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Gastric bypass-induced weight loss alters obesity-associated patterns of plasma pentraxin-3 and systemic inflammatory markers

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GASTRIC BYPASS-INDUCED WEIGHT LOSS ALTERS OBESITY-ASSOCIATED PATTERNS OF PLASMA PENTRAXIN3 AND SYSTEMIC INFLAMMATORY MARKERS

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Short Title: PTX3 and inflammation in severe obesity

GASTRIC BYPASS-INDUCED WEIGHT LOSS ALTERS OBESITY-ASSOCIATED PATTERNS OF PLASMA PENTRAXIN3 AND SYSTEMIC INFLAMMATORY MARKERS

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Abstract

Background: Systemic inflammation contributes to obesity-associated complications. The short-pentraxin C-reactive-protein (CRP) is a validated inflammatory marker, while long-pentraxin-3 (PTX3) limits inflammation and is adaptively stimulated by proinflammatory cytokines in vitro. Severely obese patients [SO:BMI>40] have highest obesity-associated complications and increasingly undergo surgical treatment. SO-associated changes in plasma PTX3 and their interactions with systemic inflammation are however unknown.

Objective: We sought to determine potential alterations in plasma PTX3 and their associations with changes in inflammatory markers prior to and following weight loss induced by laparoscopic Roux-en-Y gastric-bypass (LRYGB).

Setting: University Hospital in Trieste, Italy.

Methods: Plasma PTX3, CRP and cytokines including TNF-alpha and Interleukin-(IL)6 were measured in: 1) 24 individuals with severe, class III obesity (SO; age=42±1,F/M=18/6,BMI=45±1) before and 3,6,12 months after LRYGB; 2) age-, sex-matched normal-weight (L:n=56,BMI=22±0.2) or class I obese individuals (O:n=44,BMI=31.2±0.3).

Results: SO, but not O, had higher plasma PTX3 compared to L, associated with highest proinflammatory cytokines and CRP ($P<0.05$ vs L-MO). In all subjects, plasma IL6 and TNF-alpha were associated positively with PTX3 ($P<0.05$). Plasma CRP and proinflammatory cytokines declined during LRYGB-

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induced weight loss. In contrast, high PTX3 further increased and remained elevated ($P<0.05$ vs basal).

Conclusions: Obesity level and energy balance modulate interactions between PTX3 and systemic inflammation. Elevated PTX3 is a novel, potentially adaptive alteration associated with proinflammatory cytokines in SO. Their differential changes conversely suggest circulating PTX3 as a novel negative inflammatory marker in SO undergoing LRYGB -induced weight loss.

Keywords: obesity, LRYGB, Pentraxin3, inflammation

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Introduction

Obesity has a strong negative impact on morbidity and mortality, and severe, class III obesity [Body Mass Index (BMI) >40 kg/m2] is associated with highest disease-related complications rate and mortality rates [1-4]. Gastric bypass is increasingly performed as an effective treatment to achieve sustained weight loss and reduce cardiometabolic risks associated with severe obesity [4]. Importantly, obesity is also commonly associated with activated systemic inflammation, resulting in elevated circulating proinflammatory cytokines that include tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (IL-6). Proinflammatory changes in circulating cytokines are thought to contribute to obesity-associated morbidity by favoring the onset of insulin resistance and cardiovascular dysfunction [5-7]. Weight loss induced by caloric restriction or bariatric surgery may limit these alterations [7-12], and these effects may contribute to the positive effect of weight loss to reduce obesity-associated morbidity.

Pentraxins are acute phase reactants interacting with cytokines to modulate inflammation at both tissue and systemic levels [13], and both short and long components of the pentraxin family have been described [13]. The short pentraxin C-reactive protein (CRP) is secreted by the liver and its plasma concentration is a validated marker of systemic inflammation also in obese individuals [14.15]. On the other hand, the more recently-described long

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pentraxin3 (PTX3) may be synthesized by several cell types including adipocytes, monocytes and endothelial cells [13,16]. PTX3 has been interestingly reported to limit tissue damage and the inflammatory process in several disease models, including atherosclerosis, myocardial infarction, kidney injury and experimental carcinogenesis [17-20]. Proinflammatory cytokines have been conversely reported to positively modulate PTX3 production in adipocytes [21], and this observation suggests a potential adaptive role of PTX3 in limiting inflammation activation in adipose tissue [16,17,21,22]. Changes in circulating PTX3 in human obesity remain however controversial. Initial studies reported higher plasma PTX3 in obese than in normal weight individuals [23,24], but more recent general population reports suggest a negative impact of adiposity on plasma PTX3 [25-29]. Consistent with the latter findings, one study reported that weight loss induced by lifestyle changes enhances circulating PTX3 [29]. Importantly, the potential role of inflammatory mediators in modulating interactions between obesity and circulating PTX3 remains undefined. In addition, no studies are available on circulating PTX3 in severe, class III obesity, and its potential association with altered circulating cytokine profiles prior to and following surgically-induced weight loss in severely obese patients are unknown.

