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# The Effects of Simvastatin on Free Fatty Acids Profile in Fructose-fed Insulin Resistant Rats

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## Abstract

**Backgrounds:** Type 2 diabetes mellitus is the most common metabolic disease and free fatty acids, as signaling molecules, can play a crucial role in the development of it. Different free fatty acids, through various cell membrane receptors, induce different effects on metabolic pathways and thereby affect insulin sensitivity. Simvastatin is a cholesterol decreasing drug prescribed for hypercholesterolemic patients. Due to the observing insulin-sensitizing effect of simvastatin in our previous study, we decided to evaluate its effects on free fatty acids profile as a probable mechanism for alteration of insulin sensitivity.

**Methods:** Insulin resistance was developed in male Wistar rats by a high fructose diet for 6 weeks. After this period, animals were treated by intragastric injection of simvastatin for two weeks. Blood samples were collected in EDTA treated tubes and plasma was separated. After isolation of free fatty acids from other lipids fractions, free fatty acids profile were analyzed by gas chromatography system.

**Results:** Simvastatin increased the concentrations of medium chains free fatty acids such as hexanoic acid, octanoic acid, decanoic acid, undecanoic acid, and dodecanoic acid. Simvastatin also increased plasma total free fatty acids concentration.

**Conclusion:** The results of this study demonstrated that simvastatin by increasing the level of medium and long-chain free fatty acids can increase insulin secretion; on the other hand, simvastatin by increasing the level of total and saturated free fatty acids, could induce insulin resistance. More studies are necessary for the evaluation of the precise involved mechanisms in simvastatin effects on insulin function.



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## Introduction

Diabetes Mellitus is a metabolic disorder with high prevalence in the world resulted from deficiency of insulin secretion or insulin function (1). Type 2 diabetes is the most common type of diabetes which arises due to the insulin resistance in the muscles, adipose tissue, and liver as the main

targets of insulin functions (2). The outbreak of diabetes is increasing and 11.4% of Iranian people are diabetic (3).

Obesity is an identified risk factor for diabetes and insulin resistance (4). Chronic low-grade inflammation induced by obesity can affect insulin signaling through several mechanisms (5). Free fatty acids (FFAs) levels are increased in obesity state and can interfere with insulin signaling via Toll-Like Receptors (TLRs), alteration in phosphorylation pattern of signaling intermediates and modification of lipid messengers (6,7).

Free fatty acids act not only as a source of energy, but they are signaling molecules and according to chain length and saturation state, can play a critical role in the regulation of metabolic processes (8,9). Recent studies indicate that intestinal microbial flora, through alteration of fatty acids profile which are absorbed into the body, is one of the key factors in the development of insulin resistance (8,10).

Free fatty acids effects on metabolic regulation are exerted through G-protein coupled receptors that have been recognized as free fatty acids receptors (8). These receptors can play a key role in several processes such as secretion of leptin, insulin, and glucagon-like peptide (11). sensitivity and specificity of these receptors are dependent on the type of ligands and changed with length and degree of saturation of free fatty acids (10).

Simvastatin is a 3-hydroxy-3-methylglutaryl-coenzyme reductase inhibitor and like other statins decreases plasma level of cholesterol. Recent data have shown that statin therapy can increase the risk of diabetes (12). Statins increase insulin resistance risk by about 36% (13). Simvastatin decreases insulin induced-glucose uptake and this effect is mediated by FFAs (12). To elucidate the important role of FFAs on diabetes and insulin sensitivity, we decided to investigate the effects of this drug on plasma free fatty acids profile. For the avoidance of diet interference in the results, the study was performed in the rats with high fructose diet.

