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ORIGINAL PAPER

Investigation of Advanced Glycation End Products in Liver, Adipose, and Renal Tissue of Mice on a High-Fat Diet

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Abstract

Obesity is a complex condition associated with disruptions in carbohydrate, protein, and fat metabolism, linked to increased insulin resistance and glucose intolerance. High levels of Advanced Glycation End-products (AGEs) are associated with a range of chronic diseases, including kidney diseases, diabetic complications, cardiovascular diseases, and neurodegenerative diseases. Our study aims to investigate the accumulation of AGEs in the liver, renal and adipose tissues of mice fed a high-fat diet, contributing to a deeper understanding of obesity and its related metabolic disorders. Our study consists of three different groups fed with diets containing 60% and 10% fat. The Experiment 1 group was maintained on their diet for 12 weeks, while the obese 2 and control groups continued their diets for 24 weeks. AGEs in the liver and kidney tissues obtained were measured using the High-performance liquid chromatography grade (HPLC) method. Higher accumulation of AGEs has been observed in kidney tissue compared to adipose and liver tissues ($p < 0.05$). Moreover, the GO levels were notably higher in liver tissue than in adipose tissue of the D1 and D2 groups ($p < 0.0001$). Our results suggest that particularly in kidney tissue, increased filtration burden, functional impairment, and receptor interaction due to obesity may be effective. The lower levels of AGEs detected, especially in the obese groups compared to the control, can be attributed to the inability to metabolize AGEs due to tissue damage caused by obesity.

Keywords Obesity · HPLC · Glyoxal · Methylglyoxal · Malondialdehyde

Introduction

Obesity is among the most important health problems and is recognized by the World Health Organization (WHO) as overwhelming fat accumulation that threatens health. Epidemiological studies demonstrate that obesity have reached the status of global epidemics. It is estimated that approximately 60% of adults and one in three children are affected by obesity [1]. In the World Obesity Atlas, it is pointed out that obesity and overweight (Body Mass Index ≥25 kg/m²) will affect more than 4 billion people in 2035. According to the report in which Turkey was also evaluated, it is demonstrated that more than 50% of adults may be obese in 2035 [2].

Obesity causes oxidative stress by increasing the production of free radicals in the body. Cytokines occurring with obesity cause lipid peroxidation and subsequently oxidative stress by increasing free radical production [3]. Lipid peroxidation, which occurs in the unsaturated fatty acids of lipids in the cell membrane, is one of the most important consequences of the damage caused by free radicals. The products released because of lipid peroxidation are Malondialdehyde (MDA), 4-Hydroxynonenal (4-HNE) and F2-Isoprostane [4]. MDA is the most abundant product formed during lipid peroxidation, and it is known as a marker of oxidative stress. MDA reacts with bases in the DNA structure to form some compounds. These compounds cause mutagenic effects and are associated with many diseases such as kidney diseases, cardiovascular diseases, liver diseases, neurodegenerative diseases such as Alzheimer's and Parkinson's, and diabetes [5].

It is known that obesity increases the level of advanced glycation end products, and this is a cause of chronic

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diseases [6]. Glyoxal (GO) and methylglyoxal (MGO) products have been implicated as precursors of advanced glycation endogenes (AGEs). GO and MGO are known to consist of mechanisms such as glucose autooxidation, fat peroxidation, and polyol pathway. Products like GO and MGO are formed not only through endogenous processes, occurring sometime after the Maillard reaction, but also because of external factors such as smoking and exposure to smoke [7].

It has been revealed through research that chronic diseases are associated with the accumulation of AGEs in the body; AGEs reveal their harmful effects by activating oxidative stress through the Receptor for Advanced Glycation End Products (RAGE) pathway [6, 8]. Advanced glycation end products can cause damage to organs such as the heart and kidneys. These end products may arise not only in conditions like hyperglycemia in diabetic patients but also in individuals with diseases characterized by an excess of oxidative stress. The accumulation of advanced glycation end products increases with age, but it has been observed that the accumulation is higher in chronic diseases such as diabetes, kidney disease, cardiovascular disease, Alzheimer's disease, and rheumatoid arthritis [9].

