



## The effects of oral antidiabetics on adipogenesis related gene expressions in 3T3-L1, AML12 cell lines and their co-cultures

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**Abstract:** The objective of the study was investigated for the effects of oral antidiabetic drugs, how they effect the cells proliferation activities and how they change the expressions of FTO, CD68, NIBAN, and RAN genes which could change insulin signalization and also be effective in the adipogenetic process on 3T3-L1 adipocytes, AML12 hepatocytes and adipocyte-hepatocyte co-cultures. Cell proliferation was examined real time with iCELLigence system and measured for 96 hours each 15 min period. The time and amount of active substances of the oral anti-diabetic drugs which were applied to cells were determined real time according to IC50 value. FTO, CD68 NIBAN and RAN gene expression profiles were determined with qPCR. When single and multiple doses of glipizide and acarbose in co-culture's were compared respectively, the 24 hour IC50 values were determined as 180  $\mu$ M and 17 mg/ml in adipocytes; 72  $\mu$ M and 23 mg/ml in hepatocytes, 41.5  $\mu$ M and 5 mg/ml in coculture cells. The application of metformin for 24 hour IC50 value in single culture was determined as 175 mM in adipocytes and 2.3 mM in hepatocytes. In the Metformin administered cells. FTO, CD68 and NIBAN gene expressions were decreased in all groups. In the acarbose applied cells FTO and CD68 gene expressions were decreased in all groups. In the acarbose applied cells while NIBAN gene expression was decreased in adipocytes, it was fund to increase in co-cultured adipocytes. In the acarbose applied cells RAN gene expression was found to increase in all groups. Decreasing effects of antidiabetics on CD68 and FTO expressions may show protective effect of drug on inflammation and obesity. In conclusion, oral antidiabetic drug use may be effective in development glucose homeostasis via changing the gene expression of RAN in hepatocytes and adipocytes, this pathway may have therapeutic effect and provide novel strategies for treatment of insulin resistance.

**Keywords:** 3T3-L1 adipocyte, AML12 hepatocyte, iCELLigence system, oral antidiabetics, non-alcoholic fatty liver disease, gene expression

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### 1. Introduction

Obesity is a complex medical condition caused by excessive fat accumulation in the body caused by excessive consumption of foods and low levels of physical activity (Vasanth Rao et al., 2019). Obesity is a risk factor for type 2 diabetes mellitus (T2DM), hypertension, cardiovascular diseases, metabolic syndrome and cancer (Koldemir et al., 2012). Obesity not only contributes to other health problems, but also reduces quality of life and increases healthcare costs (Vasanth Rao et al., 2019). Type 2 diabetes mellitus (T2DM) is usually formed by the interaction of genetic and environmental factors and resulting in excessive increase in blood glucose levels, chronic, complex, and is a common metabolic disorder (Prokopenko et al., 2008). Type 2 diabetes, insulin secretion and activity of (fat, muscle and liver) is characterized by deterioration and associated with obesity (Prokopenko et al., 2008). The pathogenesis of type 2 diabetes beta cell dysfunction, insulin resistance and

increase hepatic glucose takes place (Reaven, 1995). There are several genetic disorders that cause T2DM. This genetic disorder of glucose, lipid and protein metabolism leads to the formation of T2DM (Türkün, 2008). Obesity, hyperglycemia, hypertension, and plasma lipid abnormalities accompanied by insulin resistance (Reaven, 1995). Non-alcoholic fatty liver disease (NAFLD) affects 25% of the population and treatment options are limited (Niu et al., 2019). Non-alcoholic fatty liver disease (NAFLD) is closely associated with obesity, hypertriglyceridemia and diabetes. (Sanyal, 2003). NAFLD is a costly medical disease and continues to grow in parallel with the increasing prevalence of obesity and type 2 diabetes (Younossi et al., 2016). The liver and adipose tissue originate from a multipotent cell. Thus, the key molecules and signaling systems of adipose tissue and liver are regulated by common or overlapping metabolic and immune function pathways (Wellen and Hotamisligil, 2005; Shoelson et al., 2006).

Fat mass and obesity-associated gene (FTO) located on 16 q12.2 chromosome region is highly expressed in the hypothalamus and is suggested to play an important role in controlling energy homeostasis (Yuzbashian et al., 2019). In genome wide association studies, FTO gene polymorphisms were associated with increased risk obesity (Frayling et al., 2007; Dina et al., 2007; Scuteri et al., 2007). It has been reported that FTO gene over expression induced obesity (Rösen et al., 2001; Stratigopoulos et al., 2008). CD68 is a member of the lysosome associated membrane protein family that is restricted in its expression to cells of the monocyte/macrophage lineage. Weisberg et al. (2003) reported that up to 50% of the cells in the adipose tissue were positive for CD68. Reduction of inflammatory gene expression in adipose tissue has also been associated with weight loss in obese subjects (Clement et al., 2004; Canello et al., 2005). The NIBAN gene, which is a member of the FAM129A gene family and consists of 14 exons, is located on the 13th chromosome in rats and the 1st in human and mice (Adachi et al., 2004). NIBAN regulates protein synthesis by modulating the phosphorylation of eukaryotic translation initiator factor 2 $\alpha$  (eIF2 $\alpha$ ), p70 ribosomal S6 kinase 1 subunit and eukaryotic translation initiator factor 4E binding protein 1 (Sun et al., 2007). NIBAN plays a role in adipogenesis with unknown mechanism (Ye et al., 2011). RAN (LOC100045999 GTP-binding nuclear protein Ran) protein regulates a multitude of cellular responses, including nucleo-cytoplasmic shuttling, various aspects of mitosis, and other cytoplasmic transport mechanisms in specialized cell types (Segev, 2011; Clarke and Zhang, 2008; Yudin and Fainzilber 2009). Ran signaling is highly evolutionary conserved, and is thought to be essential for cellular homeostasis (Ye et al., 2011). RAN protein plays a role in adipogenesis with unknown mechanism (Ye et al., 2011).