## Method

### Animals and Diet

For this study, male Wistar rats (weighing 250-300 g) were obtained from the animal house of Kerman University of Medical Sciences. The procedure of this study was confirmed by the Ethics Committee of Kerman University of Medical Sciences (IR.KMU.REC.1390.271). Rats were maintained in the animal room at 22±3°C under an automatic lighting schedule (08:00AM-20:00PM). After two weeks, animals were randomly divided into the three groups (n=8). Two groups were fed with a 60% fructose diet and the third one, as the healthy control group (HCG) was fed with normal chow for 8 weeks. The fructose-enriched diet contained 60% fructose, 21% protein, and 5% fat, sodium 0.49%, and potassium 0.49%. After 6 weeks, insulin resistance was confirmed by oral glucose tolerance test (OGTT) (14).

### Drug treatment

Healthy group: Animals that had been fed with normal chow were considered as the healthy group. These animals, throughout the study period (8 weeks), were fed with a normal diet without any medication.

Control group: these animals were fed high fructose diet for 8 weeks, without any medication.

simvastatin group: these animals were fed with a high fructose diet for the first 6 weeks, then received simvastatin

(25 mg/kg/day) through gavage for the remained 2 weeks (14).

### Blood and tissue collection

Animals were fasted for 12 hours overnight and blood samples were collected from the heart under ether anesthesia. The blood samples were collected into vials which contain EDTA. The vials were centrifuged at 1000 g for 10 min. at 4°C and plasma was separated immediately.

### Free fatty acid analysis

Plasma FFA was extracted and analyzed by the method explained by Kangani et al (15) with slight modifications. For this, 500 µL of plasma was mixed with 20 µL of pentadecanoic acid (1 mg/mL) as an internal standard. Lipids were extracted from plasma by a solvent which contained isopropanol-heptane-hydrochloric acid (1M) (40:10:1, v/v/v). FFAs were separated by Thin-layer Chromatography (TLC) on silica gel plates using heptane-ether-acetic acid [60:40:3] solvent system. Lipids were visualized by iodine vapor on TLC plates. FFA bands were scrapped and free fatty acid methyl esters (FAME) were prepared by a reaction with BF3 (as a catalyzer) in methanol (Sigma). FFA methyl esters were separated using an Agilent GC-7890A system equipped with a flame ionization detector and DB-225 capillary column (20m×0.1 mm I.D., 0.1µm film thickness, J&W GC columns, USA). The injection volume was 1µL in the split 30:1 injection mode.

## Results

Our results showed that simvastatin can increase the concentrations of medium chains free fatty acids such as hexanoic acid, octanoic acid, decanoic acid, undecanoic acid and dodecanoic acid in simvastatin-treated group in comparison with the control group. Some of these fatty acids were undetectable in the control and healthy groups, as it can be seen in figures 1 and 2; however, they were significantly increased after simvastatin treatment (figure 3).

