Effects of antidiabetics and exercise therapy on suppressors of cytokine signaling-1, suppressors of cytokine signaling-3, and insulin receptor substrate-1 molecules in diabetes and obesity

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SUMMARY

OBJECTIVE: Pathological destruction of insulin signaling molecules such as insulin receptor substrate, especially due to the increase in suppressors of cytokine signaling molecules, has been demonstrated in experimental diabetes. The contribution of suppressors of cytokine signaling proteins to the development of insulin resistance and the effects of antidiabetic drugs and exercise on suppressors of cytokine signaling proteins are not clearly known.
METHODS: A total of 48 Wistar albino adult male rats were divided into six groups: control group, obese group with diabetes, obese diabetic rats treated with metformin, obese diabetic rats treated with pioglitazone, obese diabetic rats treated with exenatide, and obese diabetic rats with applied exercise program. Immunohistochemical staining was performed in both the liver and adipose tissue.
RESULTS: There was a statistically significant decrease in suppressors of cytokine signaling-1, a decrease in suppressors of cytokine signaling-3, an increase in insulin receptor substrate-1, and a decrease in immunohistochemical staining in the obese group treated with metformin and exenatide compared to the obese group without treatment in the liver tissue (p<0.05). A statistically significant decrease in immunohistochemical staining of suppressors of cytokine signaling-1 and suppressors of cytokine signaling-3 was found in the obese group receiving exercise therapy compared to the obese group without treatment in visceral adipose tissue (p<0.05). Likewise, no significant immunohistochemistry staining was seen in diabetic obese groups.
CONCLUSION: Metformin or exenatide treatment could prevent the degradation of insulin receptor substrate-1 protein by reducing the effect of suppressors of cytokine signaling-1 and suppressors of cytokine signaling-3 proteins, especially in the liver tissue. In addition, exercise can play a role as a complementary therapy by reducing suppressors of cytokine signaling-1 and suppressors of cytokine signaling-3 proteins in visceral adipose tissue.
KEYWORDS: Obesity. Insulin resistance. Antidiabetic drugs. Exercise.

INTRODUCTION

Insulin resistance is an important mechanism in the development of type 2 diabetes and obesity. The effect of insulin in muscle, liver, and adipose tissue is closely related to the activity of insulin receptor substrate (IRS) proteins. Insulin resistance is characterized by a decrease in insulin signal, mainly in the IRS[1]. IRS-1 proteins are major factors in the insulin signaling pathway; it has been determined that the inhibition or degradation of these proteins leads to the decrease in the insulin signal[2]. Pathological destruction of insulin signaling molecules such as IRS, especially due to the increase in suppressors of cytokine signaling molecules (SOCS), has been demonstrated in experimental diabetes[3,4]. The SOCS are molecules that take part in the negative feedback loop to weaken the cytokine effect. Eight members of the SOCS family have been identified, sharing similar structural and functional properties [SOCS-1–7 and cytokine-inducible SH2-containing protein (CIS)][5]. Expression of SOCS family proteins can alter many different signaling pathways in different tissues. SOCS-1 and SOCS-3, in particular, are thought to play a role in the development of insulin resistance and diabetes. Decreased expression of SOCS-1 and SOCS-3 proteins, especially SOCS-3, improves insulin resistance in the liver and increases insulin sensitivity in diabetic mice[6,7]. However, there are uncertainties as to whether SOCS proteins are the cause or the result of insulin resistance.

The first molecular link between obesity and pro-inflammatory cytokines was revealed by Hotamisligil et al.[8] Since then, the relationship of some inflammatory processes with obesity

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has been a serious area of research. Obesity is now easily defined as a chronic inflammatory process[9]. At the molecular level, it has been shown that SOCS-1 and SOCS-3 bind to IRS-1 and IRS-2, disrupting the effect of insulin and leading to the development of insulin resistance in obesity[10,11]. With the inhibition of SOCS proteins, IRS could be increased and insulin resistance induced by obesity could be prevented.

Effect of antidiabetic drugs on IRS-1 degradation in the liver, vi\\sceral adipose tissue, and muscle tissue in patients with diabetes is not clear. We aimed to investigate the effect of certain antidiabetic drugs on IRS-1 protein via SOCS-1 and SOCS-3 proteins in diabetes and obesity.

