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Serum concentrations of fibroblast growth factor 19 in patients with obesity and type 2 diabetes mellitus: the influence of acute hyperinsulinemia, very-low calorie diet and PPAR-α agonist treatment

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Short title: FGF-19 in obesity and T2DM: influence of diet and fibrates

Summary

The aim of our study was to measure serum concentrations of fibroblast growth factor 19 (FGF-19) in patients with obesity (OB), obesity and type 2 diabetes mellitus (T2DM) and healthy subjects (C) at baseline and after selected interventions. We measured serum FGF-19 levels and other biochemical and hormonal parameters in **29** OB **and 19** T2DM **females** and 30 **sex- and age-matched control** subjects. The interventions were acute hyperinsulinemia during isoglycemic-hyperinsulinemic clamp **(n=11 for T2DM and 10 for C)**, very low calorie diet (VLCD, **n=12 for OB)** and 3 months treatment with PPAR-α agonist fenofibrate (**n=11 for T2DM**). Baseline serum FGF-19 levels were significantly lower in OB relative to C group **(132.1±12.7** vs. 202.2±16.7 pg/ml, $p<0.05$), while no significant difference was observed between T2DM and OB or control group. Acute hyperinsulinemia **tended to decrease** FGF-19 levels in both healthy and T2DM subjects. Three weeks of VLCD in OB group **had no significant effect on FGF-19, whereas** three months of fenofibrate treatment markedly reduced FGF-19 levels in T2DM patients (194.58±26.2 vs. 107.47±25.0 pg/ml, $p<0.05$). We conclude that FGF-19 levels in our study were at least partially dependent upon nutritional status, but were not related to parameters of glucose metabolism or insulin sensitivity.

Key words: FGF-19, obesity, type 2 diabetes mellitus, very low calorie diet, fenofibrate

Introduction

Fibroblast growth factor 19 (FGF-19) is a recently identified endocrine regulator with multiple effects on metabolic processes and energy homeostasis (Itoh and Ornitz 2004; Nishimura *et al.* 1999; Xie *et al.* 1999). Produced predominantly in enterocytes of terminal ileum under the regulation of Farnesoid X receptor **(FXR)**, a bile acid nuclear receptor, and acting mainly in liver through fibroblast growth factor receptor 4 (FGFR4), FGF-19 has been demonstrated to play a central role in

the negative feedback loop of bile acid synthesis and gallbladder refilling (Holt *et al.* 2003; Choi *et al.* 2006; Inagaki *et al.* 2005; Yu *et al.* 2005; Yu *et al.* 2000). Animal studies have also shown antiadipogenic, anti-diabetic and hypolipidemic effects of either exogenous administration or endogenous overexpression of FGF-19 with altered expression of a number of genes involved especially in lipid metabolism (Fu *et al.* 2004; Tomlinson *et al.* 2002).

In contrast to animals, only limited data on FGF-19 concentrations, regulation and function are available in humans. In the first study conducted in healthy human volunteers, serum FGF-19 levels exerted a pronounced diurnal rhythm following the rise in serum bile acids, which was abolished upon fasting. Treatment with bile acid sequestrants decreased FGF-19 concentrations, while administration of chenodeoxycholic acid had opposite effect (Lundasen *et al.* 2006). In two other studies circulating FGF-19 was found to be increased in patients with end-stage renal disease and extrahepatic cholestasis caused by tumorous obstruction of biliary ducts (Reiche *et al.*; Schaap *et al.* 2009). Chronically malnourished patients with anorexia nervosa did not show any changes in systemic FGF-19 compared to normal-weight subjects (Dostalova *et al.* 2008b). The only two trials exploring in part the relation of FGF-19 to glucose metabolism disorders could not find significant difference in its circulating levels between patients with type 2 diabetes mellitus or insulin resistance relative to healthy controls (Brufau *et al.* 2010; Schreuder *et al.* 2010).

To our best knowledge, no targeted information about the influence of obesity on circulating FGF-19 levels is available, nor are any data about the dynamic changes in FGF-19 concentrations after dietary intervention or acute hyperinsulinemia. The main regulator of FGF-19 production FXR is an important link between bile acid and triglyceride metabolism, interacting with a variety of genes and receptors, among others PPAR-α (Smelt 2010). The exact role of FGF-19 in these interactions and the influence of lipid-lowering PPAR-α agonists on FGF-19 levels are largely unknown as well. In the present study we therefore addressed the question whether circulating FGF-19 levels are influenced by increased body weight and the presence of T2DM and whether any changes in its

concentrations could contribute to positive metabolic effects of very-low calorie diet in patients with obesity or lipid-lowering therapy with PPAR-α agonists in subjects with obesity and type 2 diabetes. To this end we measured serum FGF-19 concentrations in patients with obesity and T2DM and studied its changes after above mentioned interventions.

Methods

Study subjects and interventions

Overall, 29 women with obesity **(defined as BMI ≥ 30)** without type 2 diabetes mellitus, 19 women with obesity and type 2 diabetes mellitus and 30 age-matched healthy control women were included into the study. The body weight of all study participants remained stable for at least 3 months before the beginning of the study.

Very low calorie diet sub-study

12 out of 29 obese subjects without type 2 diabetes mellitus underwent a 3-week very low calorie diet (VLCD) program. They were hospitalized in the 3rd Department of Medicine, General University Hospital, Prague and were given a very low calorie diet with energy content of 2500 kJ/day.

