.C. and Mozaffarian, D. (2009): Consumption and health effects of *trans* fatty acids: a review. Journal of AOAC International. 92:1250-1257.

[12] Saghafi, Z.; Zargaraan, A.; Tabibiazar, M.;and Hosseini, H. (2018): Is *trans* fatty acid still an issue for policy makers in Iran? A Technical Report. NFSR, 5: 47-51.

[13] Okada, Y.; Tsuzuki, Y.; Ueda, T.; Hozumi, H.; Sato, S.; Hokari, R. and Komoto, S. (2013): *Trans-fatty acids* in

diets act as a precipitating factor for gut inflammation? JGHF, 28: 29-32.

[14] Koppe, S. W.; Elias, M.; Moseley, R. H.; and Green, R. M. (2009): Trans fat feeding results in higher serum alanine aminotransferase and increased insulin resistance compared with a standard murine high-fat diet. AJPGL, 297: G378-G384.

[15] Woods, S. C.; Seeley, R. J.; Rushing, P. A.;D'Alessio, D. and Tso, P. (2003): A controlled high-fat diet induces an obese syndrome in rats. JNutr, 133: 1081-1087.

[16] Shebl, T. H.; Noor El Deen, A. A.; Younis, H. A.; Soliman, A. M; Ashmawy, A. M. and Ali, M. M. N. (2017): Relationship between serum leptin concentration and insulin resistance syndrome in patients with type 2 diabetes mellitus. J Curr Med Res Pract, 2: 125-132.

[17] Sokolowska, E. and Błachnio-Zabielska, A. U. (2019): The role of ceramides in insulin resistance. Front Endocrinol, 10: 577.

[18] Mozaffarian, D.; Pischon, T.; Hankinson, S. E. ; Rifai, N. ; Joshipura, K.; Willett,W. C. and Rimm E. B. (2004): Dietary intake of trans-fatty acids and systemic inflammation in women. Am JClin Nutri, 79: 606-612.

[19] McDonald, C. I.; Fraser, J. F.; Coombes, J. S. and Fung, Y. L. (2014): Oxidative stress during extracorporeal circulation. Eur JClin TechnolSci, 46: 937-943.

[20] Dhibi, M.; Brahmi, F.; Mnari, A.; Houas, Z.; Chargui, I.; Bchir, L.; and Hammami, M. (2011): The intake of high fat diet with different *trans* fatty acid levels differentially induces oxidative stress and non-alcoholic fatty liver disease (NAFLD) in rats. Nutr Metab (Lond), 8: 65.

[21] Makarewicz-Wujec, M.; Dworakowska, A. and Kozłowska-Wojciechowska, M. (2018): Replacement of saturated and *trans*-fatty acids in the diet v. CVD risk in the light of the most recent studies. NIH. 21:2291-2300.

[22] Mozaffarian, D.; and Clarke, R. (2009): Quantitative effects on cardiovascular risk factors and coronary heart disease risk of replacing partially hydrogenated vegetable oils with other fats and oils. Eur JClin Nutr, 63: 22-33.

[23] Berry, S.; Bruce, J.; Steenson, S.; Stanner, S.; Buttriss, J.; Spiro, A. and Farrell, L. (2019): Interesterified fats: What are they and why are they used? A briefing report from the Roundtable on Interesterified Fats in Foods. Nutr Bull, 44: 363-380.

[24] EFSA Panel on Dietetic Products, N. and Allergies. (2010): Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, *trans* fatty acids, and cholesterol. EFSA Journal, 8: 1461.

[25] Longhi, R.; Almeida, R. F.; Machado, L.; Duarte, M. M. M. F.; Souza, D. G.; Machado, P. and Souza, D. O. (2017): Effect of a *trans* fatty acid-enriched diet on biochemical and inflammatory parameters in Wistar rats. Eur JNutr, 56: 1003-1016.

[26] Segura, N.; Da Silva, R. C.; Soares, F. A. S. d. M.; Gioielli, L. A. and Jachmanián, I. (2011): Valorization of beef tallow by lipase-catalyzed interesterification with high oleic sunflower oil. JAOCS, 88: 1945-1954.

[27] Krawczynska, A.; Okreglicka, K.; Olczak, E.and Gromadzka-Ostrowska, J. (2010): Effect of dietary fat type on plasma lipid profile and leptin concentration in rats fed on high-sucrose diets.JPre Clin Clin Res, 4.

[28] Ramakrishnan, S. and Sulochana. K. (2012): Manual of Medical laboratory techniques. JP Medical Ltd.453.