There are many antidiabetic agents used in routine detavitation such as insulin, biguanides, sulfonylureas, thiazolidindiones (TZDs), sodium-glucose cotransporter type 2 inhibitors(SGLT2i), dipeptidyl peptidase 4 (DPP-4) inhibitors, glucagon-likepeptide 1 (GLP-1 receptor agonists) and  $\alpha$  glucosidase inhibitors (AGis) (American Diabetes Association, 2018). Biguanides (especially metformin) are widely used for the treatment of T2DM and exhibit hypoglycemic effects by inducing insulin sensitivity and peripheral glucose uptake, reducing hepatic gluconeogenesis, reducing glucose reabsorption of the intestine (Yaribeygi et al., 2019). Sulfonylureas is a class of hypoglycemic antidiabetic drugs that increase insulin release from cells (Yaribeygi et al., 2019). Alpha glycosidase inhibitors inhibit the  $\alpha$ -glycosidase enzymes in the small intestine and thus delay the absorption of carbohydrates (Yaribeygi et al., 2019).

In this study, the proliferative effect of AML12 hepatocytes, 3T3-L1 adipocyte and their co-culture by continuous monitoring assay was performed to investigate cytotoxicity of commonly used oral antidiabetic drug metformin, acarbose and glipizide and we sought to

determine the effects of oral antidiabetic drug metformin on FTO, CD68, NIBAN and RAN gene expressions in AML12 hepatocytes, 3T3-L1 adipocyte and their co-culture.

## 2. Materials and Method

### 2.1. Cell Culture

Murine AML12 hepatocytes and 3T3-L1 fibroblasts were purchased from the American Type Culture Collection (ATCC) (Manassas, USA). 3T3-L1 cells were cultured in glucose-free Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal calf serum, penicillin (100 units/ml), and streptomycin (100  $\mu$ g/ml) in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C, with a change of medium every 2-3 days. AML12 cells and co-culture cells were cultured in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium with 0.005 mg/ml insulin, 0.005 mg/ml transferrin, 5 ng/ml selenium, and 40 ng/ml dexamethasone, 90%; fetal bovine serum, penicillin (100 units/ml), and streptomycin (100  $\mu$ g/ml), 10% in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C with a change of medium every 2-3 days. Sub-cultured as necessary by routine trypsinization method.

### 2.2. Differentiation of 3T3-L1 preadipocytes

3T3-L1 cell differentiation was based on the protocol by Miard et al. (2008). Cells were grown in DMEM medium until 80% confluency. Two days after confluency (Day 0), cells were washed with phosphate buffered saline (PBS) and fed with adipocyte differentiation media (DMEM, 10% fetal calf serum, 1  $\mu$ M dexamethasone, 10  $\mu$ g/mL insulin, and 0.25 mM 3-methyl-1-isobutylxanthine) for 2 days. Two days (Day 2) post induction, medium was supplemented with only 10  $\mu$ g/mL insulin and replaced every 2 days until terminal differentiation.

### 2.3. Oil Red O Staining

Cells were washed with PBS and then fixed for 1 hour with 10% formalin solution. 60% isopropanol was added to each well and let sit for 5 minutes. Oil red O was diluted with water (3:2), filtered through a 0.22 $\mu$ m filter and incubated with the fixed cells for 5 min at room temperature. Cells were washed with water and the stained lipid droplets in the cells.

### 2.4. Cytotoxicity assay using iCELLigence system

The optimal cell concentration for proliferation experiments of AML12 hepatocytes, of 3T3-L1 adipocytes and their co-culture were determined. After seeding the cells in 150  $\mu$ L medium to each well of the E-plate 8, the proliferation of cells were monitored every 15 min by the iCELLigence system. Approximately 24h after seeding, the AML12 cells were subjected to 50  $\mu$ L of medium containing the following substances: acarbose (2.5 mg/ml, 5 mg/ml, 7.5 mg/ml, 10 mg/ml, 15 mg/ml, 25 mg/ml and

50 mg/ml), glipizide (100  $\mu$ M, 150  $\mu$ M and 500  $\mu$ M) and metformin (0.5 mM, 1 mM, 2 mM, 4 mM, 5 mM, 7.5 mM and 10 mM). Approximately 24h after seeding, the 3T3-L1 cells were subjected to 50  $\mu$ L of medium containing the following substances: acarbose (2.5 mg/ml, 5 mg/ml, 7.5 mg/ml, 10 mg/ml, 15 mg/ml, 25 mg/ml and 50 mg/ml), glipizide (100  $\mu$ M, 250  $\mu$ M and 500  $\mu$ M) and metformin (5mM, 10mM, 25mM, 50mM, 100mM, 150mM and 250mM). Approximately 24h after seeding, the co-culture cells were subjected to 50  $\mu$ L of medium containing the following substances: acarbose (1 mg/ml, 2 mg/ml, 4 mg/ml, 5 mg/ml, 7.5 mg/ml and 10 mg/ml), glipizide (100  $\mu$ M, 150  $\mu$ M and 500  $\mu$ M) and metformin (10 mM, 25 mM, 50 mM, 75 mM and 100 mM). Only culture medium was added to control cell group. All experiments were monitored up to 96h.

### 2.5.RNA Isolation and Real-Time PCR Analysis

Total RNA was extracted from cells using the High Pure RNA Tissue Kit (Roche, Germany) according to the manufacturers instructions. Total RNA samples were diluted in DNase–RNase free sterile water and stored at  $-80^{\circ}\text{C}$  until use. For cDNA synthesis, transcriptor HiFi cDNA synthesis kit was used (Roche, Germany). Expression of FTO, CD68, NIBAN and RAN genes were determined by quantitative real-time PCR using a LightCycler Nano (Roche Diagnostics, Germany) instrument. Measurements were carried out on at least three occasions for each sample. Cq values lower than 40 were not included to advanced statistival calculations. The expression amounts were calculated by using the reference gene (GAPDH) expression. Existence of specific gene products were confirmed with melting curve analysis. The gene expressions studies were repeated three times.

### 2.6.Statistical analysis

For the evaluation of results obtained from the iCELLigence system, statistical analyses were performed using the iCELLigence system software. The system software make a curve-fitting of elective “sigmoidal dose–response equation” to the experimental data points and calculates logarithmic half maximum inhibition concentration (log [IC50]) values. Results are presented as mean $\pm$ SE. Statistical analysis was performed with SPSS software (version 16.0; SPSS Inc., Chicago, IL). In general, statistical differences between two groups were evaluated by the Wilcoxon signed ranks test and for comparison of multiple groups Friedman test (followed by Wilcoxon signed ranks test) was used. A p-value below 0.05 was considered significant.