Scientific studies conducted on animals are utilized as valuable models in understanding the physiological and pathological mechanisms occurring in humans. In the literature review, it has been noted that AGEs are predominantly investigated in serum [10–13], while their accumulation in tissues remains inadequately explored. In this study, our study aims to detect the levels of glyoxal (GO), malondialdehyde (MDA), and methylglyoxal (MGO) in adipose, liver, and kidney tissue samples obtained from C57BL/6 J strain mice, which were induced with an obesity model by being fed with a purified diet containing 60% kcal/fat. The investigation of AGEs levels in tissues will contribute to the literature in understanding the pathophysiology of obesity-related diseases.

Material and method

Chemicals

Glyoxal (40%), methylglyoxal (40%), 1,1,3,3-Tetraethoxypropane (≥96%), trichloroacetic acid (TCA), 2-Thiobarbituric acid (TBA), 4-Nitro-o-phenylenediamine, methanol (HPLC grade), and acetonitrile (for HPLC grade) was received from Sigma-Aldrich (St. Louis, MO, USA).

HPLC Analysis of MDA

The MDA analysis method described by Aksoy et al. [14] was used by modified protocols. First, 0.25 g of tissue sample was taken into a 50 mL falcon tube and mixed with 5 mL of 10% TCA. After that, it was homogenized with an IKA ultra-thorax homogenizer for 5 min and followed by centrifugation at 8000 rpm for another 5 min. Then, 1 mL of this supernatant was mixed with 1 mL of TBA solution (0.1675 g/250 mL) for the derivatization stage and placed in a water bath at 90 °C for 30 min. After that, the derivatized sample was cooled to room temperature and filtered using 0.45 µm CA filter. This solution was injected into the HPLC device.

HPLC Determination of MDA

For the measurement of MDA, a Shimadzu Nexera-i HPLC equipped with a Shimadzu RF-20A fluorescence detector (Shimadzu Corporation, Kyoto, Japan) was used. The mobile phase was prepared using 0.05 M KH_2PO_4 buffer solution/methanol/acetonitrile (72/17/11). The separation was carried out utilizing a Gemini-NX 5 µ C18 110 Å, 4.6 mm × 250 mm column. The excitation and emission wavelengths were 530 and 550 nm, respectively. The column oven temperature was 25 °C and flow rate was 1 mL/min.

GO and MGO Analysis

The HPLC analysis for the determination of GO and MGO described by Cengiz et al. were used with some modification [15]. First, 1 mL of supernatant was taken MDA preparation into a 10 mL glass tube and 1 mL sodium acetate buffer (0.1 M, pH: 3) and 0.5 mL derivatization solution (4-nitro-1,2-phenlenediamine in 1% methanol) were added. After that, it was incubated in a water bath for 30 min at 70 °C. Then, the derivatized sample was filtered using 0.45 µm cellulose acetate (CA) filter. This solution was injected into the HPLC.

GO and MGO HPLC Parameters

The HPLC system was composed of a Shimadzu Nexera-i HPLC with a UV/VIS detector (Shimadzu Corporation, Kyoto, Japan). The mobile phase prepared using methanol: water: acetonitrile (42:56:2 v/v/v). The wavelength was set to 254 nm. The separation was achieved using an Inersil ODS-3 5 µ, 4.6 × 250 mm column. A flow rate was 1 mL/min and the column oven temperature was 30 °C.