[29] Maislos, M.; Khamaysi, N.; Assali, A.; Abou-Rabiah, Y.;Zvili, I. and Shany, S. (1993): Marked increase in plasma high-density-lipoprotein cholesterol after prolonged fasting during Ramadan. Am JClin Nutr, 57: 640-642 .

[30] Matas, C.; Cabre, M.; La Ville, A.; Prats, E.; Joven, J.; Turner, P. R.; Camps, J. (1994): Limitations of the Friedewald

formula for estimating low-density lipoprotein cholesterol in alcoholics with liver disease. Clin Chem, 40, 404-406.

[31] Rifai, N. and King. M. (1986): Immunoturbidimetric assays of apolipoproteins A, AI, AII, and B in serum. Clin Chem, 32: 957-961.

[32] Allard, J. P.; Aghdassi, E.; Chau, J.; Salit, I.and Walmsley, S. (1998): Oxidative stress and plasma antioxidant micronutrients in humans with HIV infection. Am JClin Nutr. 67: 143-147.

[33] Livak, K.J. and Schmittgen, T.D. (2001): Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods, 25: 402-408.

[34] Zhang, J.; Jin, P.; Gong, M. and Yi, O. (2018): Role of Leptin and the leptin receptor in the pathogenesis of varicocele-induced tesicular dysfunction. Mol Med Rep.17: 7065-7072.

[35] Kubant, R.; Poon, A. N.; Sanchez-Hernandez, D.; Domenichiello, A. F.; Huot, P. S.; Pannia, E. and Anderson, G. H. (2016): A Comparison of Effects of Lard and Hydrogenated Vegetable Shortening on the Development of High-Fat Diet-Induced Obesity in Rats. The Nutrition & Diabetes. 30: 915.

[36] Mohiti, J.; Afkhami, M. and Babaei, A. (2005): Relation between leptin and insulin in patients with type II diabetes mellitus. Turkish J Endocrinol Metab, 2: 55-58

[37] Taghdir, M.; Djazayeri, A.; Djalali, M.; Eshraghian, M.; Rajab, A.; Yegane, H. S. and Sepandi, M. (2010): Relationships of serum leptin concentration with insulin, glucose, HbA1c levels and insulin resistance in overweight post-menopausal diabetic women. ARYA Atherosclerosis, 5: 175-180.

[38] Amitani, M.; Asakawa, A.; Amitani, H. and Inui, A. (2013): The role of leptin in the control of insulin-glucose axis. Front neurosc, 7: 51.

[39] Hua, Y.; Fan, R.; Zhao, L.; Tong, C.; Qian, X.; Zhang, M. and Ma, W. (2020):*Trans*-fatty acids alter the gut microbiota in high-fat-diet-induced obese rats. Br J Nutr, 124: 1251-1263..

[40] Aronis, K. N.; Joseph, R. J.; Blackburn, G. L.and Mantzoros, C. (2011):*trans*-Fatty acids, insulin resistance/diabetes, and cardiovascular disease risk: should policy decisions be based on observational cohort studies, or should we be waiting for results from randomized placebo-controlled trials? Metabolism, 60: 901-905.

[41] Dhibi, M.; Mnari, A.; Brahmi, F.; Houas, Z.; Chargui, I.; Kharroubi, W. and Hammami, M. (2016): Consumption of Oxidized and Partially Hydrogenated Oils Differentially Induces *Trans*-Fatty Acids Incorporation in Rats' Heart and Dyslipidemia. JAm Coll Nutr, 35: 125-135.

[42] Estadella, D.; da Penha Oller do Nascimento, C.; Oyama, L. M.; Ribeiro, E. B.; Damaso, A. R.and de Piano, A. (2013): Lipotoxicity: effects of dietary saturated and *trans* fatty acids. Hindawi, 2013.

[43] Kris-Etherton, P. M. (2010): *Trans*-fats and coronary heart disease. NCBl-NIH, 50: 29-30.

[44] Markey, O.; Vasilopoulou, D.; Kliem, K. E.; Koulman, A.; Fagan, C. C.; Summerhill, K. and Todd, S. (2017): Plasma phospholipid fatty acid profile confirms compliance to a novel saturated fat-reduced, monounsaturated fat-enriched dairy product intervention in adults at moderate cardiovascular risk: a randomized controlled trial. Nutr J, 16: 33.

[45] Baum, S. J.; Kris-Etherton, P. M.; Willett, W. C.; Lichtenstein, A. H.; Rudel, L. L.; Maki, K. C. and Block, R. C. (2012): Fatty acids in cardiovascular health and disease: a comprehensive update. JClin Lipidol, 6: 216-234.