## 3. Results

### 3.1.Effects of Antidiabetic Drugs over Proliferation of 3T3-L1 Adipocyte

To investigate the effect of oral antidiabetic drugs on proliferation, 3T3-L1 adipocytes were cultured for 24 h in

standard medium (untreated). 100  $\mu$ M, 250  $\mu$ M and 500  $\mu$ M glipizide were applied to the cell culture medium. While 500  $\mu$ M glipizide had a lethal effect for adipocytes, 250  $\mu$ M glipizide application has an antiproliferative effect on adipocytes. IC50 values were determined as 180  $\mu$ M after 24h glipizide administration. The 3T3-L1 adipocytes were treated with 2.5, 5, 7.5, 10, 15, 25 and 50 mg / ml acarbose. The dose of acarbose applied to adipocyte cells did not have a lethal effect while producing cytotoxic effects on cells. IC50 values were determined as 17 mg/ml after 6h acarbose administration. 5, 10, 25, 50, 100, 150 and 250 mM metformin were applied to 3T3-L1 adipocyte cells. 5, 10, 25, 50 mM metformin administration had antiproliferative effect in 3T3-L1 adipocytes and 250 mM metformin had lethal effect for adipocytes. The IC50 value at 24 hours was calculated as 175 mM as a result of metformin administration.

### 3.2.Effects of Antidiabetic Drugs over Proliferation of AML12 Hepatocyte

To investigate the effect of oral antidiabetic drugs on proliferation, AML12 hepatocytes were cultured for 24 h in standard medium (untreated). 100  $\mu$ M, 250  $\mu$ M and 500  $\mu$ M glipizide were applied to the cell culture medium. 500  $\mu$ M glipizid was found to have lethal effect on hepatocytes. 250  $\mu$ M glipizide application has an antiproliferative effect on hepatocytes. IC50 values were determined as 72  $\mu$ M after 24h glipizide administration. The AML12 hepatocytes were treated with 2.5, 5, 7.5, 10, 15, 25 ve 50 mg/ml acarbose. 25 ve 50 mg/ml acarbose applied cytotoxic effects on cells. IC50 values were determined as 23 mg/ml after 24h acarbose administration. 5, 10, 25, 50, 100, 150 and 250 mM metformin were applied to 3T3-L1 adipocyte cells. Administration of 25, 50 and 100 mM metformin showed a severe cytotoxic effect on hepatocyte cells. Application of 150 and 250 mM metformin showed lethal effect on hepatocytes. IC50 value at 24 hours was calculated as 2.3 mM as a result of metformin application.

### 3.3.Effects of Antidiabetic Drugs over Proliferation of AML12 Hepatocyte and 3T3-L1 co-culture

To investigate the effect of oral antidiabetic drugs on proliferation, co-culture cells were cultured for 24 h in standard medium (untreated). 100  $\mu$ M, 250  $\mu$ M and 500  $\mu$ M glipizide were applied to the cell culture medium. 500  $\mu$ M glipizid was found to have lethal effect on cells. 250  $\mu$ M glipizide application has an antiproliferative effect on cells. IC50 values were determined as 41.5  $\mu$ M after 24h glipizide administration. Co-culture cells were treated with 1, 2, 4, 5, 7.5 and 10 mg / ml acarbose. Doses of 4, 5, 7.5 and 10 mg / ml acarbose were found to have an inhibiting effect on the cells. IC50 values were determined as 5 mg/ml after 24h acarbose administration. 10, 25, 50, 75 and 100 mM metformin were applied to the cell culture medium. The doses of metformin were not found to have a lethal effect on the cells but were found to have a anti proliferative effect on the cells.

**3.4.FTO, CD68, NIBAN and RAN Gene Expression Levels in Response to Oral Antidiabetic Drugs in 3T3-L1 Adipocytes**

24h 180 µM concentration of glipizide administered increasing effect over NIBAN and RAN gene expressions respectively as 3.05, 2.296 (p=0.005) fold compared to control adipocytes. FTO and CD68 gene expression levels were found to decrease respectively as 0.631, 0.627 (p=0.005) (Table 1.). 24h 175mM concentration of metformin administered decreasing effect over FTO, CD68, NIBAN and RAN gene expressions respectively as 0.095, 0.652, 0.874, 0.783 (p=0.005) fold compared to control adipocytes (Table 1.).

**Table 1.** The effect of oral antidiabetic drugs on FTO, CD68, NIBAN and RAN gene expression levels in 3T3-L1 adipocytes

	FTO Gene Expression Mean±SE; median (min-max)	p	CD68 Gene Expression Mean±SE; median (min-max)	p	NIBAN Gene Expression Mean±SE; median (min-max)	p	RAN Gene Expression Mean±SE; median (min-max)	p
Control	1.0 ± 0.0		1.0 ± 0.0		1.0 ± 0.0		1.0 ± 0.0	
A4	0.09 ± 0.00; 0.09 (0.09-0.10)	0.005	0.65 ± 0.00; 0.65 (0.65-0.66)	0.005	0.87 ± 0.00; 0.87 (0.87-0.88)	0.005	0.78 ± 0.00; 0.78 (0.78-0.79)	0.005*
A5	0.35 ± 0.00; 0.35 (0.34-0.35)	0.005	2.19 ± 0.00; 2.19 (2.18-2.19)	0.005	3.15 ± 0.00; 3.15 (3.15-3.16)	0.005	0.95 ± 0.00; 0.95 (0.95-0.96)	0.005*

Values are given as mean ± standart error; median (minimum-maximum). n:5 A3: Adipocyte cells treated with 180µM glipizide for 24 hours. A4: Adipocyte cells treated with 175mM metformin for 24 hours. Adjusted significanse limir p:0.010\*. (Performed with Mann-Whitney Tes. Each group was compared with control)

**3.5.FTO, CD68 and RAN Gene Expression Levels in Response to Oral Antidiabetic Drugs in AML12 Hepatocytes**

24h 72 µM concentration of glipizide administered increasing effect over FTO and RAN gene expressions were found to repectively as 2.059, 1.171 and CD68 gene expression level was found to decreasing effect 0.232 (p=0.005) fold compared to control hepatocytes (Table 2.). 24h 2.3 mM concentration of metformin administered decreasing effect over FTO, CD68 and RAN gene expressions respectively as 0.13, 0.359, 0.743 (p=0.005) fold compared to control hepatocytes (Table 2.). 24h 23 mg/ml concentration of acarbose administered increasing effect over RAN gene expression levels 3.132 fold compared to control hepatocytes. 24h 23mg/ml concentration of acarbose administered decreasing effect over FTO and CD68 gene expressions levels respectively as 0.089, 0.116 (p=0.005) fold compared to control hepatocytes (Table 2.).