METHODS

Animal models and study groups

A total of 48 Wistar albino adult male rats weighing between 180 and 220 g were used. They were divided into six groups (control, obesity + diabetes, obesity + diabetes + metformin, obesity + diabetes + pioglitazone, obesity + diabetes + exenatide, and obesity + diabetes + exercise), with eight rats in each group. The study was carried out in an internationally certified animal experimentation laboratory at Gaziantep University.

The control group was fed with standard chow for 8 weeks. The subjects in the groups other than the control group were given 1000 g: 228.0 g of casein, 2.0 g of DL-methionine, 170.0 g of maltodextrin, 175.0 g of sucrose, 25.0 g of soybean oil, 335.5 g of coconut oil, 40.0 g of mineral mix, 10.5 g of sodium bicarbonate, 4.0 g of potassium citrate (with H_2O), 10.0 g of vitamin mixture, 2.0 g of choline, and 0.1 g of FD&C Red Dye #4 containing high-fat (60% fat) special feed for 4 weeks. It was accepted that nonalcoholic fatty liver disease occurred at the end of this period in groups other than the control group[12,13].

Animals fed this diet were weighed daily, and rats with a 20% weight gain were considered obese. After that, 40 mg/kg of streptozocin (STZ) was administered to obese groups to create diabetes with obesity. Different drug treatments (metformin, pioglitazone, and exenatide) or exercise therapies were administered to these obese groups with STZ-induced diabetes for 4 weeks. The daily blood glucose levels of all animals were measured and recorded from the blood sample taken from the tail vein. The treatment lasted for 4 weeks, after which the study was terminated. During the study, the temperature of the environment where the rats were kept was maintained constant at 20–24°C and the room was provided with 12 h of light and 12 h of darkness (light between 07.00 and 19.00).

- Group 1: Control group (C): It was fed with standard feed.
- Group 2: Diabetic obese group with metformin treatment (Ob+D+M): Metformin (0.33 mg/mL/day) treatment was applied for 4 weeks (105).
- Group 3: Diabetic obese group with pioglitazone treatment (Ob+D+P): Pioglitazone (4 mg/kg/day) treatment was applied for 4 weeks (105).
- Group 4: Diabetic obese group with exenatide treatment (Ob+D+Exn): Exenatide (0.03 mg/kg/day) treatment was applied for 4 weeks (105).
- Group 5: Diabetic obese group with exercise program (Ob+D+Exc): 4 weeks of swimming exercise (1 h/day) was applied (106).
- Group 6: Diabetic obese group without any treatment (Ob+D): It was waited for 4 weeks without any application.

mRNA isolation and complementary DNA synthesis

Using the Qiagen RNeasy Plus Universal Mini Kit (50) (Hilden, Germany; cat. no. 73404) mRNA isolation kit from the liver, visceral fat, and muscle tissues obtained from the study groups, mRNAs were obtained in accordance with the kit protocol. Concentration determinations were made with the nanoDrop ND-1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA), and then the concentrations of all samples were fixed to the same range as 150 ng/μL by performing the necessary dilutions, and the stock RNAs were stored at -80°C. Complementary DNAs (cDNAs) were obtained from isolated mRNAs by reverse polymerase chain reaction (PCR) method using the Fluidigm Reverse Transcription Kit (San Francisco, CA, USA; cat. no. 100-6298), nanoDrop ND-1000 spectrophotometer (Thermo Scientific), and stock cDNAs were stored at -80°C.

Quantitative real-time (RT) PCR

BioMark qRT-PCR system (Fluidigm), a high-capacity qRT-PCR method, was used to determine the gene expression levels of SOCS-1, SOCS-3, and IRS-1. Pre-amplification process was performed using Pre-amplification Master Mix and targeted *SOCS-1*, *SOCS-3*, and *IRS-1* as well as *GAPDH* as housekeeping gene, using TaqMan™ (Thermo Scientific) gene expression primer assays. PCR mix with pre-amplified cDNA samples was loaded into Fluidigm Flex Six™ (cat. no. 100-6308) sample portion, 20X Assays diluted 1:1 with 2X Assay Loading Reagent, Flex Six ™ (Fluidigm; cat. no. 100-6308)

Gene Expression loaded into the assay portion of the IFC array. BioMark IFC Controller (Fluidigm; HX-10273) assay and sample mixes were loaded into FlexSix™ chambers using "Fluidigm's Integrated Fluidic Circuit Technology."