Fenofibrate treatment sub-study

11 obese females with type 2 diabetes mellitus and 10 non-obese women were included into fenofibrate intervention study, which was described in detail elsewhere (Haluzik *et al.* 2009) . T2DM patients were treated with diet, metformin alone or combination of metformin and glimepiride before the beginning of the study. Diabetic medication remained unchanged from three months before the start throughout the entire study. Diabetic subjects were treated with PPAR-α agonist fenofibrate

(Lipanthyl M 267) for three months. Their insulin sensitivity before and after 3 months of fenofibrate treatment was examined by isoglycemic-hyperinsulinemic clamp as described previously (Anderlova *et al.* 2007). Ten lean healthy non-diabetic subjects, who were free of any medication, underwent a single isoglycemic-hyperinsulinemic clamp and served as a control group in fenofibrate intervention study.

Written informed consent was signed by all participants before being enrolled into the study. The study was approved by the Human Ethical Review Committee, 1st Faculty of Medicine and General University Hospital, Prague, Czech Republic and was performed in accordance with the principles of the Declaration of Helsinki as revised in 2000.

Anthropometric examination, blood and tissue sampling

All subjects were measured and weighted and their body mass index (BMI) was calculated. Blood samples were withdrawn between 07:00 h and 08:00 h after overnight fasting. Blood samples were separated by centrifugation for 10 minutes at 1000x g within 30 minutes from blood collection. Serum was subsequently stored in aliquots at -70 °C until further analysis.

Clinical and hormonal parameters in obese patients on VLCD were measured one day before the beginning of the diet and at the end of the third week of VLCD. Clinical and hormonal parameters in type 2 diabetes patients **in the fenofibrate substudy** were measured at baseline and after 3 months of treatment with PPAR-α agonist fenofibrate (200mg, Lipanthyl 267M).

Hormonal and biochemical assays

Serum FGF-19 levels were measured by a sandwich enzyme immunoassay (BioVendor, Brno, Czech Republic), following the manufacturer's instructions. Serum samples for FGF-19 measurement were diluted 1:1 with a dilution buffer prior to the assay. The standard curve range for the assay was 15.0 - 960.0 ng/ml. Sensitivity was 4.8 pg/ml and the intra- and interassay variability was 3-8 % and 10-13 %, respectively. Serum insulin concentrations were measured by commercial RIA kit (Cis Bio International, Gif-sur-Yvette Cedex, France). Sensitivity was 2.0 μIU/ml. Serum adiponectin levels were measured by commercial ELISA kit (Linco Research, St. Charles, Missouri, USA). Sensitivity was 0.78 ng/ml. Serum leptin concentrations were measured by commercial ELISA kit (Biovendor, Brno, Czech Republic). Sensitivity was 0.17 ng/ml. The intra- and interassay **variability of all kits was less than** 5.0 % and 9.0 % respectively.

Biochemical parameters were measured in the Department of Biochemistry of General University Hospital, Prague by standard laboratory methods. Homeostasis model assessment (HOMA-R) index was calculated as previously described (Matthews *et al.* 1985) using the following formula:

fasting serum insulin (mIU/l) × fasting serum glucose (mmol/l)/22.5.

Statistical analysis

Statistical analysis was performed using SigmaStat software (SPSS Inc., Chicago, IL). The results are expressed as means ± standard error means (SEM). Changes in FGF-19 and hormonal parameters between studied groups were evaluated by **One-way ANOVA followed by Holm-Sidak method or ANOVA on Ranks followed by Dunn's test and Paired t-test or Wilcoxon Signed Rank Test** as appropriate. Changes of FGF-19 during the clamp were analyzed **by One-way RM ANOVA on Ranks followed by Dunn's method**. Spearman or Pearson correlation test **and multiple regression analysis** were used to calculate the relationships between FGF-19 levels and other parameters. **Statistical significance was assigned to $p < 0.05$.**

Results

Baseline characteristics of patients with obesity, patients with type 2 diabetes mellitus and control group

Anthropometric, biochemical and hormonal characteristics of obese, T2DM, and control group are shown in *Table 1*. As expected, BMI, blood glucose levels, HOMA index, serum triglyceride, leptin and insulin concentrations were significantly higher in both obese and T2DM group relative to control subjects. On the contrary, adiponectin levels were significantly lower in both of these groups. No differences in serum cholesterol was found between the groups. When comparing T2DM with obese group**,** diabetic **patients** had significantly lower BMI and leptin levels, while blood glucose, triglycerides, insulin and HOMA index were significantly higher in T2DM group relative to obese subjects.

Anthropometric and clinical characteristics of patients included into VLCD study

The influence of VLCD on anthropometric, biochemical and hormonal parameters is summarized in *Table 2*. At baseline, patients with obesity had significantly higher BMI, blood glucose, serum insulin, HOMA index and leptin and lower serum adiponectin relative to control subjects. The groups did not significantly differ in serum triglyceride and cholesterol levels. VLCD induced a significant decrease in BMI and HOMA index. Insulin, glucose and leptin concentrations tended to decrease after VLCD, but the difference did not reach the statistical significance. No significant influence of VLCD on serum cholesterol, triglyceride and adiponectin levels was found.

Anthropometric and clinical characteristics of patients included into fenofibrate treatment study

Anthropometric, biochemical and hormonal characteristics of the study sub-group have been described in detail elsewhere (Haluzik *et al.* 2009) and are briefly shown in *Table 3*. At baseline, BMI, blood glucose, serum triglyceride, insulin, glycated hemoglobin and atherogenic index were

significantly higher, while serum HDL cholesterol was significantly lower in T2DM group relative to control subjects. Three months of treatment with fenofibrate led to a significant decrease of serum triglyceride concentrations, while blood glucose, HOMA index and glycated hemoglobin slightly but significantly increased. Other parameters including BMI were not affected by fenofibrate treatment. Insulin sensitivity of T2DM group was significantly lower relative to control group and was not affected by fenofibrate treatment (data not shown here, previously described in (Haluzik *et al.* 2009).