**Table 2.** FTO, CD68 and RAN Gene Expression Levels in Response to Oral Antidyabetic Drugs in AML12 Hepatocytes

	FTO Gene Expression Mean±SE; median (min-max)	p	CD68 Gene Expression Mean±SE; median (min-max)	p	RAN Gene Expression Mean±SE; median (min-max)	p
Control	1.0 ± 0.0		1.0 ± 0.0		1.0 ± 0.0	
H1	2.06 ± 0.00; 2.06 (2.05-2.06)	0.005*	0.23 ± 0.00; 0.23 (0.23-0.24)	0.005*	1.17 ± 0.00; 1.17 (1.17-1.18)	0.005*
H2	0.13 ± 0.00; 0.13 (0.13-0.14)	0.005*	0.36 ± 0.00; 0.36 (0.35-0.36)	0.005*	0.74 ± 0.00; 0.74 (0.74-0.75)	0.005*
H3	0.09 ± 0.00; 0.09 (0.08-0.09)	0.005*	0.12 ± 0.00; 0.12 (0.11-0.12)	0.005*	3.13 ± 0.00; 3.13 (3.13-3.14)	0.005*

Values are given as mean ± standart error; median (minimum-maximum). n:5 H1: Hepatocyte cells treated with 72 µM glipizide for 24 hours. H2: Hepatocyte cells treated with 2.3mM metformin for 24 hours. H3: Hepatocyte cells treated with 23 mg/ml acarbose for 24 hours. Adjusted significanse limir p:0.010\*. (Performed with Mann-Whitney Tes. Each group was compared with control)

**3.6.FTO, CD68 and RAN gene expression levels in response to oral antidiabetic drugs in AML12 hepatocytes in co-culture**

24h 41.5 µM concentration of glipizide administered increasing effect over FTO and CD68 gene expressions respectively as 1.229, 1.716 (p=0.005) fold compared to control hepatocytes in co-culture and RAN gene expression levels decreasing effect 0.000 fold compare to control cells (p = 0.005) (Table 3.).

24h 5mg/ml concentration of acarbose administered increasing effect over RAN gene expression levels 2.385 fold compared to control hepatocytes in co-culture. 24h 5mg/ml concentration of acarbose administered decreasing effect over FTO and CD68 gene expressions levels respectively as 0.796, 0.327 (p=0.005) fold compared to control hepatocytes in co-culture (Table 3.).

**Table 3.** FTO, CD68 and RAN gene expression levels in response to oral antidiabetic drugs in AML12 hepatocytes in co-culture

	FTO Gene Expression Mean±SE; median (min-max)	p	CD68 Gene Expression Mean±SE; median (min-max)	p	RAN Gene Expression Mean±SE; median (min-max)	p
Control	1.0 ± 0.0		1.0 ± 0.0		1.0 ± 0.0	
C1	1.23 ± 0.00; 1.23 (1.22-1.23)	0.005	1.72 ± 0.00; 1.72 (1.71-1.72)	0.005	0.00 ± 0.00; 0.00 (0.00-0.00)	-
C2	0.80 ± 0.00; 0.80 (0.79-0.80)	0.005	0.33 ± 0.00; 0.33 (0.32-0.33)	0.005	2.38 ± 0.00; 2.38 (2.38-2.39)	0.005

Values are given as mean ± standart error; median (minimum-maximum). n:5 C1: Hepatocyte cells treated with 41.5 µM glipizide for 24 hours. C2: Hepatocyte cells treated with 5 mg/ml acarbose for 24 hours. Adjusted significanse limir p:0.010\*. (Performed with Mann-Whitney Tes. Each group was compared with control)

### 3.7.FTO, CD68 and RAN gene expression levels in response to oral antidiabetic drugs in 3T3-L1 adipocytes in co-culture

We demonstrated that 24h, 41.5  $\mu$ M glipizide have increasing effect over RAN gene expression as 5.287 fold to that of control cells adipocytes in co-culture whereas 41.5  $\mu$ M glipizide have decreasing effect over FTO, CD68 and NIBAN gene expressions respectively as after 0.122, 0.338, 0.525 24h administration (**Table 4.**).

We demonstrated that 24h, 5mg/ml acarbose have increasing effect over NIBAN and RAN gene expression respectively as 3.11, 1.58 fold to that of control cells adipocytes in co-culture whereas 5mg/ml acarbose have decreasing effect over FTO and CD68 gene expressions respectively as after 0.027, 0.165 24h administration (**Table 4.**).

**Table 4.** FTO, CD68 and RAN gene expression levels in response to oral antidiabetic drugs in 3T3-L1 adipocytes in co-culture

	FTO Gene Expression Mean $\pm$ SE, median (min-max)	p	CD68 Gene Expression Mean $\pm$ SE, median (min-max)	p	NIBAN Gene Expression Mean $\pm$ SE, median (min-max)	p	RAN Gene Expression Mean $\pm$ SE, median (min-max)	p
Control	1.0 $\pm$ 0.0		1.0 $\pm$ 0.0		1.0 $\pm$ 0.0		1.0 $\pm$ 0.0	
C3	0.12 $\pm$ 0.00; 0.12 (0.12-0.13)	0.005	0.34 $\pm$ 0.00; 0.34 (0.33-0.34)	0.005	0.52 $\pm$ 0.00; 0.52 (0.52-0.53)	0.005	5.29 $\pm$ 0.00; 5.29 (5.28-5.29)	0.005
C4	0.03 $\pm$ 0.00; 0.03 (0.02-0.03)	0.005	0.16 $\pm$ 0.00; 0.16 (0.16-0.17)	0.005	3.11 $\pm$ 0.00; 3.11 (3.11-3.12)	0.005	1.58 $\pm$ 0.00; 1.58 (1.58-1.59)	0.005

Values are given as mean  $\pm$  standart error; median (minimum-maximum). n:5 C3: Adipocyte cells treated with 41.5  $\mu$ M glipizide for 24 hours. C4: Adipocyte cells treated with 5 mg/ml acarbose for 24 hours. Adjusted significance limir p:0.010\*. (Performed with Mann-Whitney Tes. Each group was compared with control)

## 4. Discussion

Obesity is strongly associated with the development of insulin resistance and is thought to be responsible for the development of type 2 diabetes. Although the epidemiological relationship has been established, the cellular linkage and molecular mechanisms between obesity and insulin resistance are still unknown. Increased obesity is associated with lipid accumulation in the liver. Although the role of visceral fat in the development of systemic insulin resistance is controversial, the contribution of visceral fat is important in the development of hepatic insulin resistance (Cai et al., 2005; Kim et al., 2003). it is predicted that visceral fat depot may induce liver insulin resistance and this station stimulates systemic insulin resistance (Wang et al., 2006). Several studies have shown that hepatic insulin resistance is associated with low-grade cellular inflammation (Cai et al., 2005; Arkan et al., 2005). Paracrine interaction between fat and liver can directly elicit an inflammatory response in liver cells, but this is not clearly known.