In the current study, we therefore investigated changes in plasma PTX3, CRP and pro- (TNF-alpha, IL-6, IL-1beta) and anti-inflammatory cytokines

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[interferon-gamma (IFN-gamma), IL-10] in individuals with severe class III obesity compared to class I obese or normal-weight individuals. We hypothesized that class III obesity would be associated with higher inflammation and higher circulating PTX3 compared to class I obesity and normal weight individuals. In addition, we investigated whether surgically-induced weight loss would cause further increments in plasma PTX3, thereby potentially disrupting this putative association.

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Materials and methods

Study Protocol and Study Population

The study protocol was approved by the Ethics Committee at Trieste University Hospital. All participants were given detailed oral and written information on study aims and risks, and they gave written consent before entering the study.

Class III obese group and LRYGB

A total of 24 patients with class III, severe obesity (SO; age 43±1 years, F/M=18/6) were recruited and underwent surgery at the General Surgery Division in Trieste University Hospital, and they were followed up at the Internal Medicine Obesity and Metabolic Syndrome outpatient Clinic. Patients who participated in all time points of follow-up were included for analyses and report. In all participants, clinical history was collected with complete physical examination. BMI was calculated as weight (in kilograms) divided by height (in meters) squared. Waist circumference was measured on bare skin during mid-respiration at the natural indentation between the 10th rib and the iliac crest to the nearest 0.5 cm. Inclusion criteria were those internationally accepted for LRYGB surgery, with key role for morbid obesity and BMI >40 kg/m^2. Exclusion criteria were those for LRYGB. In addition, patients with plasma creatinine >1.5 mg/dl were not included in this study since PTX3 has been reported to be associated with acute and chronic kidney disease [30]. No patient included in this study was taking estrogen therapy that could potentially alter

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inflammation, or had thyroid disease that could potentially alter energy balance and body weight. The diagnosis of thyroid disease was based on measurement of thyroid hormones and thyroid-stimulating hormone (TSH) during routine screening before LRYGB surgery, that led to the exclusion of one subject from the current study. Type 2 diabetes mellitus was diagnosed based on clinical history or plasma glucose concentration > 126 mg/dl or hemoglobin A1c > 6.5% during pre-surgery screening. Diabetes prevalence was 4 out of 24 patients in the severely obese group.

For patients undergoing LRYGB, one blood sample was collected 1-2 weeks before surgery under 10-h fasted conditions; plasma was stored at -80 °C until biochemical and hormonal measurements were performed. Additional measurements and sampling were taken under identical conditions at 3, 6 and 12 months after surgery.

The LRYGB procedure was performed laparoscopically in all subjects as previously described [31]. Briefly, a liver retractor was used to move the left lobe laterally and visualize the hiatus and the lesser curve. A 34-French orogastric tube was inserted by the anaesthesiologist to calibrate the future gastric pouch (50-70ml). The dissection began between the first and second vascular arcades on the lesser curvature and a laparoscopic linear stapler was fired. The first suture line was horizontal, the second line ran parallel to the

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oesophagus towards the angle of His. Once the gastric pouch was completely separated from the bypassed stomach, the omentum and the transverse mesocolon were lifted upwards and the ligament of Treitz was identified. The biliary limb was measured 100 cm distal to the ligament of Treitz and the alimentary limb was measured up to 120 cm. Gastro-entero anastomosis between gastric pouch and alimentary limb was peformed. Then, the jejunojejunostomy was created between the alimentary and biliary limbs. An intraoperative test with coloured water irrigation through a naso-gastric tube was routinely performed to verify gastro-jejunal anastomosis integrity.