The influence of obesity and obesity/type 2 diabetes mellitus on FGF-19 levels and the relationship of FGF-19 levels with anthropometric and metabolic parameters

Circulating concentrations of FGF-19 reached the highest levels in lean control subjects **(202.2 ± 16.7 pg/ml)**, with a non-significant tendency towards lower levels in T2DM group **(146.4 ± 15.9 pg/ml**) and a further, statistically significant, decrease in the obese non-diabetic group **(132.1 ± 12.7 pg/ml, p<0.05)**. No significant difference in FGF-19 levels was observed between T2DM and obese group (*Figure 1*).

In a combined population of all three groups **as well as in the subpopulation of control subjects combined with obese non-diabetic patients** FGF-19 concentrations significantly negatively correlated with BMI **(r=-0.309, p<0.05 for the whole population and r=-0.343, p<0.05 for Control + Obese subgroup) and leptin (r=-0.282, p<0.05 for the whole population and r=-0.365, p=0.005 for Control + Obese subgroup) and significantly positively with** adiponectin **(r=0.289, p<0.05 for the whole population and r=0.289, p<0.05 for Control + Obese subgroup). In contrast, no such relationship could be seen in the T2DM subgroup.**. FGF-19 levels were not significantly related to age, serum blood glucose, triglycerides, insulin and cholesterol or HOMA index **in any of the studied groups**.

The influence of VLCD, fenofibrate treatment and hyperinsulinemia during the clamp on serum FGF-19 levels

Three weeks of VLCD did not significantly influence serum FGF-19 levels in obese group **(140.2 ± 19.7 vs. 176.1 ± 27.1 pg/ml, p=0.369)** (*Figure 2*), while 3 months of treatment with fenofibrate significantly decreased FGF-19 concentrations in T2DM patients **(194.6 ± 26.2 vs. 107.5 ± 25,0 pg/ml, p=0.007)** (*Figure 3*). Three hours of acute hyperinsulinemia during isoglycemic-hyperinsulinemic clamp tended to decrease FGF-19 levels in both healthy and T2DM subjects, but the difference did not reach statistical significance **(214.4 ± 15.0 vs. 145.7 ± 19.9 pg/ml**, **p=0.130 for control and 198.6 ± 18.0 vs.147.2 ± 24.8 pg/ml, p=0.519 for diabetic group**). **Nevertheless, there was a significant drop in FGF-19 concentrations in T2DM group after 90 minutes of the clamp (198.6 ± 18.0 vs. 121.1 ± 21.0 pg/ml, p<0.001), while no such change could be seen in the control group (214.4 ± 15.0 vs. 188.0 ± 41.1 pg/ml, p=0.483)** (*Figure 4*). **In T2DM group** three months of fenofibrate treatment **resulted in markedly decreased initial** FGF-19 concentrations **with no further change in its levels in the course of the isoglycemic-hyperinsulinemic clamp (95.7 ± 16.5 vs. 114.1 ± 18.0 vs. 112.5 ± 25.8 pg/ml, p=0.264)** (*Figure 4*).

Discussion

FGF-19 is a novel endocrine and paracrine regulator which plays central role in the suppression of bile acid synthesis and secretion. As bile acid metabolism is tightly connected to other metabolic pathways, especially those of lipids (Insull 2006; Lefebvre *et al.* 2009), FGF-19 was suggested to participate also in the regulation of other processes, including cholesterol, lipoprotein, triglyceride and glucose metabolism (Fu *et al.* 2004; Tomlinson *et al.* 2002). Rodents with FGF-19 overexpression have decreased body weight and improved glucose tolerance and insulin sensitivity compared to their wild-type littermates. FGF-19 over-expressing mice are also protected from high

fat diet-induced weight gain (Tomlinson *et al.* 2002). Similar results were seen in mice treated with exogenous recombinant FGF-19, which display reduced body weight and decreased serum glucose, insulin, cholesterol and triglyceride levels (Fu *et al.* 2004).

In humans, data concerning the relation of FGF-19 to metabolic parameters are less concise. In our previous study, we have demonstrated that FGF-19 levels were not affected by nutritional status in severely underweight patients with anorexia nervosa, nor were they related to any of the investigated metabolic parameters (Dostalova *et al.* 2008a). Schreuder et al did not find any influence of insulin resistance (IR) as assessed by HOMA index on intestinal FGF-19 production in patients with non-alcohol fatty liver disease (NAFLD), though the suppressing effect of FGF-19 on bile acid synthesis was impaired in IR patients (Schreuder *et al.* 2010). In a recently conducted study by Brufau e**t** al FGF-19 levels did not differ between type 2 diabetic patients and control subjects (Brufau *et al.* 2010). In contrast, Reiche et al reported a negative correlation between FGF-19 and fasting glucose in healthy subjects (Reiche *et al.* 2010). Here we show that in our patients FGF-19 levels correlated inversely with body mass index, with the most pronounced differences in serum FGF-19 concentrations being in the most obese patients with BMI over 40. T2DM group with a mean BMI of 36 kg/m^2 tended to have rather slightly decreased levels of FGF-19. A positive correlation between FGF-19 and adiponectin observed throughout the study population extends similar findings reported previously in chronically hemodialysed patients (Reiche *et al.* 2010). These findings may suggest a possible relationship between FGF-19 and the regulation of endocrine function of adipose tissue that has been in previous studies linked to the regulation of glucose metabolism and insulin sensitivity (Anderlova *et al.* 2006; Ronti *et al.* 2006).