We demonstrated that AML12 hepatocytes, 3T3-L1 adipocytes and their co-culture FTO, CD68, NIBAN and RAN gene expressions in response to metformin, glipizide and acarbose exposure.

Animal model studies showed that FTO was positively associated with fat accumulation (McCarthy et al., 2010). In our study, we found that FTO gene expression level decreased 0.631 fold in 3T3-L1 adipocyte cells treated with 180  $\mu$ M glipizide for 24 hours. Glipizide exposure may have a protective effect against insulin resistance in adipose tissue. In our study, it was found that the expression level of CD68 gene decreased by 0.627 times when the cells treated with 180  $\mu$ M 24 hours glipizide were compared with the control cells. This may cure the insulin resistance in adipocyte cells and indicate that the inflammation there decreases. The mechanism by which the NIBAN gene, which is known to be involved in the adiponic process, is involved in this process remains unclear (Ye et al., 2011). It has been found that the NIBAN gene regulates cell death signals in response to endoplasmic reticulum stress and plays an anti-apoptotic role (Sun et al., 2007). In our study to determine the relationship between NIBAN gene and glipizide use, 3T3-L1 adipocytes treated with 180  $\mu$ M glipizide for 24 hours compared to control cells were found to increase the NIBAN gene 3 fold. This may reflect the role of glipizide in the treatment of both obesity and diabetes in obese patients with T2DM by secreting insulin by trying to produce an anti-apoptotic effect. Ran protein is involved in the control of DNA synthesis and the functioning of the mitotic cell cycle, and during mitosis, regulation of molecules in microtubule polymerization may be key to signaling (Sazer et al., 2000). According to our study, when compared to control cells with adipocytes treated 24 hours 180  $\mu$ M glipizide, RAN gene expression level was increased by 2,296 times. High RAN gene expression may have a regulatory effect on glucose homeostasis.

FTO, CD68, NIBAN and RAN gene expression were decreased in all groups in metformin treated cells. FTO inhibits gluconeogenesis and adipogenesis by balancing metformin activity (Melnik, 2015). Overexpression of the FTO gene causes an increase in gluconeogenesis and adipogenesis (Melnik, 2015). Increased FTO mRNA levels in subcutaneous adipose tissue in T2DM patients and treatment with rosiglitazone decreases FTO mRNA levels in subcutaneous adipose tissue by increasing insulin sensitivity (Bravard et al., 2013). The results of our study were reported by Bravard et al. (2013) is similar to his work. FTO gene expression level was decreased by 0.095 times when compared to control cells with 24 hours 175 mM metformin treated cells. Treatment of 3T3-L1 adipocyte cells with metformin increased insulin sensitivity in the cells, resulting in decreased FTO gene expression in adipocytes. CD68 is expressed in the stromal vascular fraction in human adipose tissues (Di Gregorio et al., 2005). Studies have shown that there is a significant infiltration of fat tissue by macrophages in obesity (Weisberg et al., 2003; Xu et al., 2003). Gregorio et al. in order to better understand the relationship between macrophage infiltration and insulin resistance in adipose tissue, they measured CD68 gene expression levels by giving patients metformin and pioglitazone (Di Gregorio et al., 2005). Found a relationship between impaired glucose

tolerance and decreased expression of CD68 (Di Gregorio et al., 2005). Zulian et al. (2011) found that metformin treatment in adipose tissue decreased CD68 gene expression statistically. We found that CD68 gene expression level decreased by 0.652 fold compared to control cells treated with 175 mM metformin for 24 hours. As a result of our study similar to the literature, we found that metformin reduced CD68 gene expression level and metformin plays a role in reducing regional inflammation in adipose tissue in vitro. Reduction of FTO and CD68 gene expression in metformin treated cells may indicate that it may have a protective effect on obesity and inflammation. 3T3-L1 cells treated with 175 mM metformin for 24 hours compared to control cells, NIBAN ge decreased 0.874 fold. Reduction of NIBAN gene expression by metformin administration may indicate mature adipocytes treatment. Our study is one of the first studies conducted in the literature to explain the relationship between obesity and RAN gene in adipogenic process. Adipocytes treated with 175 mM metformin for 24 hours compared to control cells, RAN gene expression level decreased 0.783 fold. The results of our study may reflect that metformin is a regulatory mechanism against impaired glucose homeostasis by reducing RAN gene expression in adipocytes.

Glipizide is metabolized mainly by the liver. Sulfonylureas may cause chronic hepatitis with necroinflammatory changes (Bloodworth et al., 1961). In our study, we applied 72  $\mu$ M glipizide to AML12 hepatocytes for 24 hours and found that FTO gene expression level increased 2.059 times when hepatocyte cells and control cells were compared. In our study, we applied 72  $\mu$ M glipizide to AML12 hepatocytes for 24 hours and we found that CD68 gene expression levels decreased 0.232 times when hepatocyte and control cells were compared. The decrease in the level of CD68 gene expression in the hepatocytes administered in glipizide suggests that it plays a role in reducing inflammation in the liver by reducing insulin resistance in hepatocytes. We exposed AML12 hepatocytes to 72 $\mu$ M glipizide for 24 hours, and it was found that RAN gene expression level increased 1.171 ( $p = 0.005$ ) fold when hepatocyte cells and control cells were compared. Increased RAN expression with the dose administered may be due to the need for regulation of glucose homeostasis in the liver.