Animal Experimental

The research was approved by the Istanbul University Rectorate's Local Ethics Committee for Animal Experiments (Ethics Committee Approval Number: 2021/18). The research was performed at Istanbul University's Aziz Sancar Experimental Medicine Research Institute and Sabahattin

Table 1 The body and tissue weight of all mice was measured every week

Weight (gr)	Control (Mean ± SE)	D1 (Mean ± SE)	D2 (Mean ± SE)	p1 (ControlL&D1)	p2 (Control&D2)	p3 (D1&D2)
Body	26.59 ± 0.22	33.96 ± 1.37	37.49 ± 1.16	**<0.0001**	**<0.0001**	**0.0241**
Adipose	0.6 ± 0.03	1.6 ± 0.17	1.67 ± 0.12	**0.0007**	**<0.0001**	0.8686
Liver	1.24 ± 0.08	1.53 ± 0.09	1.77 ± 0.12	**0.0332**	**0.0021**	0.2529
Renal	0.3 ± 0.01	0.46 ± 0.01	0.63 ± 0.03	**<0.0001**	**<0.0001**	**0.0003**

The significance between groups was measured using the non-parametric Mann–Whitney U test, and the fold changes are presented as Mean ± Standard Error of the Mean (X ± SEM). Statistical significance is indicated by $p < 0.05$. $p1$ represents the weight of the *D1* compared to the control group; $p2$ indicates the weight of the *D2* compared to the control group, and $p3$ illustrates the weight of the *D1* compared to the *D2*. The significance levels are indicated in bold.

Zaim University (Istanbul, Turkey). Thirty female mice (6 weeks old) of the C57BL/6 J strain were utilized in the study, divided into three groups: control, Experiment 1 (D1), and Experiment 2 (D2). The control group was administered a diet containing 10% kcal fat (Purina TestDiet® F.No:#58Y2), whereas the D1 and D2 groups were provided with a high-fat diet (HFD) comprising 60% kcal fat (Purina TestDiet® F.No:#58Y1).D1 group was euthanized after 12 months, while D2 and control groups were euthanized after 24 months. The same dietary regimen was maintained for all groups until sacrified. Throughout the study, all groups were housed in sedentary experimental cages, with standard movement within the cages and no additional exercise provided. The mice were housed in an environment marked by minimal noise levels, allowing free access to food and water. The environment maintained a 12-h light/dark cycle, with a light intensity of 40 lux, a temperature of 21 °C, and a relative humidity of 50%.

Results

The evaluation was made of 30 female C57BL/6 J mice. The mice's body weight was measured weekly to investigate the effect of a high-fat diet (HFD) (%60) in study groups. Moreover, we measured tissue weight after the sacrifice of the study groups as shown in Table 1. We found that weight gain was significantly increased in mice fed a high-fat diet (D1 and D2 groups) compared to the control group and had similar weight gain in the liver and adipose tissue of the D1 and D2 groups ($p > 0.05$). However, the present study showed that a significant association with weight gain in the renal tissue of the D1 and D2 groups (respectively, $0.46 \rightarrow 0.63$; $p = 0.0003$) (Fig. 1).

We investigated levels of Glyoxal (GO), Methylglyoxal (MGO), and Malondialdehyde (MDA) in the liver, adipose, and renal tissue of the study groups. The GO levels were examined in the adipose, liver, and renal tissues of study groups, revealing a significant increase in the control and D1 group compared to the D2 group in adipose tissue (respectively, $1 \rightarrow 0.71 \rightarrow 0.42$; p(control&D2) = 0.022; p(D1&D2) = 0.0027). In the renal tissue, the GO level was found to be higher in the control and D2 group compared to the D1 group (respectively $112.3 \rightarrow 46.8 \rightarrow 89.29$; p(control&D1) = 0.0274; p(D1&D2) = 0.0115). No significant association was observed in the liver tissue regarding to GO levels in study groups ($p > 0.05$).

MDA levels were found to be elevated in both the control and D1 groups compared to the D2 group in renal tissue (respectively, $624.6 \rightarrow 633.6 \rightarrow 481.9$; p(control&D2) = 0.0007; p(D1&D2) = 0.0016). However, no statistically significant differences were observed among the groups regarding MDA levels in the liver and adipose tissue ($p > 0.05$).