Normal weight and class I obese control groups

Normal weight (N; n=56) and class I obese (O; n=44) individuals who did not undergo LRYGB were also recruited for the study as control groups. Class I obese individuals had not undertaken weight-reducing dietary treatments for at least 12 months prior to the study. They were weight-stable, defined as self-reported lack of weight changes greater than 3% of current body weight in the previous six months. In addition to sampling for hormone measurements for cross-sectional comparisons, they underwent a routine examination and biochemical work-up to assess their metabolic profile. No patients in these groups had plasma creatinine > 1.5 mg/dl, was taking estrogen therapy or had thyroid disease based on plasma thyroid hormones and TSH concentration. Class I obese individuals had BMI comparable with LRYGB patients at 12-

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month follow-up, but they were weight-stable and had not undergone bariatric surgery procedures. Pentraxin and cytokine profiles at the end of the 12-month follow-up period was therefore also compared with that of the class I obese control individuals alone, to assess the potential impact of LRYGB independently of major anthropometric and metabolic confounding variables.

Plasma analyses

Plasma glucose, total and high-density lipoprotein cholesterol, and plasma triglycerides were measured using standard methods. Plasma insulin was measured by enzyme-linked immunosorbent assay (Insulin Human Ultrasensitive ELISA; DRG Instruments, Marburg, Germany). Insulin sensitivity was calculated by the homeostatic model assessment (HOMA) [31] using the following formula: HOMA = (FPG x FPI)/22.5, where FPG and FPI are fasting plasma glucose (mmol) and fasting plasma insulin (mU/ml), respectively. Plasma PTX3 (Human Pentraxin3 /TSG-14ELISA System Perseus Proteomics Inc., Tokyo, Japan) [27] and C-reactive protein (CRP) (High sensitivity c-reactive protein, Diagnostics Biochem Canada Inc London, Ontario, Canada) were measured using commercially available ELISA kit. Plasma cytokines were measured with multiplex high-throughput xMAP technology, using analyte detection combinations commercialy available and validated by the producer (MILLIPLEX High Sensitivity Human Cytokine Magnetic Bead Panel, Millipore, Billerica, MA, USA).

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Statistical analysis

The StatView software was used for all statistical analyses. Time effects for each variable in the LRYGB group were analyzed using ANOVA and paired t-test. Differences between non- LRYGB and LRYGB individuals were analyzed using ANOVA and post-hoc tests for multiple comparisons. Linear and multiple regression analyses were used to determine associations between variables. Due to non-normal data distribution, log-transformed values for HOMA, PTX3, CRP and all cytokines were used for analyses. P values < 0.05 were considered statistically significant.

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Results

Basal

BMI, plasma insulin, glucose, HOMA index in N, O and SO (Table 1) – The three groups were comparable for age and sex. Before LRYGB, SO patients had highest BMI and waist circumference by design (Table 1). These alterations were associated with highest plasma insulin, glucose, and HOMA index compared to both normal weight and class I obese individuals (Table 1).

Plasma cytokines, CRP and PTX3 in N, O and SO (Figures 1,2) - IL-10 and IFNg were comparably lower ($P<0.05$) in O and SO than in Lean individuals (Figure 1a). In contrast, TNF-alpha and IL-6 were comparable in O and N groups while they were selectively higher in SO ($P<0.05$ vs Lean-MO) (Figure 1b). The

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TNFalpha-to-IL10 ratio consequently increased progressively from N to O to SO (all $P<0.05$) (Figure 1c). These changes were associated with stepwise increments from N to O to SO in the plasma concentration of pro-inflammatory short pentraxin CRP (all $P<0.05$). On the other hand, plasma PTX3 was comparable in N and O groups, while it was selectively higher in SO individuals ($P<0.05$ vs N-O) (Figure 2).

Associations between plasma PTX3, cytokines and clinical variables (Table 2, Figure 3) – In linear regression analysis in all subjects (n=124) PTX3 was positively associated with BMI, plasma insulin, HOMA index, CRP (Table 2) and proinflammatory IL6 and TNFalpha (Figure 3). In multiple regression analyses, plasma IL6 and TNFalpha remained positively associated with PTX3 in models including BMI and HOMA index (Table 2).