Three weeks of very-low calorie diet in our study significantly reduced body weight along with the decrease in blood glucose, insulin and HOMA index and a tendency to improved lipid profile (*Table 2*). FGF-19 levels tended to increase but the difference did not reach statistical significance. In experimental studies fasting was associated with increased production of FGF-15 (the mouse

orthologue of FGF-19) and decreased liver expression of FGF receptor 4 (FGFR4) in rodents. At the same time fasting increased the expression of CYP7A1, a key regulator enzyme in bile acid synthesis, via pathways independent of FGF-15 – FGFR4 axis (mainly through the decreased insulin-mediated suppression of CYP7A1) (Shin and Osborne 2009). Whether a similar mechanism could be applied to humans and whether FGF-19 could play a role in some adaptive responses to fasting and/or caloric restriction remains to be further determined. Collectively, these findings suggest, that serum FGF-19 is at least partially influenced by nutritional status and body weight, especially in the higher BMI ranges. In contrast, the absence of a clear association of circulating FGF-19 to parameters of glucose metabolism is in line with previous reports in humans, which so far failed to demonstrate an explicit and causal relation between FGF-19 and glucose metabolism disorders (Brufau *et al.* 2010; Schreuder *et al.* 2010).

The metabolism of bile acids on one hand and cholesterol and triglycerides on the other hand is tightly coupled. Bile acids represent the primary pathway for cholesterol catabolism and bile acid sequestration with resins is connected with decreased total and LDL cholesterol, while increasing HDL cholesterol and triglycerides (Beil *et al.* 1982; Insull 2006; Shepherd *et al.* 1980). The most important link between bile acid and lipid metabolism is thought to be Farnesoid X receptor, the activator of FGF-19 synthesis. FRX inhibits the expression of several lipogenic genes, while promoting the activity of lipoprotein lipase and stimulating VLDL receptors (reviewed in (Lefebvre *et al.* 2009; Smelt 2010). FXR also activates PPAR-α receptors promoting thus the catabolism of free fatty acids (Pineda Torra *et al.* 2003). The exact role of FGF-19 in these interplays is currently unknown. In animals, FGF-19 has been shown to inhibit the expression of several rate-limiting enzymes involved in fatty acid synthesis (Fu *et al*. 2004; Tomlinson *et al.* 2002). In humans, Brufau el al have demonstrated that treatment with bile acid sequestrant colesevelam improved diabetic **control** and decreased basal and postprandial FGF-19 concentrations in both T2DM and healthy subjects. However, no relationships between parameters of glucose metabolism and changes in FGF-

19 levels or bile acid metabolism were found (Brufau *et al*. 2010). In the present study **no correlation between FGF-19 levels and the parameters of lipid metabolism could be observed.**

As fibrates as triglyceride lowering agents act, similarly to FXR, via activation of PPAR-α, in the third part of our study we examined the influence of three months of fenofibrate administration on serum levels of FGF-19 to test the hypothesis that changes in FGF-19 may contribute to positive effects of fibrate treatment. Fenofibrate administration in hypertriglyceridemic T2DM patients decreased triglyceride levels while body weight, total and HDL cholesterol remained unchanged. FGF-19 levels after fenofibrate treatment markedly decreased. These findings argue against the hypothesis, that fibrate-induced activation of PPARα might involve also the induction of FGF-19.

A rapidly growing body of evidence is supporting the importance of bile acids and bile acid receptors in the regulation of insulin action (reviewed in (Lefebvre *et al.* 2009)). *In vitro* and animal studies suggest that FXR activation improves insulin sensitivity and insulin-stimulated glucose uptake (Cariou *et al.* 2006; Zhang *et al.* 2006). Stimulation of hepatic FXR resulted in decreased hepatic gluconeogenesis, increased glycogen synthesis and decreased plasma glucose levels (Ma *et al.* 2006; Zhang *et al*. 2006). In mice, insulin was shown to up-regulate hepatic FGF receptor 4 (FGFR4), the major hepatic FGFR isoform (Shin and Osborne 2009). Similarly to FGF-19, insulin also decreases bile acid synthesis by suppressing the expression of its rate-limiting CYP7A1 enzyme. It was demonstrated that the mouse FGF-orthologue, FGF-15, and insulin act in liver partially via the same pathways, with forkhead transcriptional factor 1 (FoxO1), a factor controlling the expression of CYP7A1 as well as several gluconeogenetic genes, being the key converging node (Shin and Osborne 2009). To examine the influence of short-term hyperinsulinemia on circulating FGF-19 levels we performed hyperinsulinemic-isoglycemic clamp in the subgroup of diabetic patients undergoing treatment with fenofibrate and in an age- and sex-matched control group. Starting at comparable levels, in T2DM group FGF-19 showed a steep dropdown at 90 minutes followed by a slow rise towards the end of the procedure. In healthy lean controls FGF-19 slowly

decreased throughout the clamp terminating at almost the exact levels as in T2DM group. Interestingly, **with basal levels of FGF-19 almost halved after 3 months of fenofibrate treatment, no significant effect of acute hyperinsulinemia on serum FGF-19 during the clamp was observed.** Our current data do not allow us to unravel the exact mechanisms of insulin-mediated FGF-19 regulation and its modulation by fibrate therapy, **but these findings suggest the existence of other regulatory pathways, independent of intestinal bile acid actions.** A direct effect of insulin on FGF-19 production might be one of the options. However, other factors affected by both procedures (clamp and fenofibrate treatment) could constitute further links between insulin and FGF-19 production. One of the candidates might be free fatty acids (FFA), as they are almost completely inhibited during hyperinsulinemic-isoglycemic clamp (Stumvoll *et al.* 2000) and they are also reduced by fibrate-mediated PPARα activation. As we did not assess FFA levels during the clamp substudy, this and other possible mechanisms remain subject of further investigations.

Taken together, our study demonstrated that FGF-19 levels correlated inversely with BMI and were significantly increased in severely obese patients compared to lean healthy controls. Very low calorie diet **showed no significant effect on** FGF-19 levels, while PPARα agonist treatment decreased them significantly. Acute hyperinsulinemia **tended to** reduce FGF-19 suggesting the existence of other regulatory pathways besides the well-established intestinal bile acid regulation. Though no significant relationship of FGF-19 to the presence of T2DM or various measures of glucose metabolism and insulin sensitivity could be established, the role of FGF-19 in the complex net of reactions integrating bile acid, lipid and glucose metabolism remains an appealing challenge for further investigations.

