

BETATROPHIN – A NEW INSIGHT INTO LIPID HOMEOSTASIS

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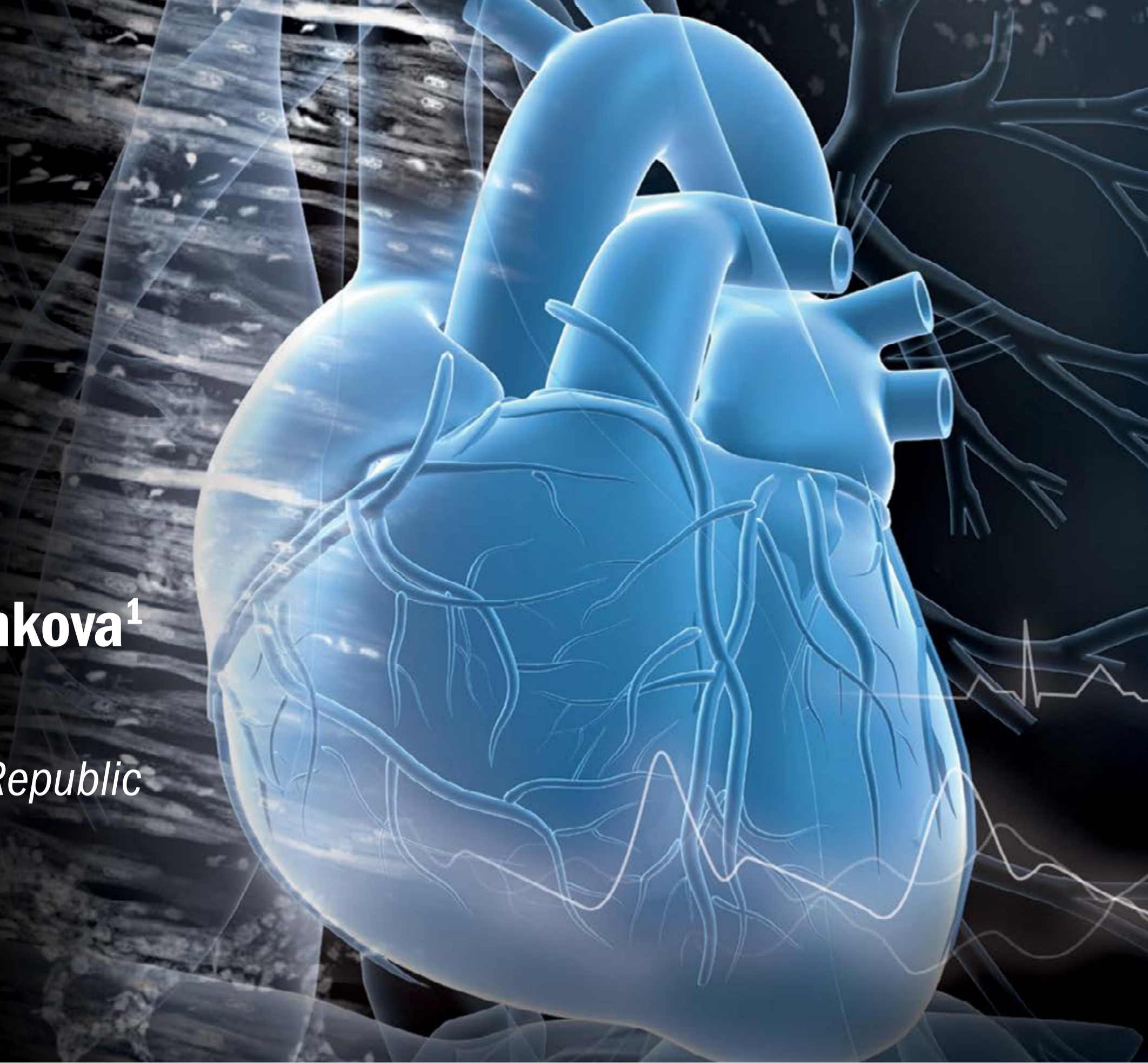
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Introduction