LRYGB

BMI and biochemical profile in SO prior to and following LRYGB (Table 3) - LRYGB expectedly led to a substantial, progressive reduction in BMI and waist circumference, with substantial excess BMI loss (EBMIL), total weight body loss (TWBL), excess weight loss (EWL). These changes were also expectedly associated with lower plasma glucose and plasma insulin, resulting in major reduction in HOMA index (Table 3). No early reductions were observed for plasma total cholesterol and triglycerides, whereas HDL cholesterol was lower 1 month after LRYGB than prior to LRYGB. Lowering of plasma triglycerides

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with higher HDL cholesterol was observed 6 and 12 months after LRYGB (Table 3). 12 months after LRYGB, patients had BMI, waist circumference, plasma, free fatty acids, and HOMA comparable to those in O individuals that had not undergone surgery (all P = not significant).

Plasma cytokines, CRP and PTX3 in SO following LRYGB (Table 3, Figures 4,5) - LRYGB did not alter plasma cytokine concentrations during the first six months of follow-up. At the 12-month follow-up, plasma TNF-alpha, IL-6, IL-1beta as well as IFNgamma were lower compared to basal values prior to LRYGB (Figure 4a-e). The TNFalpha-IL10 ratio was also lower at the 12-month follow-up compared to basal value ($P<0.05$, 12-month vs t=0) (Figure 4f). Plasma CRP underwent earlier, progressive reduction from the first follow-up evaluation ($P<0.05$) (Figure 5). In contrast, high plasma PTX3 observed prior to LRYGB further increased at the first follow-up and remained comparably elevated at subsequent measurements compared to basal value (Figure 5). No statistically significant associations were observed between changes in PTX3 over the 12-month follow-up and corresponding changes in BMI, CRP and cytokines (not shown). All results at all time points are from 24 patients enrolled in the study.

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Discussion

The current study provides novel information on the impact of severe obesity on plasma PTX3, and on its potential associations with inflammatory markers prior to and following gastric bypass-induced weight loss. In particular, we here demonstrated that: 1) associations between PTX3 and systemic inflammation are modulated by obesity level and changes in energy balance; 2) patients with severe obesity have high plasma PTX3 compared to both class I obese and normal weight individuals, associated with increasing pro-inflammatory cytokines; 3) LRYGB-induced weight loss profoundly alters pentraxin-cytokine patterns, with further increments in circulating PTX3 in the presence of lower inflammation activators and markers.

BASAL

Under basal conditions, circulating PTX3 was higher in severe, class III obesity than in both normal weight and class I obese individuals. This alteration was associated with higher circulating inflammatory markers, and plasma IL6 and TNFalpha were positive predictors of PTX3 in the whole study population. The current results therefore indicate that PTX3 may represent a marker of systemic inflammation in class III obese individuals. In vitro studies notably demonstrated a direct stimulatory effect of TNF-alpha on PTX3 production in adipocytes [21]. The current findings are therefore in agreement with the hypothesis that higher circulating pro-inflammatory cytokines directly

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contribute to higher circulating PTX3 in class III obese individuals, and this putative effect could be aimed at limiting inflammation activation at adipose tissue and systemic level [16-18]. It should be pointed out that these combined observations could contribute to explain existing controversial reports on the impact of obesity on circulating PTX3, since high or preserved circulating PTX3 were notably mostly reported in obese patients affected by chronic complications that commonly involve inflammation activation, such as metabolic syndrome and atherosclerosis [23] or diabetes [28]. Additional studies with simultaneous measurement of PTX3 and inflammatory markers in obese individuals with various levels of obesity-associated complications are needed to further confirm this interaction.

SURGERY

LRYGB-induced weight loss was associated with profoundly altered patterns of circulating pentraxins and cytokines. Early reduction in body mass lead to early lowering of CRP, although reductions in pro-inflammatory cytokines were only observed 6-12 months after surgery. At variance with CRP, high basal PTX3 further increased and remained elevated throughout the 12 month follow-up period. Surgically-induced weight loss is therefore associated with differential changes in plasma CRP, cytokines and PTX3 concentrations. At variance with pre-surgical conditions, the current findings indicate that PTX3 may become a

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clinically relevant negative marker of systemic inflammation in the increasing population of severely obese patients undergoing LRYGB.