MGO levels could not be detected in liver tissue of study groups therefore it could not be assessed. However, there were significantly higher MGO levels in adipose tissue of the control group compared to the D1 and D2 groups. Additionally, MGO levels were significantly higher in the D1 group compared to the D2 group (respectively $0.84 \rightarrow 0.53 \rightarrow 0.38$; p(control&D1) = 0.0068; p(control&D2) = 0.0147; p(D1&D2) = 0.0232). The MGO levels in the control group were determined to be higher in renal tissue than in both the D1 and D2 (respectively, $40.43 \rightarrow 23.14 \rightarrow 25.28$; p(control&D1) = 0.0011, p(control&D2) = 0..0095). However, no significant difference was observed between the D1 and D2 groups regarding MGO levels. The comparison of GO, MGO, and MDA tissue levels among the study groups is shown in Fig. 2 (data shown in Table 2).

GO and MGO are reactions that can occur during glucose auto-oxidation, lipid peroxidation, and the polyol pathway in the human body. Therefore, these two compounds are generally detected in similar amounts. This is because both are produced in the same metabolic pathways and play roles in various biological processes in the body [16–18]. Whereas no significant difference was observed in the levels of GO and MGO in the adipose tissue of the D1 group ($p > 0.05$), the GO levels were significantly higher than MGO levels in the D2 groups (respectively, $89.29 \rightarrow 25.28$ $p = 0.0336$). Furthermore, in renal tissue, the GO levels were found to be higher than the MGO level in