IMMUNOHISTOCHEMICAL ANALYSIS

For immunohistochemical study, 4-μm-thick sections from paraffin blocks obtained from formalin-fixed liver, visceral adipose tissue, and muscle tissues were taken on polylysine-coated slides. The slides were first incubated at 37°C for 15 min. Afterward, automatic staining with SOCS-1, SOCS-3, and IRS-1 polyclonal antibodies]SOCS-1 (Bios, bs-0113R, 1:50), SOCS-3 (Bios, bs-0580R, 1:50), and IRS-1 (Bios, bs-0319R, 1:50)] (Ventana® Bench Mark Ultra, SN:316054) immunohistochemical staining was performed. All the stained sections were evaluated under the Olympus BX46 light microscope for the extent and intensity of staining at 40×, 100×, and 200× magnifications.

Statistical analysis

The IBM Statistics SPSS version 20.0 was used for statistical analysis. While analyzing the data, it was determined whether or not the data of the groups showed normal distribution, and as a result, one-way ANOVA or Kruskal-Wallis test was applied to compare the groups. Data were summarized as mean±standard deviation. Gene expression analyses were evaluated with the QIAGEN Globe. In the analysis, the *GAPDH* was used as a housekeeping gene. In this analysis, the program calculates the analysis of raw data results (Ct values) with the $2^{-\Delta\Delta Ct}$ method, and after these values are calculated, it uses the basic Student's t-test method to make comparisons between the groups (calculation of p-values).

RESULTS

Body weight and HbA1c alteration in obesity and diabetes models

At the end of the first 11 weeks, obesity was induced by applying a high-fat diet to 48 animals (according to the Lee index).

There was a statistically significant difference between the control group and the obese group. This difference demonstrated the presence of obesity (control group 297.40±53.23 vs. obese group 354.10±75.91; p<0.01). At the end of the 16th week, when different interventions were applied, the mean weight changes of obese and diabetic obese animals were examined. The mean intracardiac plasma glucose value was 395.8±70.7 in the control group and 547.0±133.4 in the diabetic group. The HbA1c values of the obese and diabetic obese groups were 6.5±0.22% and 9.9±0.53%, respectively (p<0.01).

Gene expression analysis and immunohistochemical evaluation of SOCS-1, SOCS-3, and IRS-1 in liver tissue in experimental obesity and obesity with diabetes models

There was a statistically significant decrease in SOCS-3 gene expression in the control group compared to the obese control group (p<0.05). Compared to the obese control group, the difference in SOCS-1 gene expression in the obese exenatide group was statistically significant, while the expression of the SOCS-1 gene was found to be decreased in the obese exenatide group (p<0.05). There was no statistical significance in the analyses of the obesity + metformin, obese + pioglitazone, and obese + exercise groups compared to the obese control group (p>0.05).

SOCS-1 gene expression was found to be decreased in the ob+STZ+P group compared to the control group (p<0.05) (Table 1).

Table 1. Gene expression analysis of SOCS-1, SOCS-3, and IRS-1 in liver tissue in experimental obesity and obesity with diabetes models.

	Control		Ob+Metformin		Ob+Pioglitazone		Ob+Exenatide		Ob+Exercise	
	X fold	p-value	X fold	p-value	X fold	p-value	X fold	p-value	X fold	p-value
SOCS-1	1.212	0.372	1.036	0.514	0.751	0.285	0.570	0.028[B]	0.6461	0.246
SOCS-3	0.393	0.016[A]	1.061	0.758	0.587	0.102	1.780	0.187	0.753	0.317
IRS-1	0.912	0.850	1.237	0.333	0.693	0.157	0.611	0.066	0.775	0.387
	Control		Ob+STZ+Metformine		Ob+STZ+Pioglitazone		Ob+STZ+Exenatide		Ob+STZ+Exercise	
	X fold	p-value	X fold	p-value	X fold	p-value	X fold	p-value	X fold	p-value
SOCS-1	1.187	0.279	0.673	0.059	0.542	0.019[C]	0.576	0.109	0.627	0.413
SOCS-3	0.498	0.053	1.703	0.304	0.816	0.467	1.773	0.136	0.814	0.496
IRS-1	1.094	0.541	0.908	0.952	0.805	0.463	1.011	0.647	0.977	0.959

SOCS: suppressors of cytokine signaling; IRS: insulin receptor substrate; Ob: obese; STZ: streptozocin. Glyceraldehyde-3-phosphate dehydrogenase was used as the housekeeping gene for normalization. [A]Statistically significant difference with obese control group, p<0.05. [B,C]Statistically significant difference with control, p<0.05.