Betatrophin (ANGPTL8, lipasin, RIFL) is a new member of angiopoietin-like protein family. ANGPTLs exhibit multiple functions, playing a role in lipid and glucose metabolism, inflammation, hematopoiesis, and cancer. Betatrophin is predominantly expressed in liver and adipose tissue. Betatrophin is an atypical member of the ANPGTL family since it lacks fibrinogen-like domain and coiled-coil domain. ANGPTL3 and ANGPTL4 play essential role in lipid metabolism [1]. Betatrophin is involved in triglyceride metabolism through its interaction with ANGPTL3 and regulation of lipoprotein lipase activity [2,3]. Lipoprotein lipase binds to the surface of capillary microvascular endothelial cells and hydrolyzes triglycerides in chylomicrons and VLDL, yielding free fatty acids, which are then taken up by peripheral tissues, including fat, muscle and heart [4]. Betatrophin inhibits lipoprotein lipase and suppresses triglyceride clearance which leads to increase in serum triglyceride [1]. Betatrophin is thought to be a potential player in dyslipidemia with a strong association with HDL-cholesterol and also a potential therapeutic tool for treatment of dyslipidemia [6].

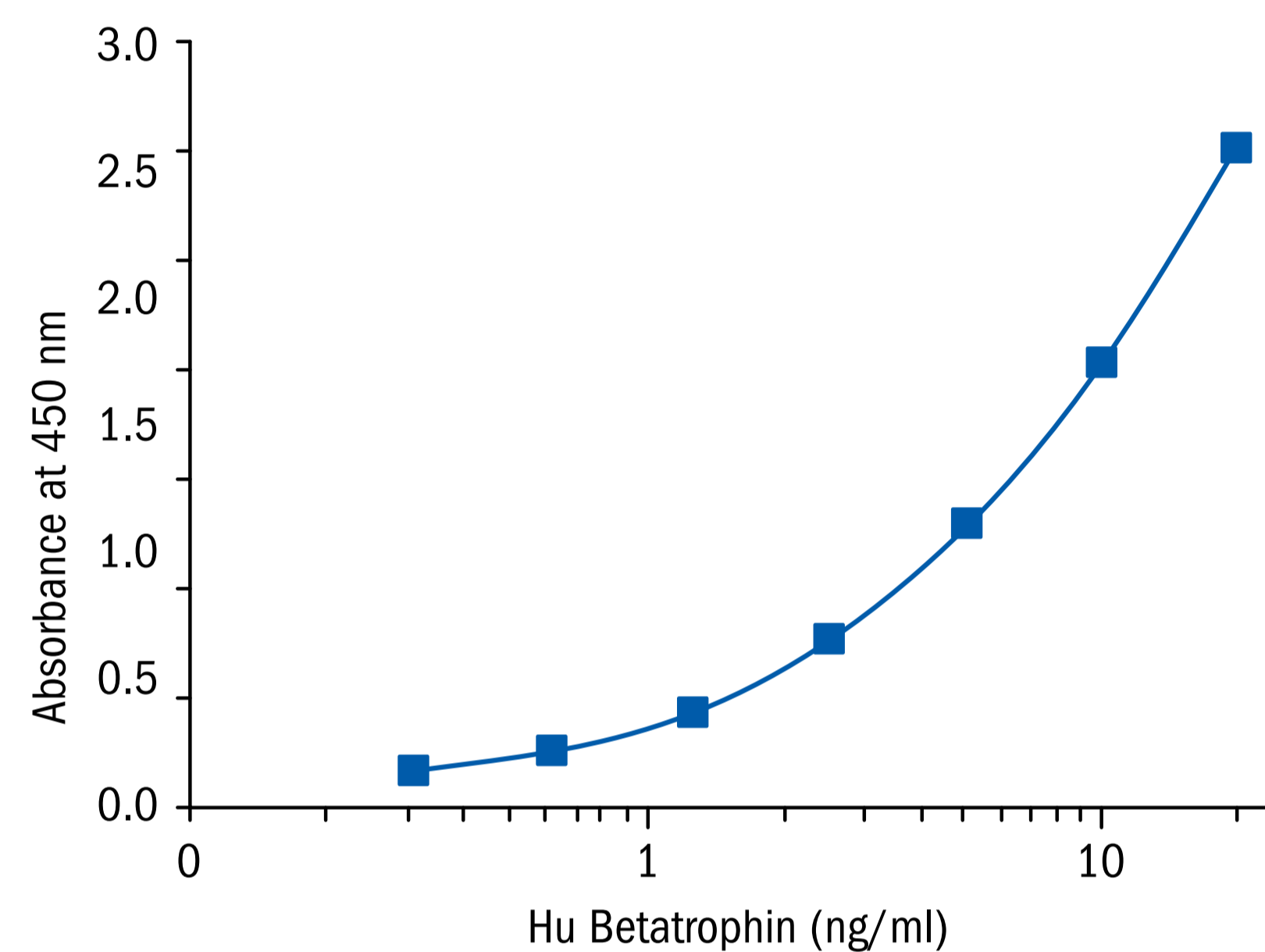
Elevated circulating levels of betatrophin have been described in patients with metabolic syndrome and diabetes [3] and in patients after surgically induced weight loss, but not after diet-induced weight loss [5]. Elevated betatrophin was reduced after exercise training in obese [7]. However, the changes in circulating betatrophin levels during food absorption and their impact have not been completely described yet.