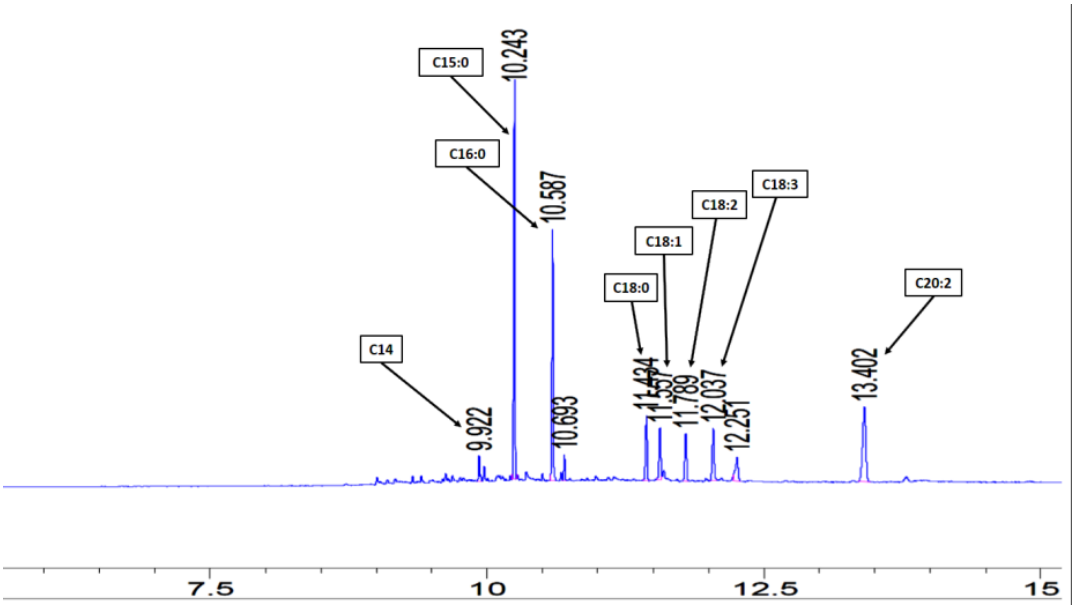


**Figure 1.** Chromatogram of free fatty acids in the healthy group

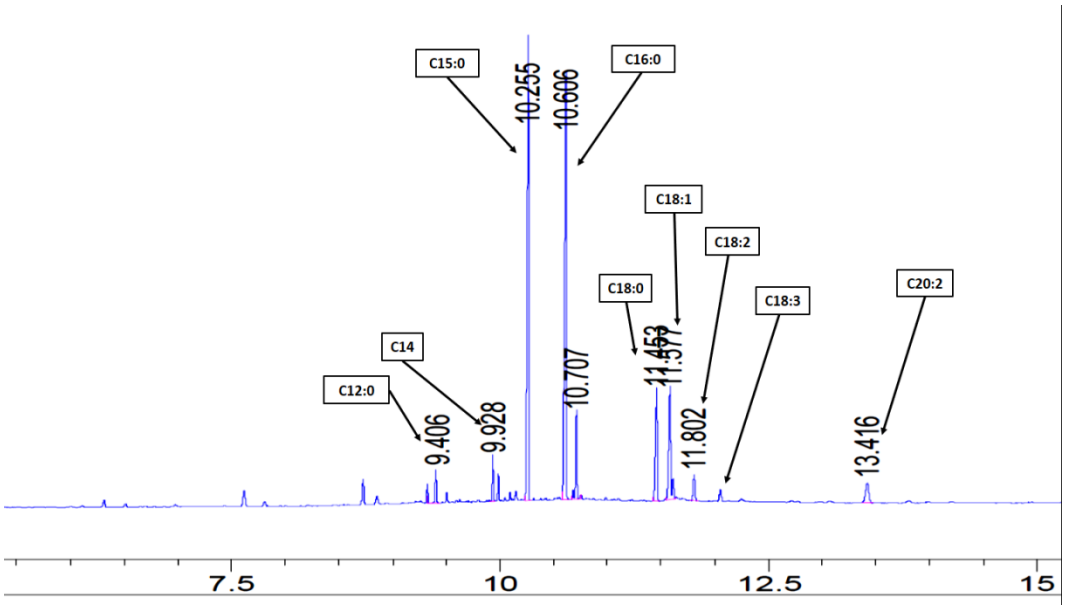


**Figure 2.** Chromatogram of free fatty acids in the control group

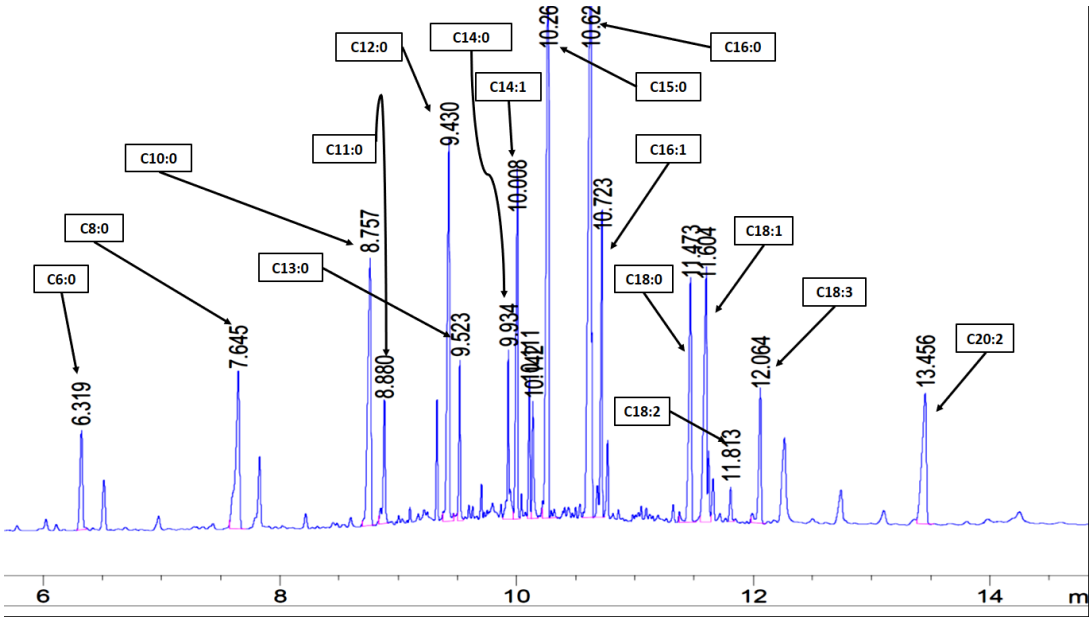


**Figure 3.** Chromatogram of free fatty acids after simvastatin treatment

We did not see any difference in palmitic acid (Hexadecanoic acid), as the main plasma free fatty acid, among groups. Results have been summarized in table 1.

**Table 1.** Concentrations of different plasma free fatty acids in insulin resistant rats

| | C6 | C8 | C10 | C11 | C12 | C13 | C14 |
|---|---|---|---|---|---|---|---|
| **Simvastatin** | 2.94±0.21* | 3.93±0.38* | 5.49±0.85* | 2.87±0.39* | 6.03±1.08* | 2.35±0.27* | 3.89±0.9* |
| **Control** | 0 | 0 | 0.69±0.21 | 0 | 1.07±0.07 | 0 | 1.11±0.06 |
| **Healthy** | 0 | 0 | 0.35±0.23 | 0 | 0 | 0 | 0.53±0.2 |
| | **C16** | **C16:1** | **C18** | **C18:1** | **C18:2** | **C18:3** | **C20:2** |
| **Simvastatin** | 9.96±0.62 | 2.02±0.32 | 3.95±0.41 | 3.5±0.31 | 0.97±0.46* | 1.43±0.38* | 3.24±0.27 |
| **Control** | 11.16±2.6 | 1.1±0.25 | 2.73±0.12 | 2.55±0.41 | 0.03±0.01 | 0.065±0.021 | 1.45±0.12 |
| **Healthy** | 3.73±1.14 | 0.66±0.19 | 1.47±0.43 | 1.37±0.42 | 0.036±0.012 | 0.042±0.016 | 1.38±0.4 |

All data have been shown as Mean± SEM (n=8)

* P-value demonstrate the significant difference in comparison with the control group

Based on the degree of saturation of fatty acids, the most changes in the fatty acids profile were seen in saturated fatty acids. Even though, in unsaturated free fatty acids, the difference was also significant (figure 4).