Higher circulating PTX3 following LRYGB-induced weight loss is in good agreement with results from a general population cohort undergoing lifestyle-induced weight reduction [29], and these combined results are consistent with the concept that negative energy balance enhances circulating PTX3 in obese humans. The current study design does not allow to directly identify underlying mechanisms for this effect, but the time-course of PTX3 modifications suggests that different factors could have contributed at different time points. Although no statistically significant associations were observed between overall changes in circulating PTX3 and changes in body weight markers at one-year follow-up, the initial rapid, substantial weight loss could have facilitated early PTX3 increments through yet undefined mechanisms. Circulating pro-inflammatory cytokines were persistently elevated at early follow-up, and they could have played a relevant permissive role in the rise of plasma PTX3. Sustained PTX3 elevation despite declining pro-inflammatory cytokines at later stages of follow-up could have been mediated at least in part by increasing HDL-cholesterol and by the relative increase in anti-inflammatory cytokines, including IL-10. Both HDL-cholesterol and IL-10 were indeed previously reported to stimulate PTX3 production from non-adipose sources in experimental models [32,33], and they could have therefore prevented a decline

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in circulating PTX3 despite declining pro-inflammatory mediators. On the other hand, available knowledge does not support a role of lower plasma glucose and insulin resistance in LRYGB-induced PTX3 elevation, since insulin resistance and high plasma glucose may indeed increase circulating PTX3 in humans [34]. Further studies are needed to confirm these hypotheses and to identify potential additional mechanisms underlying PTX3 elevation during surgically-induced negative energy balance and weight loss.

The LRYGB-induced decline in biomarkers of systemic inflammation is well-established in available literature [9-12]. Our results identify a time-course of changes in pro- and anti-inflammatory cytokines with potentially relevant pathophysiological implications. In particular, as stated above lack of early decline in pro-inflammatory cytokines could have played a permissive role in early increments in circulating PTX3. Importantly, cytokine profiles at 12-month follow-up were superimposable to those in matched individuals with class I obesity who had not undergone bariatric surgery, thereby confirming the effectiveness of LRYGB in normalizing systemic inflammatory responses.

Overall, the observed results provide novel information on the impact of severe obesity and LRYGB on circulating PTX3, its interactions with systemic inflammation and potential underlying regulatory mechanisms. It should be pointed out that the study sample does not allow to directly extend these

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findings to potentially important patient groups, including older people and people with type 2 diabetes. Additional studies will be necessary to directly confirm the current conclusions under these clinical conditions.

Conclusion: the current study demonstrated that interactions between PTX3 and systemic inflammation are modulated by obesity level and changes in energy balance. High plasma PTX3 is a novel, potentially adaptive alteration associated with elevated proinflammatory cytokines in SO. Opposite changes in PTX3 and systemic inflammation suggest circulating PTX3 as a novel negative inflammation marker in severely obese individuals undergoing LRYGB -induced weight loss.

Conflict of Interest: The authors declare no conflict of interests

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References

[1] Flegal KM, Kit BK, Orpana H, Graubard BI. Association of all-cause mortality with overweight and obesity using standard body mass index categories: a systematic review and meta-analysis. *JAMA* 2013;309:71-82.

[2] Executive summary: Guidelines (2013) for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the Obesity Society published by the Obesity Society and American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Based on a systematic review from the The Obesity Expert Panel, 2013. *Obesity* 2014;Suppl 2:S5-S39.

[3] Carr DB, Utzschneider KM, Hull RL, et al. Intra-abdominal fat is a major determinant of the National Cholesterol Education Program Adult Treatment Panel III criteria for the metabolic syndrome. *Diabetes* 2004;53:2087-2094.

[4] Sjöström L, Lindroos AK, Peltonen M, et al; Swedish Obese Subjects Study Scientific Group. Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. N Engl J Med 2004;351:2683-2693

[5] Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444:860-867

Accepted manuscript

ACCEPTED MANUSCRIPT

[6] Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 1995;95:2111–2119.

[7] Kopp HP, Kopp CW, Festa A, et al. Impact of weight loss on inflammatory proteins and their association with the insulin resistance syndrome in morbidly obese patients. Arterioscler Thromb Vasc Biol 2003;23:1042-1047.

[8] Richard C, Couture P, Desroches S, Lamarche B. Effect of the Mediterranean diet with and without weight loss on markers of inflammation in men with metabolic syndrome. Obesity 2013;21:51-57.

[9] Holdstock C, Lind L, Engstrom BE, et al. CRP reduction following gastric bypass surgery is most pronounced in insulin-sensitive subjects. Int J Obes 2005;29:1275-1280

[10] Viana EC, Araujo-Dasilio KL, Miguel GP, et al. Gastric bypass and sleeve gastrectomy: the same impact on IL-6 and TNF-α. Prospective clinical trial. Obes Surg 2013;23:1252-1261

[11] Arismendi E, Rivas E, Agustí A, et al. The systemic inflammome of severe obesity before and after bariatric surgery. PLoS One 2014;9:e107859.