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***Table 1*:** Anthropometric, biochemical and hormonal characteristics of control group of healthy women(Control), obese women without type 2 diabetes mellitus (Obese) and type 2 diabetic women (T2DM). Values are means ± SEM. Statistical significance is from **One-way ANOVA or ANOVA on Ranks** as appropriate. *p<0.05 vs. control group; ° p<0.05 T2DM vs. Obese.

Group	**Control**	**Obese**	**T2DM**
Number of subjects	30	29	19
Age (years)	48.4 ± 2.2	45.9± 1.4	51.4± 1.5
BMI (kg/m^2)	23.8 ± 0.3	44.6± 1.5*	36.7± 1.2*°
Blood glucose (mmol/l)	4.30 ± 0.23	5.72± 0.33*	10.07 ± 0.84*°
Cholesterol (mmol/l)	5.07 ± 0.17	4.99 ± 0.23	4.38± 0.21
Triglycerides (mmol/l)	1.16 ± 0.11	1.68 ± 0.15*	2.82 ± 0.26*°
Insulin (mIU/l)	14.72 ± 1.65	24.59 ± 2.34*	46.60± 4.02*°
HOMA index	2.50 ± 0.48	6.06± 0.66*	18.05± 1.46*°
Leptin (ng/ml)	12.57 ± 1.54	47.67± 2.19*	28.1± 3.30*°
Adiponectin (µg/ml)	22.23 ± 1.88	14.94± 1.19*	13.02± 2.11*

***Table 2*:** Anthropometric, biochemical and hormonal characteristics of control group of healthy women (Control) and obese female subjects before (Obese **Baseline**) and after (Obese **after VLCD**) three weeks of VLCD. Values are means ± SEM. Statistical significance is from **One-way ANOVA or ANOVA on Ranks** (Obese **Baseline** or Obese **after VLCD** vs. Controls) and from Paired t-test or Wilcoxon Signed Rank Test (Obese **Baseline** vs. Obese **after VLCD**) **as appropriate**. *p<0.05 vs. control group; ° p<0.05 **Obese Baseline vs. Obese after VLCD**.

Group	**Control**	**Obese**	
		Baseline	**After VLCD**
Number of subjects	30	12	12
BMI (kg/m^2)	23.8 ± 0.3	49.5 ± 2.7*	47.1 ± 2.5*°
Blood glucose (mmol/l)	4.30 ± 0.23	5.91 ± 0.47*	5.26 ± 0.26*
Cholesterol (mmol/l)	5.07 ± 0.17	4.50 ± 0.23	4.12 ± 0.22
Triglycerides (mmol/l)	1.16 ± 0.11	1.37 ± 0.01	1.17 ± 0.10
Insulin (mIU/l)	14.72 ± 1.65	34.03 ± 3.56*	30.34 ± 4.11*
HOMA index	2.50 ± 0.48	8.53 ± 1.09*	6.57 ± 0.88*°
Leptin (ng/ml)	12.57 ± 1.54	45.83 ± 3.89*	42.47 ± 4.77*
Adiponectin (µg/ml)	22.23 ± 1.88	13.66 ± 1.45*	14.33 ± 1.52*

***Table 3*:** Anthropometric, biochemical and hormonal characteristics of control group of healthy females (Control) and obese type 2 diabetic women before (T2DM baseline) and after three months of treatment with PPAR-α agonist fenofibrate (T2DM fenofibrate). Values are means ± SEM. Statistical significance is from **One-way ANOVA or ANOVA on Ranks as appropriate** (T2DM/Baseline or T2DM/Fenofibrate vs. Controls) and from Paired t-test or Wilcoxon Signed Rank Test (T2DM/Baseline vs. T2DM/Fenofibrate) *p<0.05 vs. control group; ° p<0.05 T2DM/Fenofibrate vs. T2DM/baseline

Group	**Control**	**T2DM**	
		Baseline	Fenofibrate
Number of subjects	10	11	11
BMI (kg/m^2)	23.7 ± 0.8	36.7 ± 2.9*	36.5±2.7*
Blood glucose (mmol/l)	4.97 ± 0.19	8.48 ± 0.68*	9.43±0.84*°
Glycated Hemoglobin (%)	3.81 ± 0.08	5.77 ± 0.56*	6.10 ± 0.58*°
Cholesterol (mmol/l)	5.39 ± 0.24	5.22 ± 0.32	5.09±0.25
HDL-cholesterol (mmol/l)	1.55 ± 0.28	1.17 ± 0.11*	1.20 ± 0.11*
Triglycerides (mmol/l)	0.98 ± 0.12	2.96 ± 0.39*	2.17±0.24*°
Insulin (mIU/l)	23.0 ± 2.9	44.2 ± 7.1*	47.5±8.0*
HOMA index	5.08 ± 0.56	16.66 ± 0.21*	20.12 ± 0.30*°
Adiponectin (µg/ml)	22.41 ± 1.82	17.46 ± 4.04	16.53 ± 2.56
Atherogenic index	1.88 ± 0.09	3.17 ± 0.37*	3.02 ± 0.75*

Figure legend

Figure 1. Serum FGF-19 concentrations in control group (black bar, **n=30**), obese nondiabetic group (light grey bar, **n=29**) and type 2 DM group (dark grey bar, **n=19**). Values are means ± SEM. Statistical significance is from **One-way ANOVA or ANOVA on Ranks** as appropriate. *p<0.05 vs. control group

Figure 2. Serum FGF-19 concentrations in control group (black bar, **n=30**), obese patients before (light grey bar, **n=12**) and after (dashed bar, **n=12**) three weeks of very low calorie diet (VLCD). Values are means ± SEM. Statistical significance is from **One-way ANOVA or ANOVA on Ranks and from paired t-test or Wilcoxon Signed Rank Test** as appropriate. *p<0.05 vs. control group