Materials and Methods

This study was focused on circulating betatrophin levels in healthy and obese subjects. Serum betatrophin was measured in healthy subjects and in obese patients who underwent three months of prescribed exercise training. In another group of healthy donors, preprandial and postprandial betatrophin levels were analyzed.

We used new Human Betatrophin ELISA (BioVendor Brno, Czech Republic) to measure serum betatrophin levels. In this assay, standards and samples are incubated in microplate wells pre-coated with polyclonal anti-human betatrophin antibody. After incubation and washing, biotin labelled polyclonal anti-human betatrophin antibody is added and incubated with captured human betatrophin. After another washing, streptavidin-HRP conjugate is added. After incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (tetramethylbenzidine - TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of betatrophin. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of samples are determined using this standard curve. The total assay time is less than 4 hours.

Standard Curve for Human Betatrophin ELISA



Human Betatrophin ELISA characteristics:	
Intra-assay CV (n=8)	5.4 - 9.4 %
Inter-assay CV (n=6)	2.4 - 10.0 %
Dilution Linearity	91.4 - 103.6 %
Spiking Recovery	95.1 - 100.1 %
Sensitivity	0.244 ng/ml
Specificity	no crossreactivity with human ANPTL3 and ANGPTL4

Results

Serum betatrophin levels were increased in obese compared to healthy subjects (mean: 24.3 ng/ml vs 12.8 ng/ml). After three month exercise training, circulating betatrophin in obese subjects dropped to 17.3 ng/ml. Mean fasting serum betatrophin level in healthy subjects was 10.1 ng/ml. Two hours after meal, mean betatrophin level raised to 12.2 ng/ml while four hours after meal, it fell to 9.4 ng/ml. Both these changes in betatrophin levels were significant ($p < 0.001$).