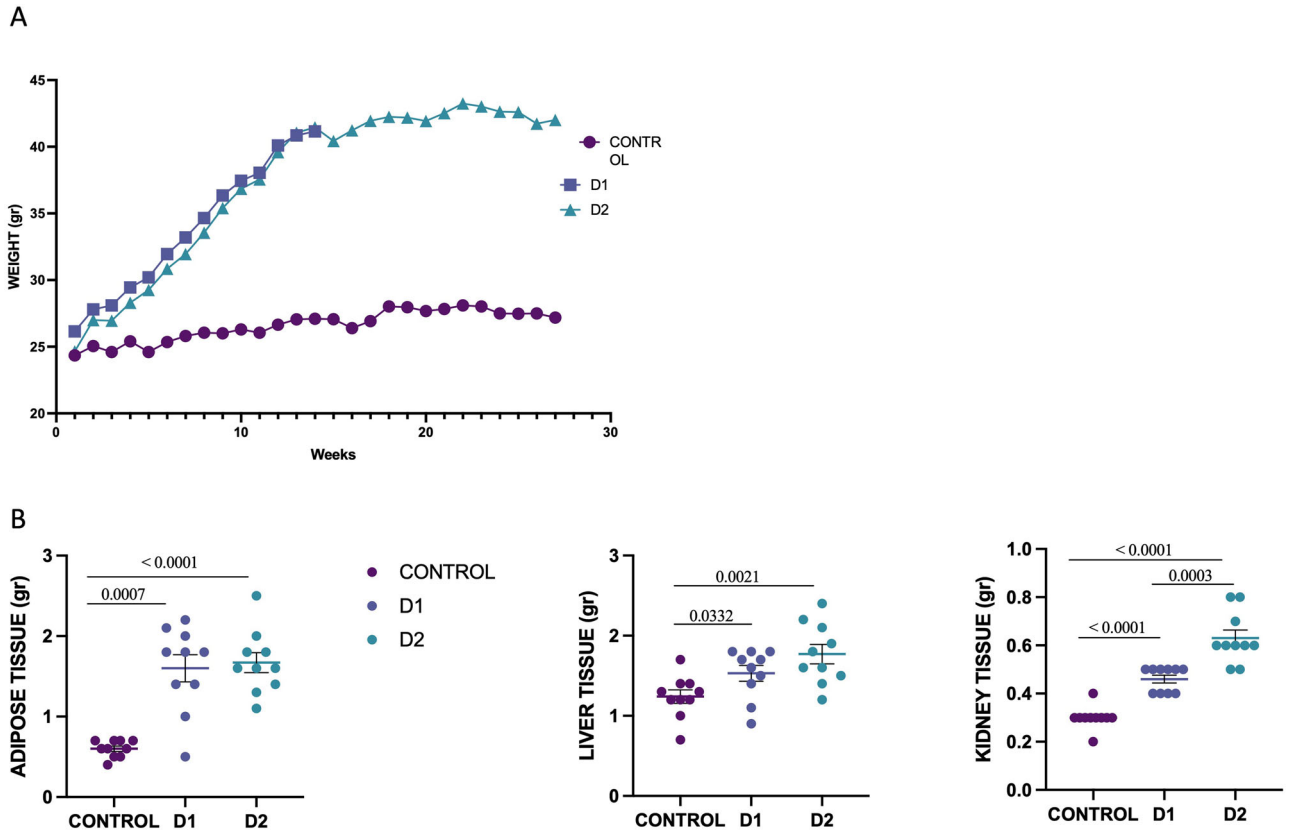

Fig. 1 The weights of the experimental and control groups. The body and tissue weight of all mice was measured every week. **A** After 12 and 24 weeks of feeding, the body and tissue weight of the D1 and D2 groups were dramatically higher than control group. Morever, the body weight of the D2 shows a statistically significant difference compared to group D1 (control&D1 $p < 0.0001$; control&D2 $p < 0.0001$; D1&D2 $p = 0.0241$). **B** The statistically significant differences were observed in kidney tissue weight among all three groups, but significant differences were detected between experimental and control groups in liver and adipose tissue (renal tissue: control&D1 $p < 0.0001$, control&D2 $p < 0.0001$ and D1&D2 $p = 0.003$; liver tissue: control&D1 $p = 0.0332$, control&D2 $p = 0.0021$; adipose tissue: control&D1 $p = 0.0007$, control&D2 $p < 0.0001$). GraphPad Prism version 8 program was used to create Fig. 1

both the D1 and D2 groups were shown in Table 3. When comparing the GO, MGO and MDA levels in the three tissues of the D1group, the renal tissue exhibited higher levels of GO, MGO and MDA. Moreover, the GO levels were notably higher in liver tissue than in adipose tissue of the D1 and D2 groups (data shown in Table 4).

Discussion

Obesity is the main cause of chronic diseases such as Type 2 diabetes, cardiovascular disease, NAFLD, and some types of cancer [19]. Fat acid and increased circulating concentrations of advanced glycation end products (AGEs) are associated with increased levels of oxidative stress and inflammatory markers. Advanced glycation end products (AGEs) represent a diverse and extensive collection of biologically active compounds resulting from the non-enzymatic interaction between reducing sugars and proteins, lipids, and nucleic acids [11]. Several studies have highlighted some dietary aspects that can influence extra- and intra-cellular accumulation of AGEs [20–24]. The present study was designed to examine the GO, MGO, and MDA levels in the liver, renal, and adipose tissue of mice fed a high-fat diet.

Elevated levels of circulating free fatty acids are associated with high-fat consumption, and a notable rise in tissue triglyceride levels can be the most detrimental outcome of a high-fat diet., which leads to protein nitration and lipid peroxidation [25] Li et al. observed a significantly increased level of AGEs in the liver of the obese group compared to the control group, with no notable change observed in the kidney [26]. On the other hand, we found a significant relationship between AGEs and MDA levels in renal tissue. Moreover, Tezuka et al. reported that has a close clinical association between higher AGEs serum concentrations and chronic renal disease [27]. As an unexpected finding in our study, we observed higher levels of AGEs in the control tissues, contrary to lower AGE levels observed in mice fed a high-fat diet. These results indicate a reversal of the expected effects of high-fat diets. It is known that AGEs are