Figure 3. Serum FGF-19 concentrations in control group (black bar, **n=10**), type 2 DM group before (dark grey bar, **n=11**) and after three months of fenofibrate treatment (dashed bar, **n=11**). Values are mean± SEM. Statistical significance is from **One-way ANOVA or ANOVA on Ranks and from paired t-test or Wilcoxon Signed Rank Test** as appropriate. *p < 0.05 vs. control group, °p <0.05 vs. T2DM group **before** treatment

Figure 4. Changes of **FGF19** concentrations during isoglycemic-hyperinsulinemic clamp in control group of healthy females (filled circles, **n=10**) and obese females with type 2 DM before (open circles, **n=11**) and after three months of treatment with PPAR-α agonist fenofibrate (filled triangles**,** **n=11**). Statistical significance is from **One-way** RM ANOVA on Ranks. * p<0.05 vs. baseline value of the respective group

Figure 1

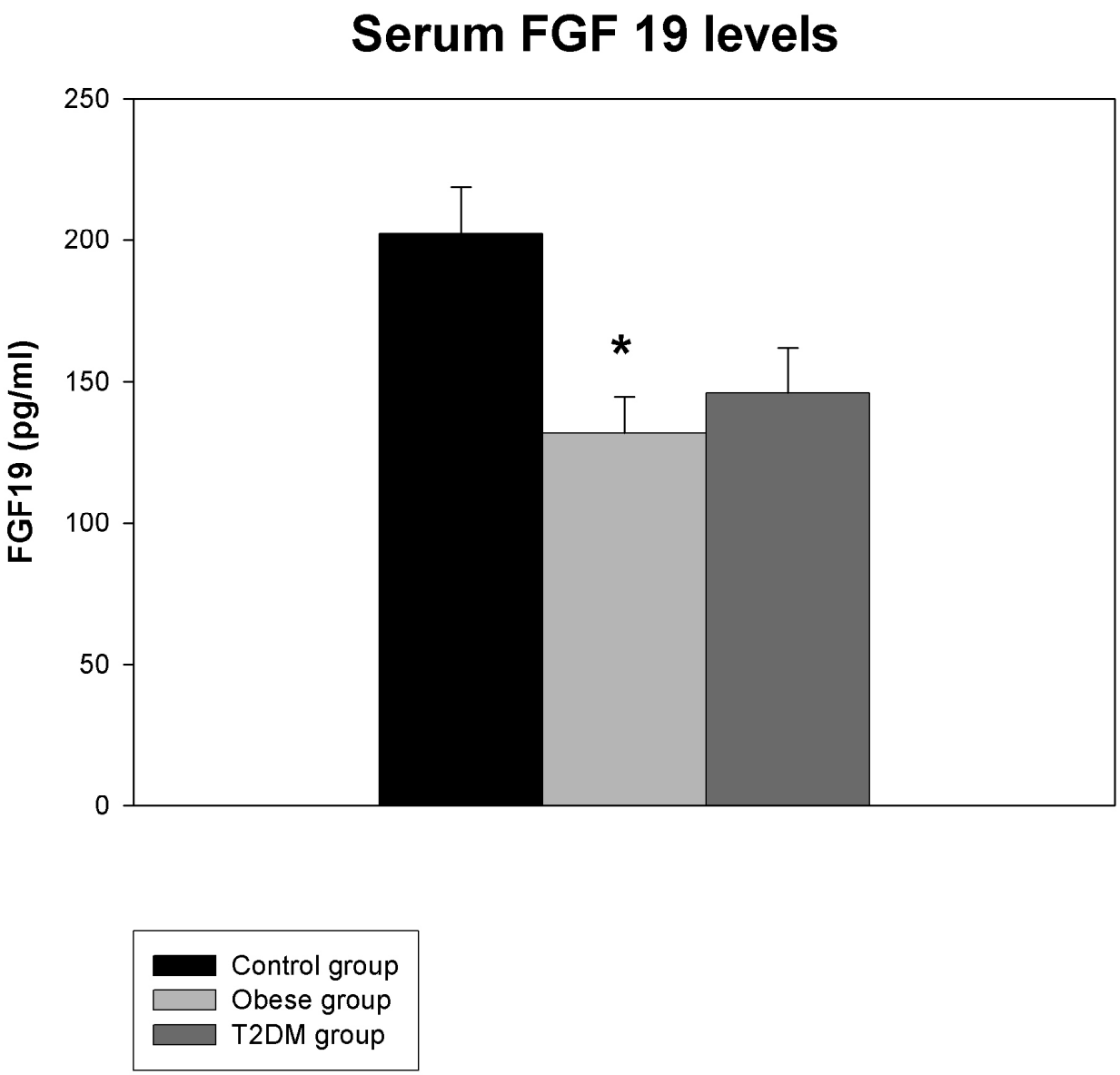

Serum FGF 19 levels
FGF19 (pg/ml)
250
200
150
100
50
0
*
Control group
Obese group
T2DM group