Accepted manuscript

ACCEPTED MANUSCRIPT

[12] Dalmas E, Venteclef N, Caer C, et al. T cell-derived IL-22 amplifies IL-1β-driven inflammation in human adipose tissue: relevance to obesity and type 2 diabetes. Diabetes 2014;63:1966-1977.

[13] Garlanda C, Bottazzi B, Moalli F, Deban L, Molla F, Latini R, Mantovani A. Pentraxins and atherosclerosis: the role of PTX3. Curr Pharm Des 2011;17:38–46.

[14] Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest 2003;111:1805-1812.

[15] Emerging Risk Factors Collaboration, Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet 2010;375:132-140.

[16] Norata GD, Garlanda C, Catapano AL. The long pentraxin PTX3: a modulator of the immunoinflammatory response in atherosclerosis and cardiovascular diseases. Trends Cardiovasc Med 2010;20:35-40

[17] Salio M, Chimenti S, De Angelis N, et al. Cardioprotective function of the long pentraxin PTX3 in acute myocardial infarction. Circulation 2008;117:1055–1064

[18] Norata GD, Marchesi P, Pulakazhi Venu VK, et al. Deficiency of the long pentraxin PTX3 promotes vascular inflammation and atherosclerosis. Circulation 2009;120:699–708

Accepted manuscript

ACCEPTED MANUSCRIPT

[19] Lech M, Römmele C, Gröbmayr R, et al. Endogenous and exogenous pentraxin-3 limits postischemic acute and chronic kidney injury. Kidney Int 2013;83:647-661.

[20] Bonavita E, Gentile S, Rubino M, et al. PTX3 is an extrinsic oncosuppressor regulating complement-dependent inflammation in cancer. Cell 2015;160:700-714

[21] Abderrahim-Ferkoune A, Bezy O, Chiellini C, Maffei M, et al. Characterization of the long pentraxin PTX3 as a TNFalpha-induced secreted protein of adipose cells. J Lipid Res 2003;44:994-1000.

[22] Alberti L, Gilardini L, Zulian A, et al. Expression of long pentraxin PTX3 in human adipose tissue and its relation with cardiovascular risk factors. Atherosclerosis 2009;202:455-460

[23] Zanetti M, Bosutti A, Ferreira C, et al. Circulating pentraxin 3 levels are higher in metabolic syndrome with subclinical atherosclerosis: evidence for association with atherogenic lipid profile. Clin Exp Med 2009;9:243-248

[24] Miyaki A, Maeda S, Yoshizawa M, et al. Is pentraxin 3 involved in obesity-induced decrease in arterial distensibility? J Atheroscler Thromb 2010;17:278-284.

[25] Ogawa T, Kawano Y, Imamura T, et al. Reciprocal contribution of pentraxin 3 and C-reactive protein to obesity and metabolic syndrome. Obesity 2010; 18:1871-1874

Accepted manuscript

ACCEPTED MANUSCRIPT

[26] Osorio-Conles O, Guitart M, Chacón MR, et al. Plasma PTX3 protein levels inversely correlate with insulin secretion and obesity, whereas visceral adipose tissue PTX3 gene expression is increased in obesity. Am J Physiol Endocrinol Metab 2011;301:E1254-E1261.

[27] Barazzoni R, Aleksova A, Carriere C, et al. Obesity and high waist circumference are associated with low circulating pentraxin-3 in acute coronary syndrome. Cardiovasc Diabetol 2013;12:167.

[28] Miyazaki T, Chiuve S, Sacks FM, Ridker PM, Libby P, Aikawa M. Plasma pentraxin 3 levels do not predict coronary events but reflect metabolic disorders in patients with coronary artery disease in the CARE trial. PLoS One 2014;9:e94073

[29] Witasp A, Carrero JJ, Michaëlsson K, et al. Inflammatory biomarker pentraxin 3 (PTX3) in relation to obesity, body fat depots and weight loss. Obesity 2014;22:1373-1379.

[30] Witasp A, Ryden M, Carrero JJ, et al. Elevated circulating levels and tissue expression of pentraxin 3 in uremia: a reflection of endothelial dysfunction. PLoS One 2013;8:e63493.