Metformin is not involved in hepatic metabolism and is excreted unchanged in the urine. Biguanides, such as metformin hydrochloride, have not been reported associated with liver damage (Sulkin et al., 1997). In our study, AML12 hepatocytes cells treated with 24 hours 2.3 mM metformin and compared to control cells, FTO gene expression level decreased 0.13 fold. This may indicate that metformin administration to hepatocytes decreases the amount of fat in the liver and that metformin has a protective effect on the liver. Treatment of hepatocytes with 2.3 mM metformin for 24 hours revealed that CD68 gene expression level decreased by 0.359 times. As a result of the application of metformin to the hepatocyte

cells decreased CD68 gene expression level showed that the inflammation in that region decreased and metformin in the treatment benefit. It was determined that the expression level of RAN gene decreased by 0.743 times when hepatocytes treated with 24 hours 2.3 mM metformin were compared with control cells. According to the study of transgenic mice, RAN signaling is essential for pancreatic islet development and glucose homeostasis (Xia et al., 2011). The dose of metformin administered may reflect the activity of regulating decreased glucose homeostasis in hepatocytes.

Alpha-glycosidase inhibitors may be particularly useful in patients with liver disease (Tolman et al., 2007). Rudovich et al. (2010) found that in combination with ezetimibe and acarbose to treat non-alcoholic fatty liver disease, liver fat, inflammation and fibrosis were significantly reduced. In our study, compared to AML12 hepatocyte cells treated with 23 mM acarbose for 24 hours and control cells, FTO gene expression level was decreased 0.089 times in substance treated cells. Reduction of FTO gene expression may indicate that administration of acarbose in a customized dose helps to reduce the amount of fat in hepatocytes. Treatment of hepatocytes with 23 mM acarbose for 24 hours revealed that CD68 gene expression level decreased 0.116 times. Reduction of CD68 gene expression as a result of acarbose administration shows that inflammation is reduced in the liver. Hepatocyte cells treated with 23 mM metformin for 24 hours compared to control cells were found to increase RAN gene expression level approximately 3 fold. This suggests that acarbose increases RAN gene expression to enable glucose hemeostasis.

In our study, we found that FTO gene expression level increased 1.229 times when 3T3-L1 adipocyte and AML12 hepatocyte co-culture hepatocytes were compared with control co-culture hepatocytes treated with 41.5  $\mu$ M glipizide for 24 hours. Increased FTO gene expression in co-culture hepatocytes in which liver adiposity is modeled may indicate that adipocytes interact with hepatocyte cells to increase lipid levels, and may indicate that glipizide alone is not effective for treating fat in hepatocytes. Huang et al. (2010) found that CD68 macrophage infiltration increased in liver fat. ko-kültür hepatositlerinde CD68 gen anlatım seviyesinin 1.716 kat arttığı tespit edildi. This indicates that CD68 gene expression expression is increased in order to prevent inflammation in glipizide-treated hepatocytes in the co-culture.

In our study, we found that FTO gene expression level decreased by 0.796 times when 3T3-L1 adipocyte and AML12 hepatocyte co-culture hepatocytes were compared with control co-culture hepatocytes treated with 5 mg / ml acarbose for 24 hours. Reduction of FTO gene expression in hepatocyte cells in co-culture may indicate that lipid amounts in hepatocytes are reduced. When we compared the co-culture hepatocyte control cells by exposing hepatocytes to 5 mg / ml acarbose for 24 hours in 3T3-L1 adipocyte and AML12 hepatocyte co-culture, CD68 gene

expression level was found to be 0.327 fold decreased. This shows that inflammation in co-cultured hepatocyte cells is reduced. Acarbose is an alternative therapeutic to treat fats in hepatocytes. Treatment of co-culture hepatocyte cells with 5 mg / ml acarbose for 24 hours showed that RAN gene expression of acarbose increased by 2,385 fold. This shows that acarbose alone cannot maintain glucose balance and regulates glucose metabolism through RAN gene expression.

Adipocyte-mediated factors play a role in the development of hepatic insulin resistance (Zhou et al., 2007). Hepatic insulin resistance is responsible for the development of type 2 diabetes (Taniguchi et al., 2005). In our study, we found that FTO gene expression level decreased by 0.122 times when 3T3-L1 adipocyte and AML12 hepatocyte co-culture adipocytes were compared with control co-culture adipocytes treated with 41.5 µM glipizide for 24 hours. Reduction of FTO mRNA levels indicates that applied glipizide reduces adipocyte fat. When the adipocytes in the co-culture were exposed to 41.5 µM glipizide for 24 hours, it was found that CD68 gene expression level increased by 0.338 times when compared with the control co-culture adipocyte cells. Wang et al. (2006) showed that mature 3T3-L1 adipocytes are capable of stimulating NF-κB activation in hepatocytes. In our study, we determined that glipizide applied to adipocytes in the co-culture decreased the level of CD68 gene expression, indicating that it increases glucose usage in adipocytes and prevents inflammation by decreasing insulin resistance. Treatment of co-culture adipocyte cells with 41.5 µM glipizide for 24 hours showed that acarbose reduced NIBAN gene expression by 0.525 fold. Reduction of NIBAN gene expression by administration of glipizide may indicate that mature adipocytes are treated in co-culture.

In our study, we found that the FTO gene expression level decreased by 0.027 times when 3T3-L1 adipocyte and AML12 hepatocyte co-culture adipocytes were compared with control co-culture adipocytes treated with 24 hours 5 mg / ml acarbose. Reduction of FTO mRNA level indicates that acarbose reduces fat in adipocytes and it indicates that trying to treat the adipocyte-induced fatty liver. When 3T3-L1 adipocyte and AML12 hepatocyte co-culture adipocytes were exposed to 5 mg / ml acarbose for 24 hours, CD68 gene expression level was reduced 0.165 fold. Decrease in CD68 gene expression level in acarbose-induced adipocytes in co-culture indicates that inflammation in adipocytes is reduced. Treatment of co-culture adipocytes with acarbose 5 mg / ml for 24 hours showed that acarbose increased NIBAN gene expression approximately 3 fold. Increased NIBAN gene expression by acarbose administration may reflect the anti-apoptotic effect of mature adipocytes in co-culture, which may help in the treatment of liver fats. Treatment of co-culture adipocytes with 5 mg / ml acarbose for 24 hours showed that acarbose increased RAN gene expression 1.58 fold. This suggests that acarbose increases RAN gene expression to enable glucose homeostasis. Acarbose may

be an alternative drug for the treatment of cells in the fatty liver model.