There were statistically significant SOCS-1, SOCS-3, and IRS-1 immunohistochemical staining changes in the obese groups treated with metformin and exenatide compared to the obese group (p<0.05). There was a statistically significant immunohistochemical staining decrease in only SOCS-1 and SOCS-3 in the diabetic obese group treated with metformin compared to the obese group (p<0.05), and also there was a statistically significant decrease in only SOCS-1 in the diabetic obese group treated with exenatide compared to the obese group (p<0.05) (Table 2).

Immunohistochemistry analysis bar graph and histological staining of SOCS-1, SOCS-3, and IRS-1 in obese and diabetic obese groups in liver tissue are shown in Figures 1 and 2, respectively.

Gene expression analysis and immunohistochemical evaluation of SOCS-1, SOCS-3, and IRS-1 in visceral adipose tissue in experimental obesity and obesity with diabetes models

Compared to the obese control group, *SOCS-1* and *SOCS-3* genes were found to be statistically significant in the control group, while the expressions of these genes were found to be increased in the control group (p<0.05). Compared to the obese control group, the *SOCS-1* gene was found to be statistically significant in the obese + exenatide group, while the expression of the SOCS-1 gene was increased in the obese + exenatide group (p<0.05). Compared to the obese control group, the *SOCS-1* gene was found to be statistically significant in the obese +

Table 2. Immunohistochemical evaluation of SOCS-1, SOCS-3, and IRS-1 in liver tissue in experimental obesity and obese diabetes models.

	Obese control		Ob+Metformin		Ob+Pioglitazone		Ob+Exenatide		Ob+Exercise	
	Score	p-value	Score	p-value	Score	p-value	Score	p-value	Score	p-value
SOCS-1	2.80		1.22	0.001[A]	1.75		1.20	0.001[A]	1.38	0.003[A]
SOCS-3	2.75		1.40	0.001[B]	1.75		1.33	0.001[B]	1.87	
IRS-1	1.40		2.75	0.001[C]	2.38		2.75	0.001[C]	2.25	
	Ob+Streptozotocin		Ob+STZ+Metformine		Ob+STZ+Pioglitazone		Ob+STZ+Exenatide		Ob+STZ+Exercise	
	Score	p-value	Score	p-value	Score	p-value	Score	p-value	Score	p-value
SOCS-1	2.75		1.50	0.004[D]	1.70		1.30	0.001[D]	1.75	
SOCS-3	2.73		1.50	0.003[E]	1.45	0.001[E]	1.70	0.020	1.63	0.016[E]
IRS-1	1.25		2.25		2.50		2.70	0.005[F]	2.50	0.021[F]

SOCS: suppressors of cytokine signaling; IRS: insulin receptor substrate; Ob: obese; STZ: streptozocin. [A,B,C]Statistically significant difference with obese control, p<0.05. [D,E,F]Statistically significant difference with obese control with diabetes, p<0.05.
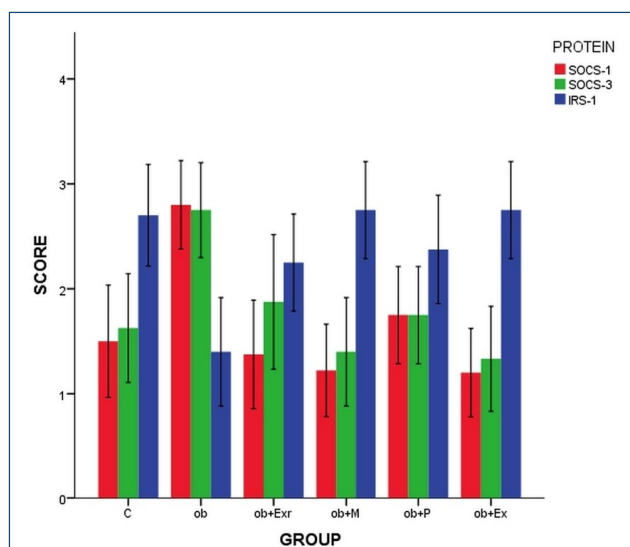