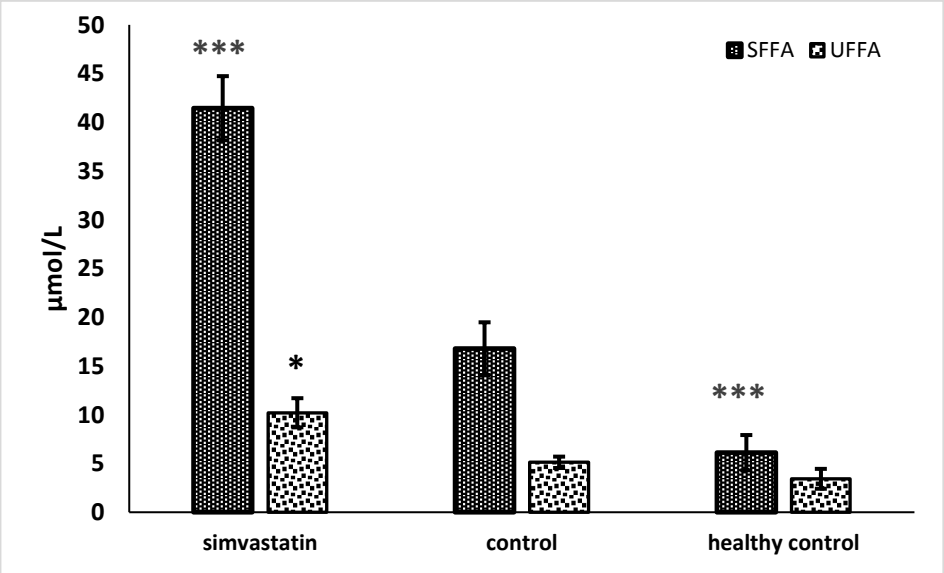


**Figure 4.** Concentrations of saturated and unsaturated free fatty acids in the studied groups

SFFA: saturated free fatty acids and UFFA: unsaturated free fatty acids
*: P<0.05 and ***: P<0.0001 in comparison to the control group

In relation to the length of fatty acids, we observed sharp increase in short and medium-chain free fatty acids level in the simvastatin-treated group compared to the control group, while the long-chain free fatty acids concentration did not show any significant differences among groups (figure 5).

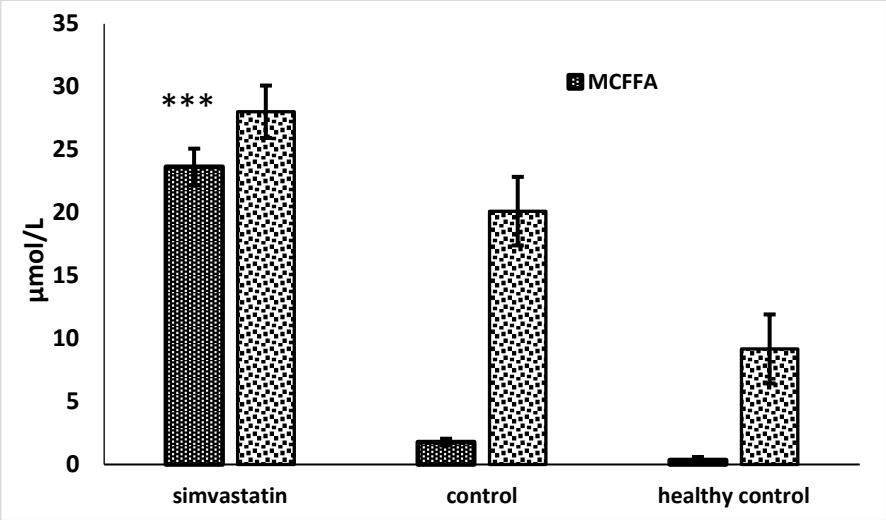


**Figure 5.** Concentrations of medium and long chain free fatty acids in the studied groups

All data have been shown as Mean± SEM
MCFFA: medium chain free fatty acids and LFFA: Long chain free fatty acids
*: P<0.05 and ***: P<0.0001 in comparison to the control group

In addition to the profile of free fatty acids, total free fatty acids also increased with simvastatin therapy. Our results demonstrated significant increase of total free fatty acids in the control group compared to the healthy animals (figure 6).