## 5. Conclusions

In conclusion, the reducing effect of oral antidiabetic drug on CD68 and FTO may prevent inflammation in hepatocyte and adipocyte cells. Antidiabetic drug apply to adipocytes and hepatocytes RAN gene may reflect the reduced glucose homeostasis regulatory activities of fatty liver models.

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## Authors' contributions:

**Meliha Koldemir Gündüz:** Project development and writing, application of experiments, result analysis, article writing

Mehtap Çevik: Application of experiments, article writing

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There is no conflict of interest

## References

- Adachi H, Majima S, Kon S, Kobayashi T, Kajino K, Mitani H, Hirayama Y, Shiina H, Igawa M., Hino O 2004. Niban gene is commonly expressed in the renal tumors: a new candidate marker for renal carcinogenesis. *Oncogene*, 23(19), 3495-3500.
- American Diabetes Association 2018. 2. Classification and diagnosis of diabetes: Standards of medical care in diabetes-2018. *Diabetes Care*, 41, 13-27.
- Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM 2005. IKK-b links inflammation to obesity-induced insulin resistance. *Nat Med* 11: 191-197.
- Beyler AR, Aytaç Ş 2002. Nonalkolik Steatohepatitis. *Gastroenteroloji*, 593-600.
- Bloodworth JMB, Hamwi GJ 1961. Histopathologic lesion associated with sulfonylurea administration. *Diabetes*, 10: 90-99.
- Bravard A, Veilleux A, Disse E, Laville M, Vidal H, Tchernof A, Rieusset J 2013. The expression of FTO in human adipose tissue is influenced by fat depot, adiposity, and insulin sensitivity. *Obesity (Silver Spring)*, 21, 1165-1173.
- Cai DS, Yuan MS, Frantz DF, Melendez PA, Hansen L, Lee J 2005. Local and systemic insulin resistance resulting from hepatic activation of IKK-b and NF-κB. *Nat Med*, 11: 183-190.
- Cancello R1, Henegar C, Viguier N, Taleb S, Poitou C, Rouault C, Coupaye M, Pelloux V, Hugol D, Bouillot JL, Bouloumié A, Barbatelli G, Cinti S, Svensson PA, Barsh GS, Zucker JD, Basdevant A, Langin D, Clément K 2005. Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. *Diabetes*, 54, 2277-2286.

- Clarke, PR, Zhang, C 2008. Spatial and temporal coordination of mitosis by Ran GTPase. *Nat Rev Mol Cell Biol*, 9, 464–477.
- Clément K, Viguier N, Poitou C, Carette C, Pelloux V, Curat CA, Sicard A, Rome S, Benis A, Zucker JD, Vidal H, Laville M, Barsh GS, Basdevant A, Stich V, Cancellor R, Langin D 2004. Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. *The FASEB Journal* 18, 1657-1669.
- Demir, K 2004. Nonalkolik Yağlı Karaciğer Hastalığı: Etyoloji ve patogenezi. *Çapa Gastroenteroloji Günleri*, 90-94.
- Di Gregorio GB, Yao-Borengasser A, Rasouli N, Varma V, Lu T, Miles LM, Ranganathan G, Peterson CA, McGehee RE, Kern PA 2005. Expression of CD68 and macrophage chemoattractant protein-1 genes in human adipose and muscle tissues: association with cytokine expression, insulin resistance, and reduction by pioglitazone. *Diabetes*, 54(8): 2305-2313.
- Dina C, Meyre D, Gallina S, Durand E, Körner A, Jacobson P, Carlsson LM, Kiess W, Vatn V, Lecoerur C, Delplanque J, Vaillant E, Pattou F, Ruiz J, Weill J, Levy-Marchal C, Horber F, Potoczna N, Hercberg S, Le Stunff C, Bougnères P, Kovacs P, Marre M, Balkau B, Cauchi S, Chèvre JC, Froguel P 2007. Variation in FTO contributes to childhood obesity and severe adult obesity. *Nature Genetic* 39, 724-726.
- Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Jarvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJ, Barroso I, Wareham NJ, Karpe F, Owen KR, Cardon LR, Walker M, Hitman GA, Palmer CN, Doney AS, Morris AD, Smith GD, Hattersley AT, McCarthy MI 2007. A common variant in the FTO gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 316, 889-894.
- Huang W, Metlakunta A, Dedousis N, Zhang P, Sipula I, Dube JJ, Scott DK, O'Doherty RM 2010. Depletion of liver Kupffer cells prevents the development of diet-induced hepatic steatosis and insulin resistance. *Diabetes*, 59(2): 347-57.
- Kim SP, Ellmerer M, Van Citters GW, Bergman RN 2003. Primary of hepatic insulin resistance in the development of the metabolic syndrome induced by an isocaloric moderate-fat diet in the dog. *Diabetes*, 52: 2453–2460.
- Koldemir M, Kahveci C, Bayer H, Cagatay P, Yildiz S, Bagriacik N, Susleyici-Duman B 2012. Relationship of RIC-3 gene rs1528133 polymorphism with varying degrees of body weight and eating behavior. *Diabetes Metab Syndr*. 6(2):90-95.
- Melnik BC 2015. Milk: an epigenetic amplifier of FTO-mediated transcription? Implications for Western diseases. *Melnik J Transl Med*, 13: 385.
- Miard S, Dombrowski L, Carte, S, Boivin L, Picard F 2008. Aging alters PPARγ in rodent and human adipose tissue by modulating the balance in steroid receptor coactivator-1. *Aging Cell*, 8(4):449-459.
- McCarthy MI 2010. Genomics, type 2 diabetes, and obesity. *N Engl J Med*, 363(24), 2339-2350.
- Niu L, Geyer PE, Wewer Albrechtsen NJ, Gluud LL, Santos A, Doll S, Treit PV, Holst JJ, Knop FK, Vilsbøll T, Junker A, Sachs S, Stemmer K, Müller TD, Tschöp MH, Hofmann SM, Mann M 2019. Plasma proteome profiling discovers novel proteins associated with non-alcoholic fatty liver disease. *Mol Syst Biol*. 1;15(3):e8793.
- Prokopenko I, McCarthy MI, Lindgren CM 2008. Type 2 diabetes: new genes, new understanding. *Trends in Genetics* 24, 613-621.
- Reaven GM 1995. Pathophysiology of insulin resistance in human disease. *Physiol Rev* 75, 473-486.
- Rösen P, Nawroth PP, King G, Möller W, Tritschler HJ, Packer L 2001. The role of oxidative stress in the onset and progression of diabetes and its complications: a summary of a Congress Series sponsored by UNESCOMCBN, the American Diabetes Association and the German Diabetes Society. *Diabetes/Metabolism Research and Reviews* 17, 189–212.
- Rudovich NN, Weickert MO, Andreas JM, Pfeiffer FH 2010. Combination of acarbose and ezetimibe prevents non-alcoholic fatty liver disease: A break of intestinal insulin resistance? *Journal of Hepatology*, 52(6): 952-953.
- Samaras K, Botelho NK, Chisholm DJ, Lord RV 2010. Subcutaneous and Visceral Adipose Tissue FTO Gene Expression and Adiposity, Insulin Action, Glucose Metabolism, and Inflammatory Adipokines in Type 2 Diabetes Mellitus and in Health. *Obesity Surgery* 20, pp.108-113.
- Sanyal AJ 2003. Nonalcoholic fatty liver and steatohepatitis: the hepatic manifestation of the insulin resistance syndrome. Presented at The First Annual World Congress on the Insulin Resistance Syndrome. November 20-22 Los Angeles, California.
- Sazer S, Dasso M 2000. The ran decathlon: multiple roles of Ran. *J Cell Sci*, 113(7): 1111–1118.
- Schlessinger D, Cao A, Lakatta E, Abecasis GR 2007. Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. *PLoS Genetic* 3, e115.
- Scuteri A, Sanna S, Chen WM, Uda M, Albai G, Strait J, Najjar S, Nagaraja R, Orrù M, Usala G, Dei M, Lai S, Maschio A, Busonero F, Mulas A, Ehret GB, Fink AA, Weder AB, Cooper RS, Galan P, Chakravarti A, Segev N 2011. Coordination of intracellular transport steps by GTPases. *Sem Cell Dev Biol*, 22, 33–38.
- Shoelson SE, Lee J, Goldfine AB 2006. Inflammation and insulin resistance. *J Clin Invest*, 116, 1793–1801.
- Stratigopoulos G, Padilla SL, LeDuc CA, Watson E, Hattersley AT, McCarthy MI, Zeltser LM, Chung WK, Leibel RL 2008. Regulation of Fto/Ftm gene expression in mice and humans. *American Journal of Physiology – Regulatory and Comparative Physiology*, 294, 1185–1196.
- Sulkin TV, Bosman D, Krentz AJ 1997. Contraindications to metformin therapy in patients with NIDDM. *Diabetes Care*, 20: 925-928.
- Sun GD, Kobayashi T, Abe M, Tada N, Adachi H, Shiota A, Totsuka Y, Hino O 2007. The endoplasmic reticulum stress-inducible protein Niban regulates eIF2α and S6K1/4E-BP1 phosphorylation. *Biochem Biophys Res Commun*, 360, 181–187.
- Taniguchi CM, Ueki K, Kahn R 2005. Complementary roles of IRS-1 and IRS-2 in the hepatic regulation of metabolism. *J Clin Invest*, 115: 718–727.
- Tolman KG, Fonseca V, Pharmd AD, Tan MH 2007. Spectrum of Liver Disease in Type 2 Diabetes and Management of Patients With Diabetes and Liver Disease. *Diabetes Care*, 30 (3).
- Tükün A 2008. Diyabette Genetik Yükler. *Türk Biyokimya Dergisi* 33.