Figure 1. Immunohistochemistry analysis bar graph of SOCS-1, SOCS-3, and IRS-1 in obese group in liver tissue. C: control; Ob: obese control; Ob+Exr: obese + exercise; Ob+M: obese + metformin; Ob+P: obese + pioglitazone; Ob+Ex: obese + exenatide; Ob+STZ: obese + streptozocin; Ob+STZ+Exr: obese + streptozocin + exercise; Ob+STZ+P: obese + streptozocin + pioglitazone; Ob+STZ+Ex: obese + streptozocin + exenatide.
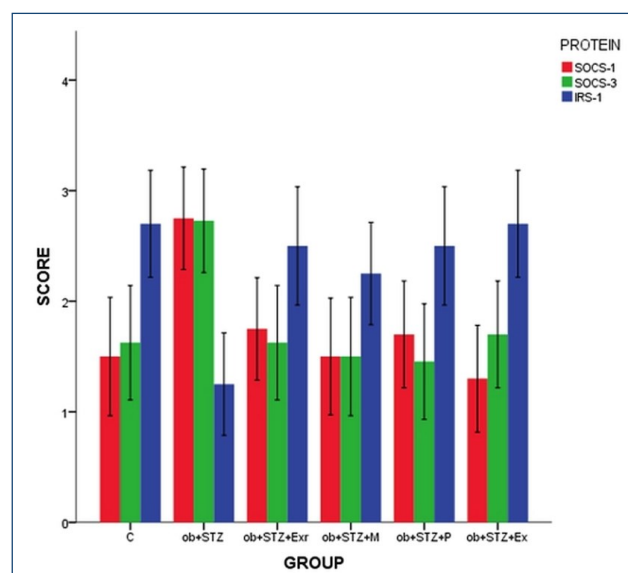

Figure 2. Immunohistochemistry analysis bar graph of SOCS-1, SOCS-3 and IRS-1 in diabetic obese groups. C: control; Ob: obese control; Ob+Exr: obese + exercise; Ob+M: obese + metformin; Ob+P: obese + pioglitazone; Ob+Ex: obese + exenatide; Ob+STZ: obese + streptozocin; Ob+STZ+Exr: obese + streptozocin + exercise; Ob+STZ+P: obese + streptozocin + pioglitazone; Ob+STZ+Ex: obese + streptozocin + exenatide.

exercise group, while the expression of the SOCS-1 gene was increased in the obese + exercise group (p<0.05). Compared to the diabetic control group, the *IRS-1* gene was found to be statistically significant in the obese + STZ + metformin group, and the expression of the IRS-1 gene was found to be decreased in the obese + STZ + metformin group (p<0.05) (Table 3).

A statistically significant decrease in immunohistochemical staining of SOC-1, SOCS-3, and IRS-1 was found in the obese group receiving exercise therapy compared to the obese group (p<0.05).

Gene expression analysis and immunohistochemical evaluation of SOCS-1, SOCS-3, and IRS-1 in muscle tissue in experimental obesity and obesity with diabetes models

Compared to the control group in muscle tissue samples, no difference was found in SOCS-1, SOCS-3, and IRS-1 gene expression levels in neither obese nor diabetic obese groups. Likewise, no significant immunohistochemistry staining was seen on these proteins.

DISCUSSION

In this study, we evaluated the effect of several antidiabetic drugs and exercise on the alteration of SOCS-1, SOCS-3, and IRS-1 in the liver, adipose, and muscle tissues. SOCS proteins, mainly SOCS-1 and SOCS-3, have been associated with insulin resistance, obesity, and the development of diabetes[2]. However, there is still no clear conclusion that these SOCS proteins are the cause or consequence of insulin resistance. SOCS proteins should not be evaluated only on the basis of insulin effect. SOCS proteins play different roles in the functioning of many systems, such as immune, hematopoietic, and hormone receptor signaling pathways[12]. In our study, it was observed that some drugs and exercise used in the treatment of type 2 diabetes could affect the gene expressions of SOCS-1,

SOCS-3, and IRS-1, as well as the degree of immunohistochemical staining of these proteins in different tissues.