[31] Barazzoni R, Zanetti M, Nagliati C, et al. Gastric bypass does not normalize obesity-related changes in ghrelin profile and leads to higher acylated ghrelin fraction. Obesity 2013;21:718-722.

Accepted manuscript

ACCEPTED MANUSCRIPT

[32] Norata GD, Marchesi P, Pirillo A, et al Long pentraxin 3, a key component of innate immunity, is modulated by high-density lipoproteins in endothelial cells. Arterioscler Thromb Vasc Biol 2008;28:925-931

[33] Doni A, Michela M, Bottazzi B, et al. Regulation of PTX3, a key component of humoral innate immunity in human dendritic cells: stimulation by IL-10 and inhibition by IFN-gamma. J Leukoc Bio. 2006;79:797-802

[34] Todoric J, Handisurya A, Knapp B, Tura A, Pacini G, Kautzky-Willer A. Relationship of pentraxin 3 with insulin sensitivity in gestational diabetes. Eur J Clin Invest 2013;43:341-349.

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Figure Legend:

Figure 1: a) Plasma interferon-gamma (IFNg) and Interleukin-10 (IL10); b) plasma Interleukin-1beta (IL1beta), Interleukin-6 (IL6) and TNF-alpha; c) plasma TNF-alpha to IL10 ratio in Normal weight, Class I Obese and Class III Obese groups. *: P<0.05 vs Normal weight, **: P<0.05 vs Normal weight and Class I Obese by ANOVA and Wilcoxon test.

Figure 2: a) Plasma C-reactive Protein (CRP); b) plasma Pentraxin-3 (PTX3) in Normal weight, Class I Obese and Class III Obese groups. *: P<0.05 vs Normal weight, **: P<0.05 vs Normal weight and Class I Obese by ANOVA and Wilcoxon test.

Figure 3: Correlations between plasma PTX3 and plasma TNF-alpha or IL-6 in the three study groups (n=124).

Figure 4: Changes in plasma (a) Interferon-gamma (IFN-gamma), (b) Interleukin-10 (IL10), (c) Interlleukin-1beta (IL1beta), d) Interleukin-6 (IL6), e) TNF-alpha and f) TNF-alpha to IL10 ratio over 12 months of observation in severely obese patients undergoing gastric bypass (LRYGB) surgery (n=24 at all time points), compared to moderately obese patients not treated with LRYGB (Ob-Non LRYGB). *: P<0.05 vs 0 by Paired t-test (0 vs follow-up time points) or ANOVA and post-hoc tests (0 vs Class I Obese). No statistically significant differences were observed for any variable between 0 and 3-month or between 0 and 6-month follow-up by Paired t-test. No statistically significant differences were also observed for any

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variable between 12-month and Class I Obese – No-LRYGB by ANOVA and post-hoc tests.

Figure 5: Changes in plasma a) CRP and b) PTX3 over 12 months of observation in severely obese patients undergoing gastric bypass surgery (LRYGB) (n=24 at all time points), compared to Class I Obese patients not treated with LRYGB (Class I Obese – No-LRYGB). *: P<0.05 vs 0 and Class I Obese; **: P<0.05 vs 0, 3-month, 12-month; ***; P<0.05 vs 0, 3-month, 6-month, Class I Obese by Paired t-test (0 vs follow-up time points) or ANOVA and post-hoc tests (0 vs Class I Obese).