Serum betatrophin in healthy, obese and obese after exercise training

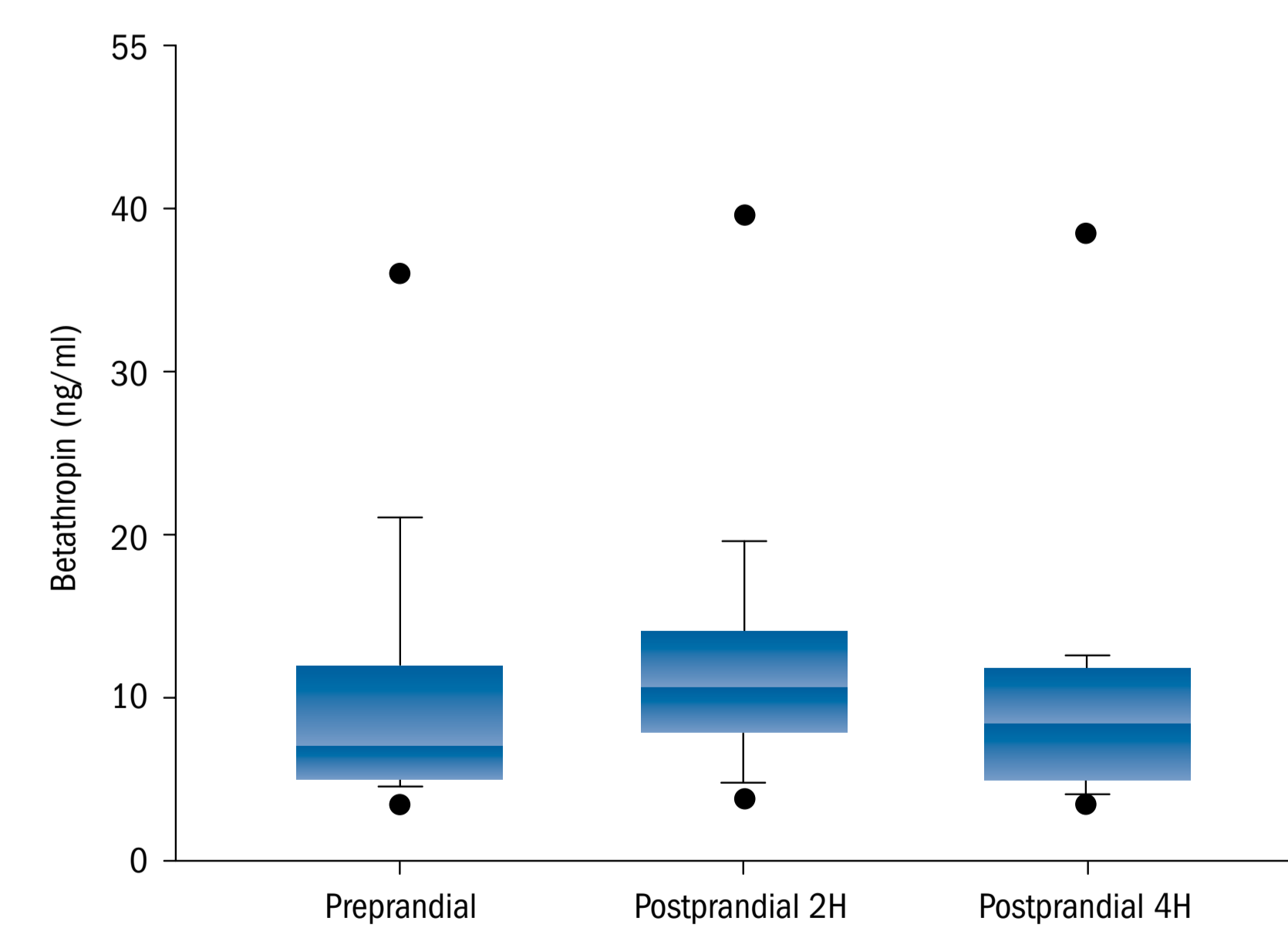
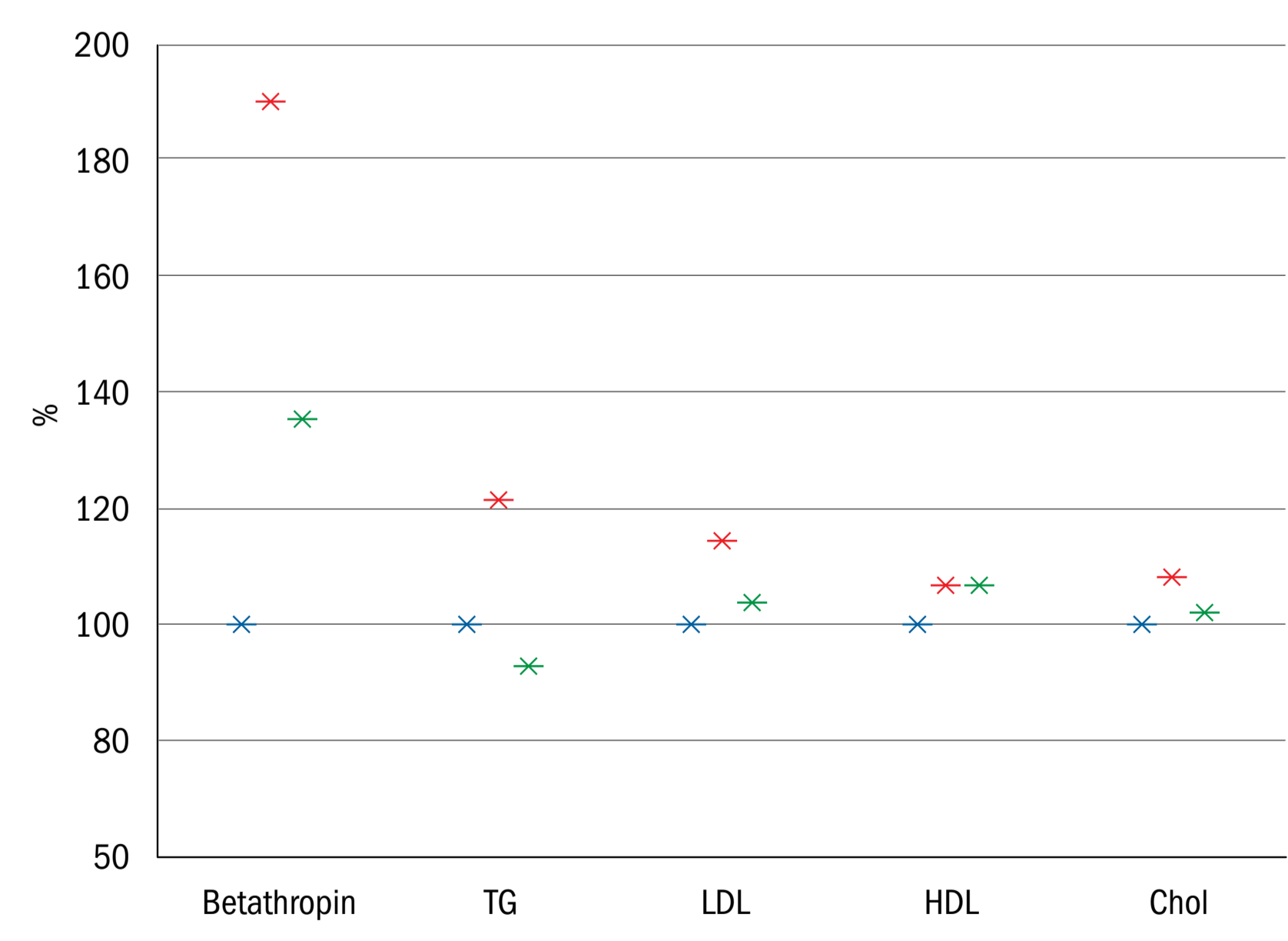
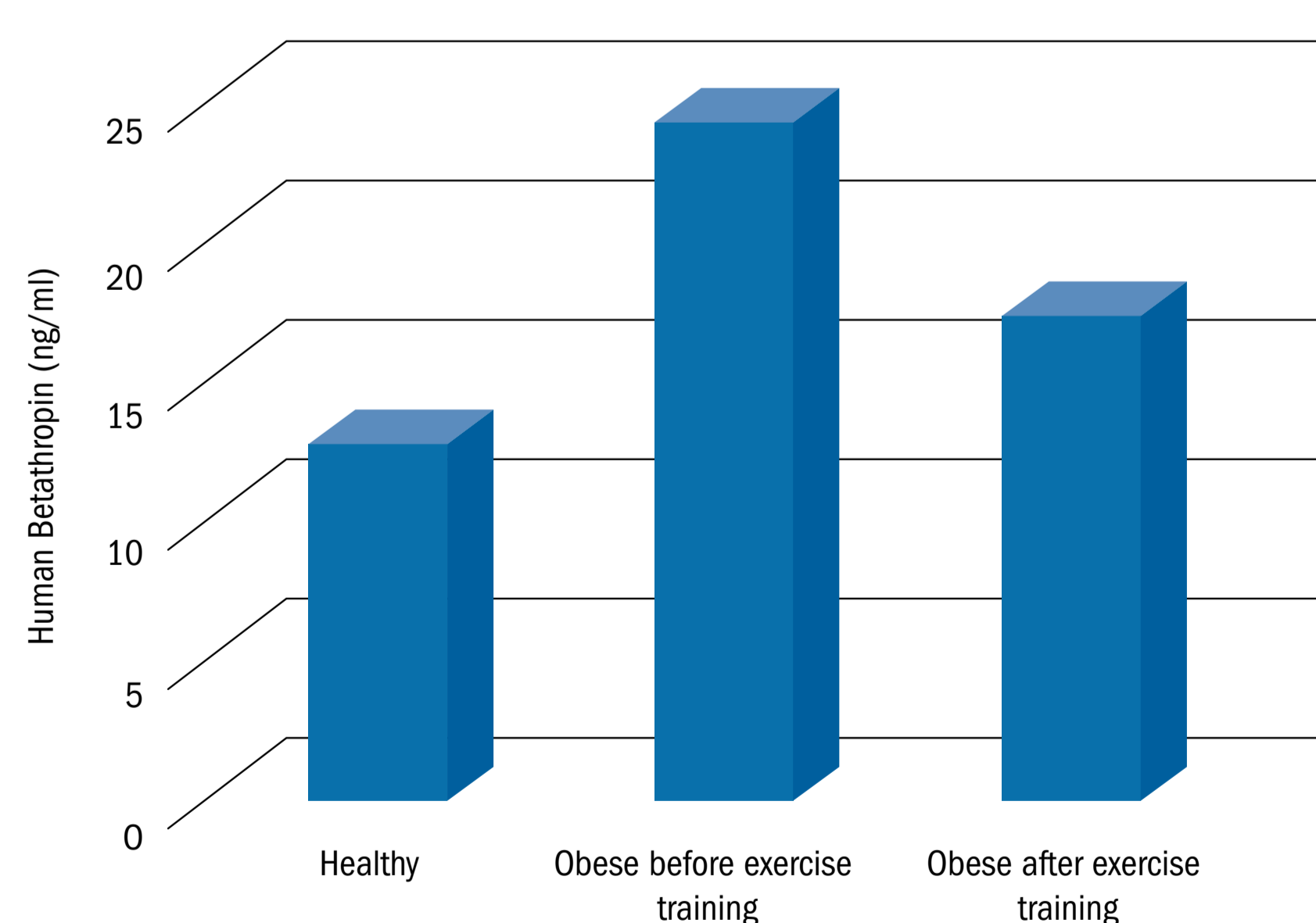
	n	Serum Betatrophin (ng/ml)				
		Mean	Median	SD	Min	Max
Healthy	155	12.8	10.4	8.9	2.7	72.0
Obese before exercise training	26	24.3	19.0	19.3	6.5	104.9
Obese after exercise training	26	17.3	14.6	8.7	6.5	41.7

Lipid profile in healthy, obese and obese after exercise training

	BMI	Weight (kg)	TG (mmol/l)	LDL (mmol/l)	HDL (mmol/l)	Chol (mmol/l)
Healthy	27.0	80.3	1.4	2.8	1.5	5.0
Obese before exercise training	32.4	88.5	1.7	3.2	1.6	5.4
Obese after exercise training	30.5	83.3	1.3	2.9	1.6	5.1

Comparison of mean preprandial and postprandial serum betatrophin levels

	n	Serum Betatrophin (ng/ml)				
		Mean	Median	SD	Min	Max
Healthy	19	10.1	6.9	7.4	3.2	35.8
Obese before exercise training	19	12.2	10.5	7.7	3.6	39.4
Obese after exercise training	19	9.4	8.3	7.4	3.2	38.2



Conclusion

The results confirm significant increase in betatrophin levels two hours after meal and significant decrease in circulating betatrophin four hours after meal. Our data also show that circulating betatrophin is elevated in obese and can be reduced by exercise training independently of the diet. Our findings indicate that betatrophin plays a role in regulating plasma LDL and triglyceride. Moreover, it has been shown that betatrophin level reflects the effects induced by exercise more sensitively than common lipid profile and could be used in diagnostics.

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