Figure 2

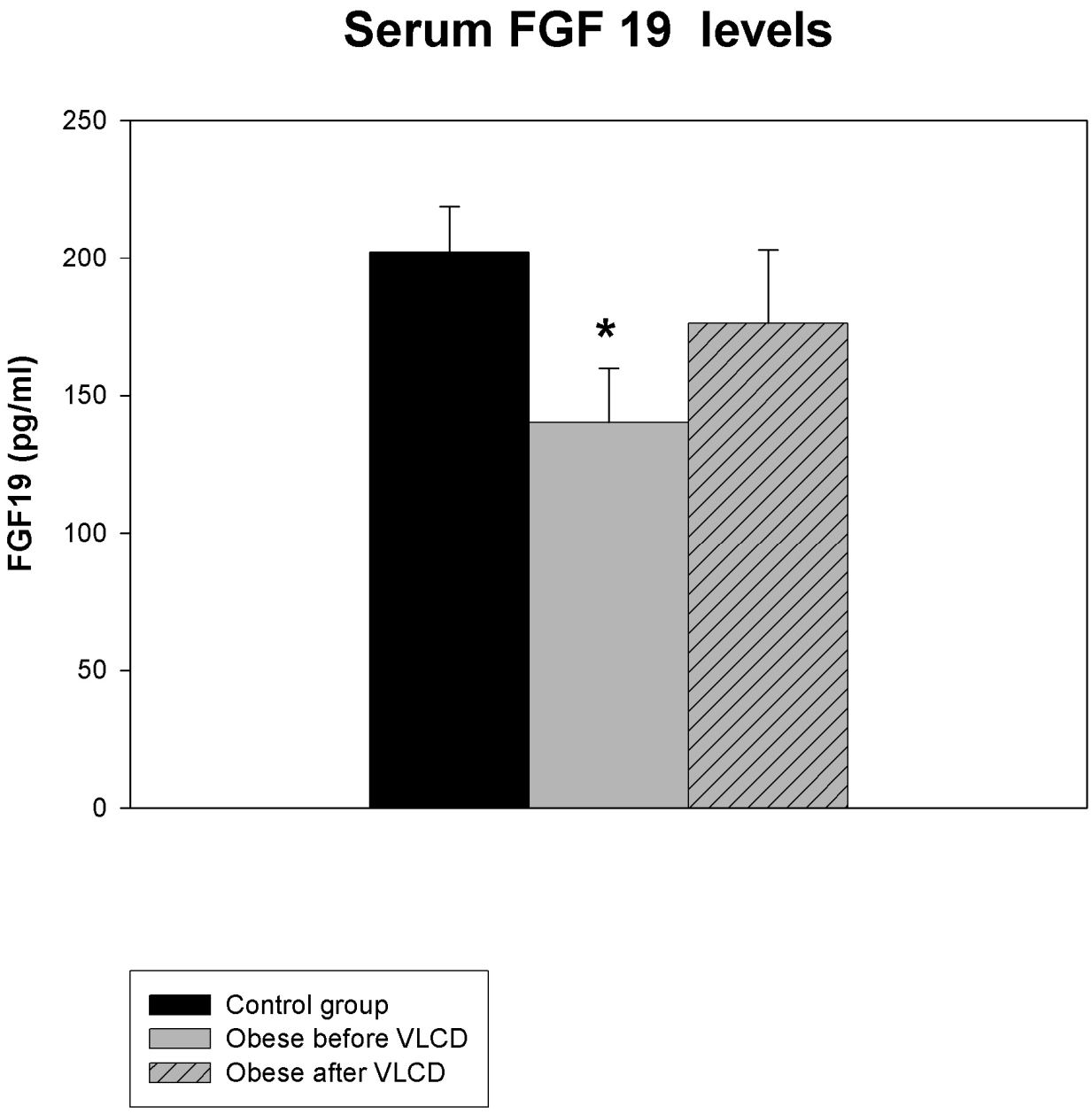

Serum FGF 19 levels
FGF19 (pg/ml)
250
200
150
100
50
0
*
Control group
Obese before VLCD
Obese after VLCD