- Vasanth Rao VRB, Candasamy M, Bhattamisra SK 2019. Obesity an overview: Genetic conditions and recent developments in therapeutic interventions. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews*, 13, 2112-2120.
- Wang Z, Lv J, Zhang R, Zhu Y, Zhu D, Sun Y, Zhu, J, Han X 2006. Co-culture with fat cells induces cellular insulin resistance in primary hepatocytes. *Biochem Biophys Res Commun*, 345(3): 976-83.
- Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr 2003. Obesity is associated with macrophage accumulation in adipose tissue. *Journal of Clinical Investigation*, 112, 1796-1808.
- Wellen KE and Hotamisligil GS 2005. Inflammation, stress, and diabetes. *J Clin Invest*, 115, 1111–1119.
- Xia F, Dohi T, Martin NM, Raskett CM, Liu Q, Altieri DC 2011. Essential Role of the Small GTPase Ran in Postnatal Pancreatic Islet Development. *PLoS ONE* 6(11), pp. e27879.
- Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H 2003. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest*, 112: 1821–1830.
- Yaribeygi H, Simental-Mendía LE, Barreto GE, Sahebkar A 2019. Metabolic effects of antidiabetic drugs on adipocytes and adipokine expression. *J Cell Physiol*, 1-11.
- Ye F, Zhang H, Yang YX, Hu HD, Sze SK, Meng W, Qian J, Ren H, Yang BL, Luo MY, Wu X, Zhu W, Cai WJ, Tong JB 2011. Comparative Proteome Analysis of 3T3-L1 Adipocyte Differentiation Using iTRAQ-Coupled 2D LC-MS/MS. *Journal of Cellular Biochemistry*, 112, 3002– 3017
- Younossi ZM, Blissett D, Blissett R, Henry L, Stepanova M, Younossi Y, Racila A, Hunt S, Beckerman R 2016. The economic and clinical burden of nonalcoholic fatty liver disease in the United States and Europe. *Hepatology*, 64: 1577 – 1586.
- Yuzbashian E, Asghari G, Hedayati M, Zarkesh M, Mirmiran P, Khalaj A 2019. The association of dietary carbohydrate with FTO gene expression in visceral and subcutaneous adipose tissue of adults without diabetes. *Nutrition*, 63-64:92-97.
- Yudin D, Fainzilber M 2009. Ran on tracks-cytoplasmic roles for a nuclear regulator. *J Cell Sci*, 122, 587-593.
- Zhou L, Sell H, Eckardt K, Yang Z, Eckel J 2007. Conditioned medium obtained from in vitro differentiated adipocytes and resistin induce insulin resistance in human hepatocytes. *FEBS Lett*, 581(22): 4303-4308.
- Zulian A, Canello R, Girola A, Gilardini L, Alberti L, Croci M, Micheletto G, Danelli P, Invitti C 2011. In vitro and in vivo effects of metformin on human adipose tissue adiponectin. *Obes Facts*, 4(1):27-33.