Cytokines and growth factors regulated by SOCS-1 and SOCS-3 are important in both physiologic and neoplastic growth of hepatocytes[13]. Studies showed that SOCS-1 and SOCS-3 may be a therapeutic target in hepatic insulin resistance[14,15]. There are publications reporting that overexpression of SOCS-3 is associated with insulin resistance, particularly in hepatic tissue. In addition, some mutations in the *SOCS-1* gene have been associated with obesity and insulin resistance and obesity in the literature[2]. However, there is still a lack of sufficient and quality data to establish the effect of antidiabetic drugs and exercise on SOCS proteins and IRS-1. In our study, the decrease in immunohistochemical staining of SOCS-1 and SOCS-3 in the liver was accompanied by a significant increase in IRS-1 in groups who were obese and were treated with metformin or exenatide. In the liver tissue of diabetic obese group, the decrease in the staining of SOCS-1 and SOCS-3 proteins was accompanied by a significant increase in IRS-1 staining only in the exenatide-treated group.

In addition, three different IRS molecules were detected in visceral adipose tissue (IRS-1-2-3). It is thought that the chemical structures of IRS molecules and the signal pathways they cause are similar[16]. In our study, there was a significant decrease in SOCS-1 and SOCS-3 staining only in the obese adipose tissue group receiving exercise therapy (p=0.001). Interestingly, these decreases were accompanied by significant decrease in the staining of IRS-1. Other treatment modalities caused almost no change in IRS-1 staining. In the diabetic obese groups, a decrease was observed in SOCS-3 staining in the exenatide and exercise groups, while the decrease in only exenatide treatment was significant. Change in IRS-1 level did not observe in the groups receiving these treatments. In different studies, it has been shown that changes in IRS molecules in adipose tissue may vary according to the causes of insulin resistance. Different alterations

Table 3. Gene expression analysis of SOCS-1, SOCS-3, and IRS-1 in visceral adipose tissue in experimental obesity and obesity with diabetes models.

	Control		Ob+Metformin		Ob+Pioglitazone		Ob+Exenatide		Ob+Exercise	
	X fold	p-value	X fold	p-value	X fold	p-value	X fold	p-value	X fold	p-value
SOCS-1	20.452	0.049[A]	5.305	1.870	1.870	0.671	9.101	0.029[C]	4.265	0.031[D]
SOCS-3	6.567	0.008[B]	0.776	1.736	1.736	0.215	1.249	0.211	1.302	0.305
IRS-1	0.927	0.670	0.888	0.458	0.458	0.695	0.746	0.108	0.685	0.153
	Control		Ob+STZ+Metformin		Ob+STZ+Pioglitazone		Ob+STZ+Exenatide		Ob+STZ+Exercise	
	X fold	p-value	X fold	p-value	X fold	p-value	X fold	p-value	X fold	p-value
SOCS-1	2.584	0.208	0.490	0.754	1.141	0.337	1.089	0.614	1.763	0.277
SOCS-3	9.315	0.051	5.853	0.063	7.053	0.122	1.235	0.573	2.031	0.302
IRS-1	1.097	0.653	0.494	0.025[E]	1.242	0.342	1.066	0.804	1.227	0.500

Ob : obese; STZ: streptozocin. [A,B,C,D]Statistically significant difference with obese control, p<0.05. [E]Statistically significant difference with obese control with diabetes, p<0.05. Glyceraldehyde-3-phosphate dehydrogenase was used as the housekeeping gene for normalization.

in IRS molecules have been observed in different insulin resistance models. Modulation and signaling pathway of IRS and SOCS in the case of insulin resistance in adipose tissue is still not clearly defined. SOCS proteins can inhibit insulin signaling by five different mechanisms. The mechanism underlying SOCS-3-mediated insulin resistance involves the following:

1. Competition for binding to the activated insulin receptor
2. IRS protein degradation
3. Inhibition of the tyrosine kinase activity of the insulin receptor;
4. Negative feedback regulation of the Janus kinase 2 (JAK2)/STAT3 signal transduction pathway
5. Regulation of leptin signal transduction[17]. In addition, the presence and functionality of the IRS-1 molecule is necessary for the effectiveness of insulin in tissues. The changes in these molecules should be examined separately according to each different insulin resistance scenario.