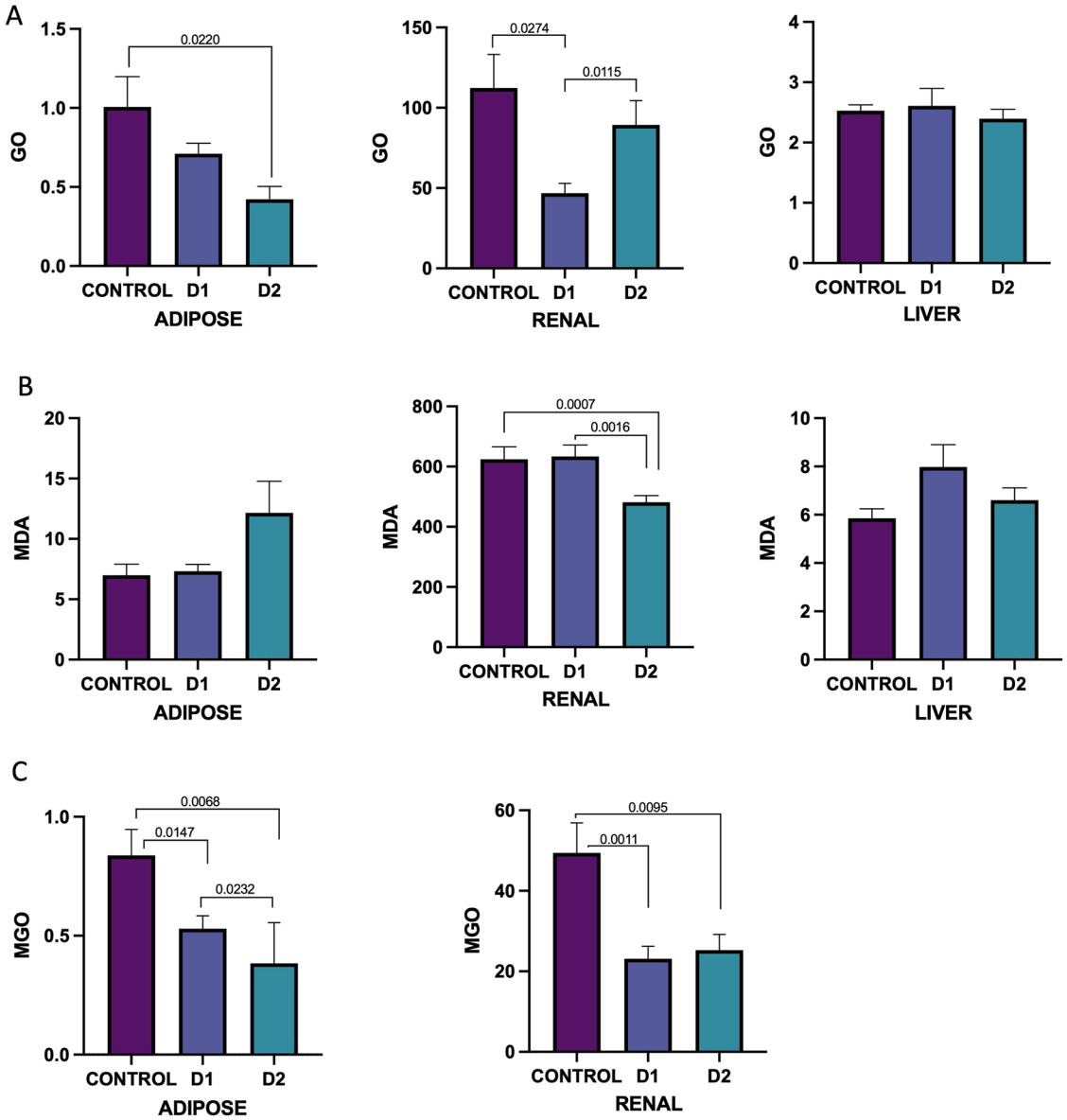

Fig. 2 Tissue levels of GO, MGO, and MDA in the study groups. Specially, the levels of GO, MDA, and MGO were found to be very high in renal tissue. **A** The GO levels in different tissues of the study groups (adipose tissue: control&D2 $p = 0.022$, D1&D2 $p = 0.0027$; renal tissue: control&D1 $p = 0.0274$, D1&D2 $p = 0.0115$). **B** The MDA levels in different tissues of the study groups. We found statistically significant differences in terms of MDA levels in renal tissue (control&D2 $p = 0.0007$, D1&D2 $p = 0.0016$). **C** The MGO levels in different tissues of the study groups (adipose tissue: control&D1 $p = 0.0147$, control&D2 $p = 0.0068$, D1&D2 $p = 0.0232$; renal tissue: control&D1 $p = 0.0011$, control&D2 $p = 0.0095$). GraphPad Prism version 8 program was used to create Fig. 2