Figure 3

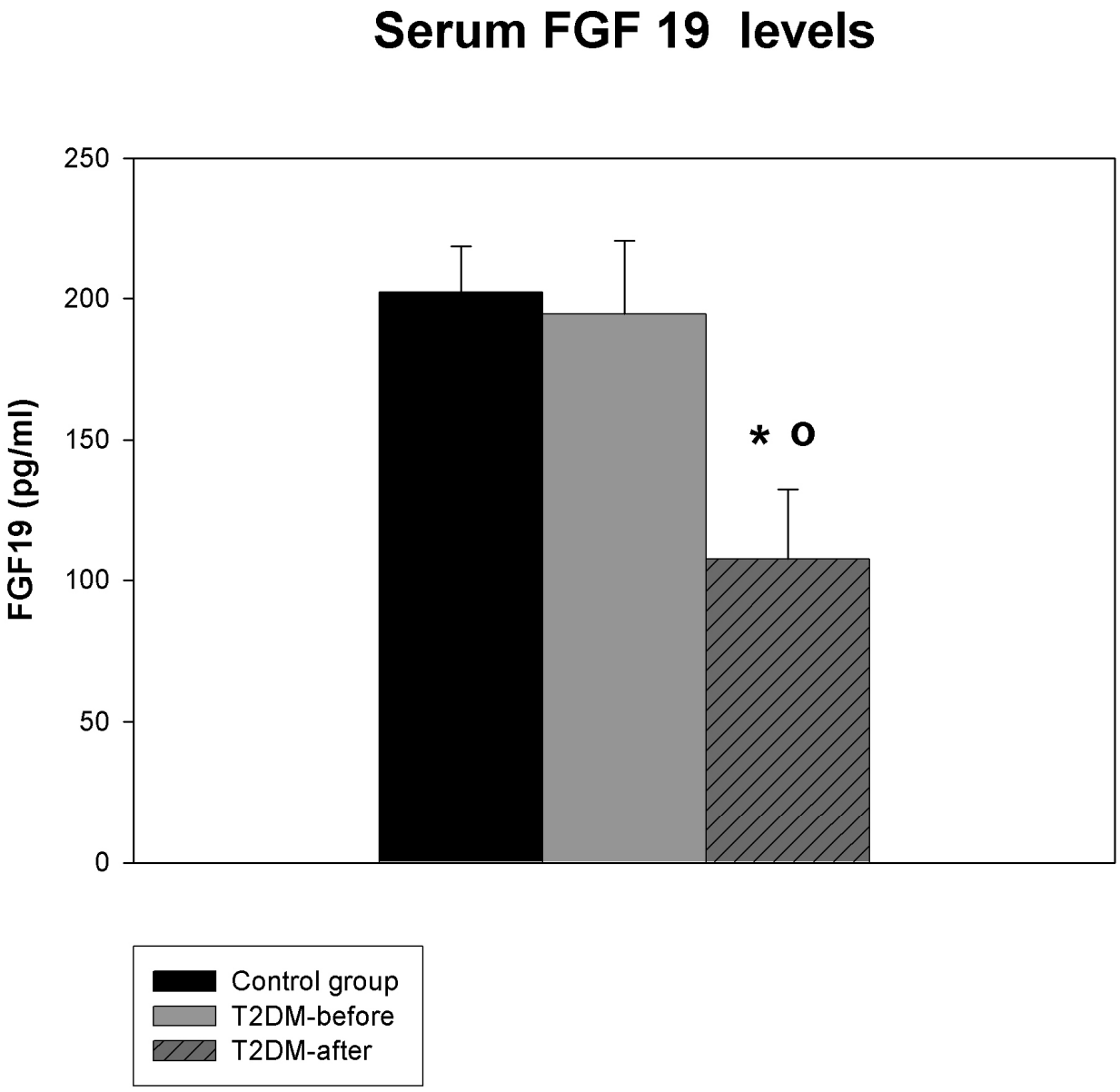
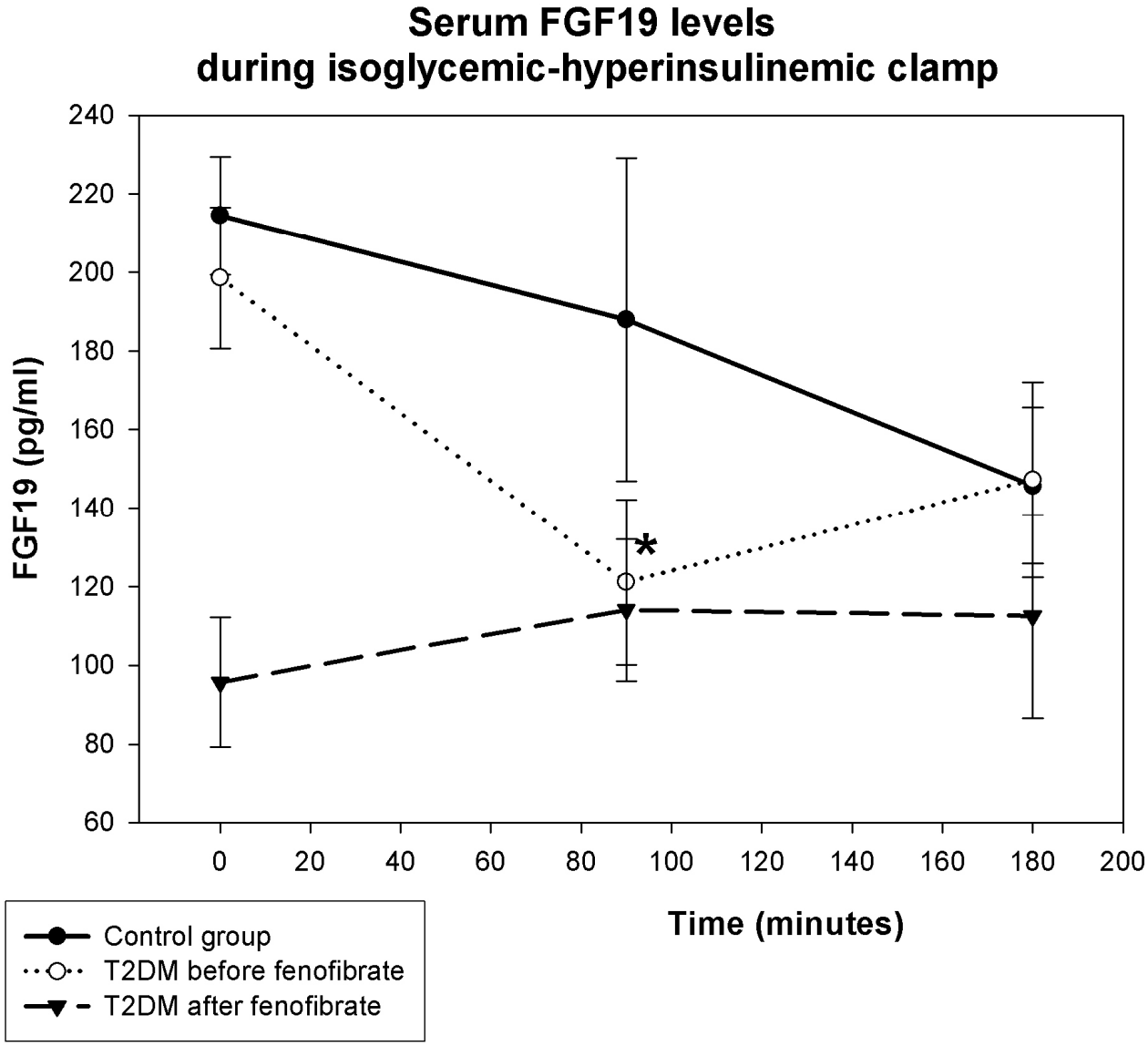

Serum FGF 19 levels
FGF19 (pg/ml)
250
200
150
100
50
0
* o
Control group
T2DM-before
T2DM-after

Figure 4


Serum FGF19 levels
during isoglycemic-hyperinsulinemic clamp
FGF19 (pg/ml)
240
220
200
180
160
140
120
100
80
60
0
20
40
60
80
100
120
140
160
180
200
Time (minutes)
*
Control group
T2DM before fenofibrate
T2DM after fenofibrate