[46] Manzur, F.;Alvear, C.and Alayón, A. (2009): Consumo de ácidos grasos trans y riesgo cardiovascular. Rev. colomb. cardiol, 103-111.

[47] Czernichow, S.; Thomas, D. and Bruckert, E. (2010): n-6 Fatty acids and cardiovascular health: a review of the evidence for dietary intake recommendations. Br JNutr, 104: 788-796.

[48] Nagao, K. and Yanagita,T. (2008): Bioactive lipids in metabolic syndrome. NIH, 47:127-146.

[49] Kaur, N.; Chugh, V. and Gupta. A.K. (2014): Essential fatty acids as functional components of foods-a review. JFood Sci Technol, 51:2289-2303.

[50] Zanwar, A.A.; Hegde, M.V. and Bodhankar, S.L. (2013): Protective role of concomitant administration of flax lignan concentrate and omega-3-fatty acid on myocardial damage in doxorubicin-induced cardiotoxicity. FSHW, 2: 29-38.

[51] Tan, B.L. and Norhaizan, M.E. (2019): Effect of high-fat diets on oxidative stress, cellular inflammatory response and cognitive function. Nutrients. 11:2579.

[52] Zhou, Y. and Rui, L. (2013): Leptin signaling and leptin resistance. Front med, 7: 207-222.

[53] Zambonin, L.; Ferreri, C.; Cabrini, L.; Prata, C.; Chatgilialoglu, C.and Landi, L. (2006): Occurrence of trans-fatty acids in rats fed a trans-free diet: a free radical-mediated formation? SFRBM, 40: 1549-1556.

الملخص العربي

تأثير الأحماض الدهنية المتحولة علي البروتينات الدهنية (أ) والأكسدة والتعبير عن جينات الليبتن ومستقبلات الليبتن في الجرذان البيضاء

خليفة الضوى[1]،دعاء محمد عبدالفتاح[1]، احمد حامد عريشه[2]،محمد بدوي[3]، ياسمين علي عبدالعال [4]
[1]قسم الكيمياء الحيوية- كلية الطب البيطري - 44511جامعة الزقازيق
[2]قسم الفسيولوجي والكيمياء الحيوية - كلية الطب البيطري - 44511جامعة الزقازيق
[3] قسم التغذية الاكلينكية - كلية الطب البيطري-جامعة الزقازيق
[4]قسم المختبرات الطبية-كلية العلوم الطبية التطبيقية- جامعة 6 اكتوبر

ان تناول الكثير من الاحماض الدهنية المتحولة مرتبط بالسمنة، واضطراب مستوي البروتين الدهني في الدم، والنوع الثاني من داء السكري، وأمراض القلب التاجية مما يؤدي الي تصلب الشرايين.

وكان الهدف من هذا العمل تقييم التأثير السلبي للاحماض الدهنية المتحولة علي حالة الدهون والتركيز علي التغيرات الفسيولوجية علي الحيوانات مقارنة بمجموعةcis

وأجريت هذة الدراسة لمده 12 اسبوعاً علي عدد ٦٠ ذكر من ذكور الجرذان البيضاء والتى تم تأقلمها على بيئة التجربة لمدة اسبوعين ثم تم تقسيم الجرذان الي اربعة مجموعات ١٥ فأر في كل مجموعة على النحو التالي:المجموعه الأولي : مجموعه ضابطة تغذت علي عليقة طبيعية.المجموعه الثانية : استبدال360 جرام من الزيت النباتي المهدرج إلي كل كيلو جرام من العليقة. المجموعة الثالثة : استبدال360 جرام من الشحم البقري إلي كل كيلوعليقة.المجموعة الرابعة : استبدال360 جرام من زيت بذر الكتان إلي كل كيلوعليقة.وقد كشفت النتائج التي توصلنا اليها الي ما يلي:أدى تغذية الزيت النباتي المهدرج جزئيًا وشحم البقر إلى زيادة ملحوظة في وزن الجسم في كل من مجموعتي TFAs و HFD التي تصل إلى حالة السمنة مع زيادة معنوية في جينات مستقبلات اللبتين واللبتين. أيضا ، زيادة كبيرة في Malondialdehyde و Lipoprotein-a. مما سبق استنتج ان الدهون النباتيه تؤدي الي زياده الوزن وتسبب خلل في الاكسده, عسر شحميات الدم و ارتفاع البروتين الدهني (أ) التي تؤدي الي تصلب الشرايين وامراض القلب التاجية