formed during lipid peroxidation and auto-oxidation reactions, so the elevated AGE levels in the control group may be influenced by different metabolic mechanisms. The other study showed that higher MGO serum concentrations were negatively related to the estimated glomerular filtration rate and may lead to renal function deterioration [28]. Zhao et al. reported that the AGEs levels in plasma, liver, and kidney of very high-fat diet mice were higher than in the low-fat diet group [29]. However, our study showed no significant relation between AGEs and MDA in liver tissue was found.

Our study showed that GO and MGO levels were higher in the control group compared to the D1 and D2 groups of renal and adipose tissue. Even if our current study shows that high-fat diet-induced obesity isn't associated with increased tissue AGE levels in tissue, previous studies reported that AGEs in blood leaked from some organs [30]. Moreover, MGO and GO are a potent glycating agent, that reacts with amino acid residues of protein, nucleic acids, and lipids and this process influences various proteins which is important for cell metabolism. The lower detection

Table 2 The levels of GO, MGO and MDA tissue levels among the study groups

AGEs	Tissues	Control (Mean ± SE) (μg/100 g)	D1 (Mean ± SE) (μg/100 g)	D2 (Mean ± SE) (μg/100 g)	*p1 (Control&D1)*	*p2 (Control&D2)*	*p3 (D1&D2)*
GO	Adipose	1 ± 0.19	0.71 ± 0.06	0.422 ± 0.08	0.1655	**0.022**	**0.0027**
	Liver	2.53 ± 0.1	2.609 ± 0.3	2.396 ± 0.16	0.7394	0.5288	0.5787
	Renal	112.3 ± 20.88	46.8 ± 6.17	89.29 ± 15.14	**0.0274**	0.3821	**0.0115**
MDA	Adipose	6.99 ± 0.92	7.33 ± 0.55	12.16 ± 2.62	0.1576	0.1377	0.2086
	Liver	5.86 ± 0.39	7.98 ± 0.92	6.605 ± 0.51	0.072	0.2393	0.2713
	Renal	624.6 ± 40.96	633.6 ± 37.93	481.9 ± 21.56	0.6833	**0.0007**	**0.0016**
MGO	Adipose	0.84 ± 0.11	0.53 ± 0.05	0.38 ± 0.17	**0.0068**	**0.0147**	**0.0232**
	Renal	49.43 ± 7.43	23.14 ± 3.08	25.28 ± 3.9	**0.0011**	**0.0095**	0.5787

The significance between groups was measured using the non-parametric Mann–Whitney U test, and the fold changes are presented as Mean ± Standard Error of the Mean (X ± SEM). Statistical significance is indicated by $p < 0.05$. $p1$ represents the weight of the D1 compared to the control group; $p2$ indicates the weight of the D2 compared to the control group, and $p3$ illustrates the weight of the D1 compared to the D2

The significance levels are indicated in bold.

Table 3 The levels of GO and MGO in the same tissues of study group

Group/Tissue	GO Mean ± SE (μg/100 g)	MGO Mean ± SE (μg/100 g)	*p value (GO&MGO)*
D1/Adipose	0.71 ± 0.06	0.53 ± 0.05	**0.0524**
D2/Adipose	0.422 ± 0.08	0.38 ± 0.17	**0.0336**
D1/Renal	46.8 ± 6.17	23.14 ± 3.08	**0.0039**
D2/Renal	89.29 ± 15.14	25.28 ± 3.91	**<0.0001**

The significance between groups was measured using the non-parametric Mann–Whitney U test, and the fold changes are presented as Mean ± Standard Error of the Mean (X ± SEM). Statistical significance is indicated by $p < 0.05$

The significance levels are indicated in bold.