One study showed that SOCS-1, SOCS-3, and IRS-1 proteins showed different alterations according to the relevant tissue. In addition, SOCS-1 binds to the IRS-2 recognition site in the IR kinase domain (IR) and primarily inhibits IRS-2-mediated insulin signaling, whereas SOCS-3 binds to IR and inhibits both IRS-1 and IRS-2[2]. SOCS-1 has been reported to increase the ubiquitin-mediated degradation of some proteins as part of the ubiquitin-ligase complex[18]. In another study, it was also shown that SOCS-1 and SOCS-3 bind IRS proteins in cultured cells in the liver and direct them to ubiquitin-mediated protein degradation[19]. However, there are also studies in which no interaction could be detected between SOCS and IRS. Chronic insulin therapy or long-term exposure to hyperinsulinemia has been shown to reduce IRS-2 mRNA in hepatocyte cell cultures, whereas IRS-1 is reduced mainly through protein degradation[2]. Pro-inflammatory cytokines, such as TNF-α, have been shown to inhibit insulin signaling via IR and IRS proteins[20]. It has been suggested that SOCS protein-mediated inhibition of IRS phosphorylation is also involved in TNF-α-mediated inhibition of insulin signaling[21].

The findings we obtained at different levels in different tissues differed according to the drug treatments and exercise status we used. It is not surprising that different interventions yield different results in various tissues. Because the mechanism of action of each drug is different, it is known that the main site of action of metformin is the liver. The reason is the dominance of the presence of organic cation transporter-1 in the liver. Therefore, the uptake of metformin in the liver is higher than in other organs[22].

Studies have shown that metformin increases AMP-activated protein kinase (AMPK) activation and decreases lipopolysaccharide-induced inflammatory responses[23]. In another study, it was shown that activation of AMPK significantly suppressed the acute-phase response and decreased SOCS-3 gene expression[24]. These findings are consistent with the data in our study. Metformin treatment significantly reduces SOCS-1–3 in liver tissue in the obese and diabetic obese groups. In our study, the fact that metformin treatment did not cause an increase in IRS-1 in the diabetic obese group, but also preserved the level of IRS-1, may suggest that metformin is more effective in the liver tissue by creating an anti-inflammatory effect.

The antioxidant effects of GLP-1 analogs are known. GLP-1 analogs are known to reduce free radicals and inflammation-induced oxidative stress. There are also studies in the literature showing that it reduces lipotoxicity and glucotoxicity[25]. Increasing the level of inflammation has a negative effect on IRS-1. In addition, the contribution of SOCS proteins to the inflammatory process is known. In our study, the fact that the most effective treatment was exenatide in both the obese group and the diabetic obese group can be attributed to the antioxidant effects of GLP-1 analogs.

The difference in the normal expression levels of these molecules in different tissues in the normal physiological process and the different mechanisms of action of the drugs used may have revealed these results. Comprehensive studies, including the mechanisms of action of drugs, may yield clearer results.

In light of the current literature, the decrease in SOCS-1 and SOCS-3 and the increase in the IRS-1 molecule are extremely important in terms of preventing diabetes-related complications due to better glycemic control in obese and especially obese patients with diabetes. In our study, exenatide was found to be the most effective drug in liver tissue in both obese and diabetic obese patients. Metformin treatment had similar effects to exenatide in the obese group. Although it caused a decrease in SOCS-1–3 in the diabetic obese group and an increase in IRS-1, this increase was not as significant as the increase caused by exenatide. When the effects on visceral adipose tissue were evaluated, it was determined that the most effective treatment was exercise. There was a decrease in both SOCS-1 and SOCS-3 in the obese group. These findings can be accepted as molecular evidence of the expected benefit of adding exercise to drug therapy in diabetes and obesity.

CONCLUSION

Metformin or exenatide treatment could prevent the degradation of IRS-1 protein by reducing the effect of SOCS-1 and SOCS-3 proteins, especially in liver tissue. Drugs that alter the SOCS effect and/or IRS-1 protein may be new agents for the treatment of obesity, insulin resistance, and type 2 diabetes. It also suggests that the use of exercise therapy as a complement to medical treatments may be beneficial.

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AUTHORS' CONTRIBUTIONS

EA, ZB: Conceptualization, Methodology, Software, Writing – original draft, and Writing – review & editing. **ZAS:** Conceptualization, Writing – original draft, and Writing – review & editing. **SOB, CD:** Conceptualization, Formal Analysis, Methodology, Software, Writing – original draft, and Writing – review & editing. **MK, IY:** Formal Analysis, Writing – original draft, and Writing – review & editing.

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