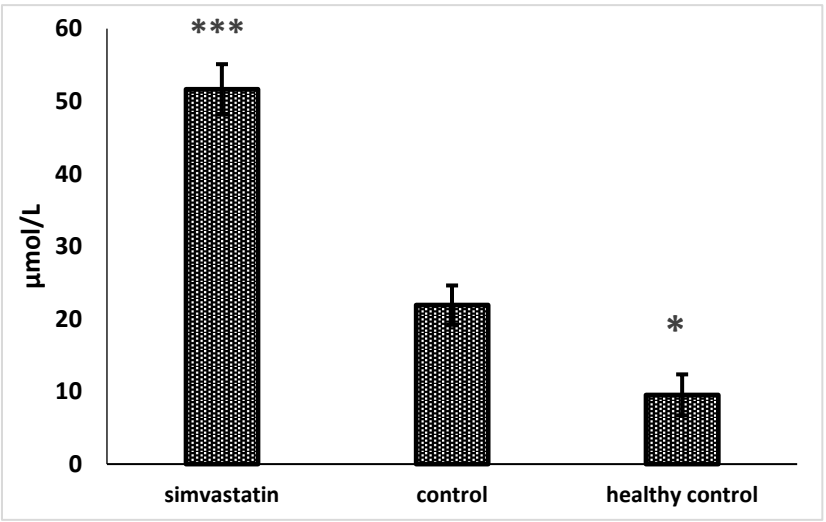


**Figure 6.** Total concentration of free fatty acids in the studied groups

*: P<0.05 and ***: P<0.0001 in comparison to the control group

## Discussion

Statins are a class of drugs that inhibit HMG-CoA reductase and thereby diminish the level of blood cholesterol and LDL-c and consequently decrease the risk of atherosclerosis (16). Surprisingly, we observed that simvastatin can significantly change the quantity and quality of plasma free fatty acids.

The effects of free fatty acids on metabolic processes are induced by a series of G-protein coupled receptors which are known as Free Fatty Acids Receptors (FFAR). The sensitivities of these receptors are different based on the length and saturation status of distinct free fatty acids (8,10).

Among free fatty acid receptors, FFAR1/GPR40 and FFAR4/GPR120 are targeted by medium and long chain free fatty acids, while FFAR3/GPCR41 and FFAR2/GPCR43 are specific for short-chain free fatty acids (10).

Simvastatin significantly increased the medium and long chain free fatty acids in this study. Therefore, it is possible this drug in this way activates FFAR1 and by increasing the level of Ca2+, induces GSIS in pancreatic cells (8,17,18). Also, simvastatin significantly increased the level of linolenic acid which is a strong ligand for FFAR4 and thereby, can increase the GLP secretion from L cells in the colon (8). In addition to these, free fatty acids, through increasing the ATP level in the β cells, can promote insulin secretion (19).

FFAR2 and FFAR3 are coupled with Gi type of G-proteins and by inhibition of cAMP accumulation, prevent the incretin effect and GSIS and diminish insulin secretion (20,21). Simvastatin increased the level of short-chain free fatty acids in this study and it is possible that this pathway is activated by simvastatin.

In addition to the above, inflammation can develop insulin resistant and FFAs can affect inflammation. Saturated FFA and ω6 FFA act as pro-inflammatory molecules and ω3 FFA as anti-inflammatory agents (22,23). Thus, we can propose that although simvastatin can induce the insulin secretion from β cells, this drug can promote insulin resistance by Toll-Like Receptors and synthesis of long-chain acyl CoA, ceramides, and diacylglycerol (22,23).

## Conclusion

This study revealed that simvastatin, through increasing the level of medium and long-chain free fatty acids can increase insulin secretion: on the other hand, simvastatin, by increasing the level of total and saturated free fatty acids, can induce insulin resistance in liver and muscles. These results are in contradiction with our previous study that we concluded that simvastatin increases insulin resistance. This inconsistency may be because of another mechanism affected by simvastatin. Correct understanding of this process requires more studies about molecular mechanisms of simvastatin effects such as adiponectin secretion or PPARγ activity and expression.

## Acknowledgments

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