Table 4 The levels of GO, MGO, and MDA in the different tissues of the study group

STUDY GROUPs	AGEs	Tissues	MEAN ± SX (μg/100 g)	p1 (Adipose&Liver)	p2 (Adipose&Renal)	p3 (Liver&Renal)
D1	GO	Adipose	0.71 ± 0.06	**<0.0001**	**<0.0001**	**<0.0001**
		Liver	2.61 ± 0.29			
		Renal	46.8 ± 6.17			
	MDA	Adipose	7.331 ± 0.55	0.7229	**<0.0001**	**<0.0001**
		Liver	7.98 ± 0.92			
		Renal	633.6 ± 37.93			
	MGO	Adipose	0.5294 ± 0.05	–	**<0.0001**	**–**
		Renal	23.14 ± 3.07			
D2	GO	Adipose	0.422 ± 0.08	**<0.0001**	**<0.0001**	**<0.0001**
		Liver	2.396 ± 0.16			
		Renal	89.29 ± 15.14			
	MDA	Adipose	12.16 ± 2.62	0.1008	**<0.0001**	**<0.0001**
		Liver	6.605 ± 0.51			
		Renal	481.9 ± 21.56			
	MGO	Adipose	0.38 ± 0.17	–	**<0.0001**	–
		Renal	25.28 ± 3.9			

The significance between groups was measured using the non-parametric Mann–Whitney U test, and the fold changes are presented as Mean ± Standard Error of the Mean (X ± SEM). Statistical significance is indicated by $p < 0.05$. $p1$ represents the AGEs and MDA levels of the adipose tissue compared to the liver tissue; $p2$ indicates the AGEs and MDA levels of the adipose tissue compared to the renal tissue, and $p3$ illustrates the AGEs and MDA levels of the liver tissue compared to the renal tissue

The significance levels are indicated in bold.

in the D1 and D2 groups compared to the control group may be attributed to interactions with various proteins such as hemoglobin, insulin, some growth factors and ECM proteins [31–34]. However, the study reported that AGEs and MDA plasma levels were not significantly altered after fat-feeding [34]. We additionally observed, in concordance with the literature that the lower accumulation is present in adipose tissue compared to liver and renal tissue [35].

AGEs (Advanced Glycation End-products) interact with proteins, and in obese mice, this interaction might have occurred more intensely, leading to a higher accumulation of AGEs in tissues. The interaction of AGEs with proteins may disrupt their function and alter their metabolism, affecting the clearance or accumulation of AGEs in tissues. Especially, renal tissue play an important role in filtering and removing waste products from the body, including AGEs. The increased accumulation in the kidneys might indicate a higher burden on these organs to process and eliminate AGEs, leading to their accumulation. Additionally, increased inflammation and oxidative stress in obese mice may lead to faster metabolism or detoxification of AGEs and the proteins they interact with. This could contribute to lower measured levels of AGEs than expected. In summary, the present study was the first to assess of long-term high-fat diet on tissue accumulation of AGEs. Our results can be utilized to develop dietary strategies to inhibit the formation of AGEs to prevent the development of obesity and obesity related metabolic diseases. Moreover, more studies are needed to elucidate the biological basis of AGEs's relationship with biochemical parameters.

Data availability

The datasets utilized and/or analyzed during the present study are accessible from the corresponding author upon reasonable request.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s12013-024-01260-6.

Author contributions All authors contributed to the conception and design of the study; data acquisition: Ş.D. and Y.Y.; data analysis and interpretation: M.Y. and F.Ç.; manuscript drafting: S.D. and Y.Y.; manuscript critical revision: Ü.Z. and Ş.D.; administrative, technical, and material support: E.Y., D.K., S.D., and S.U.; study supervision: Ü.Z. and S.D.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Ethics approval This study followed the principles outlined in the Declaration of Helsinki. Approval for the study was granted by the Istanbul University Rectorate's Local Ethics Committee for Animal Experiments (Approval Number: 2021/18).

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