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Fig 1

Figure 1

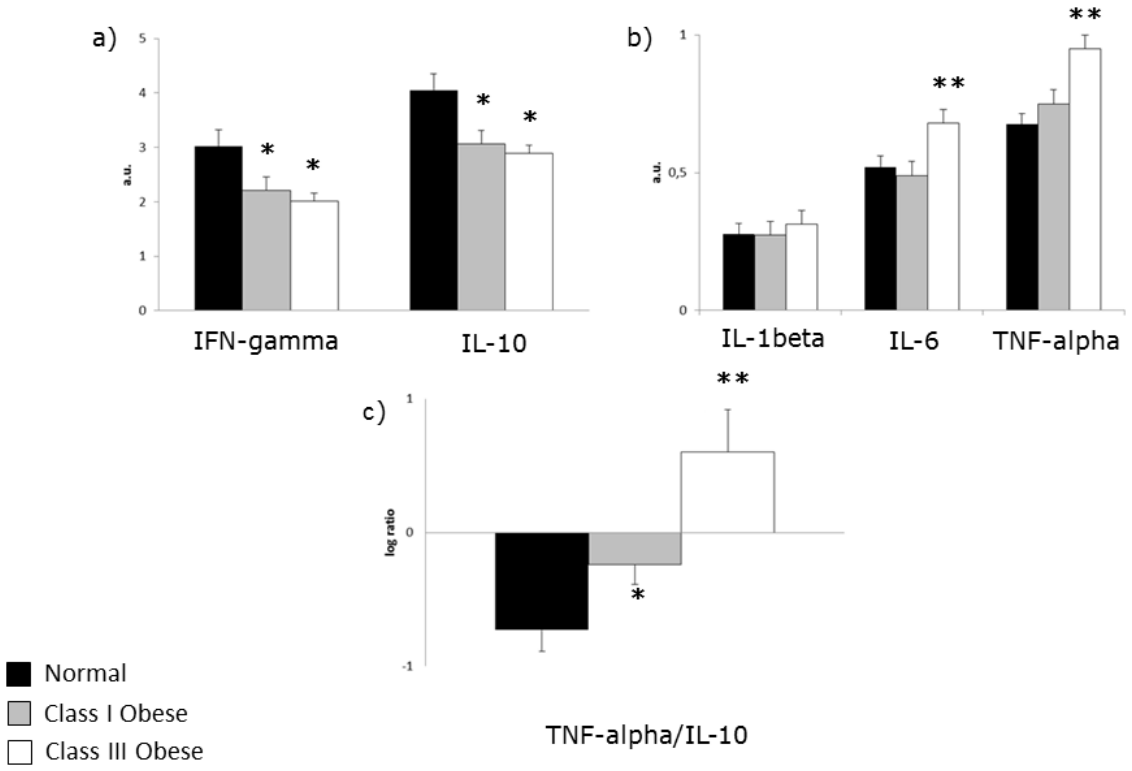

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Fig 2

Figure 2

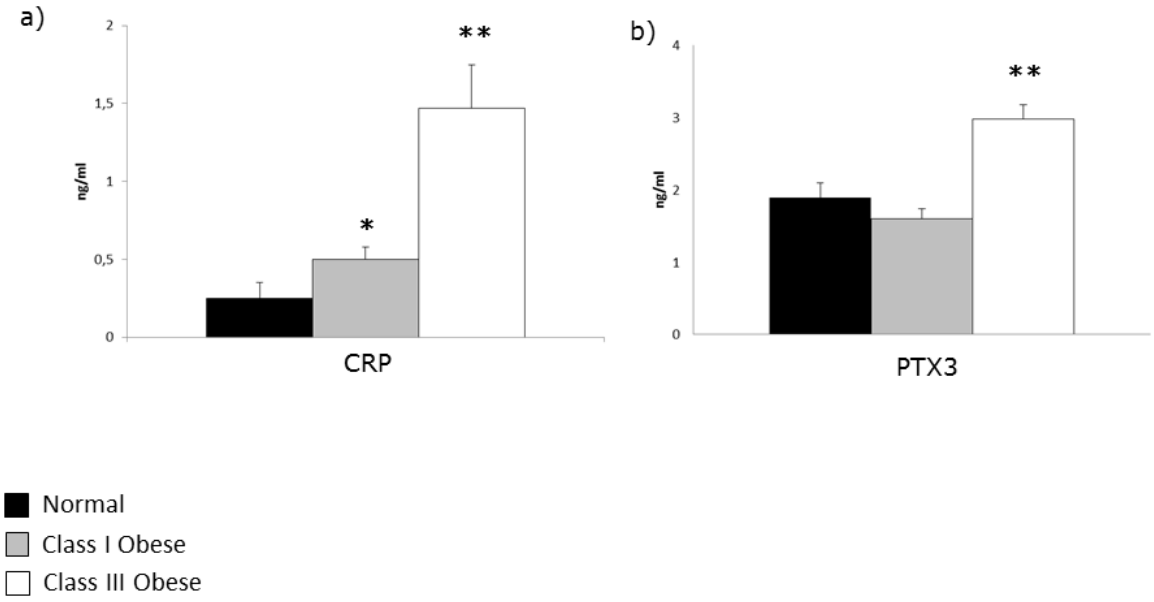

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Fig 3

Figure 3

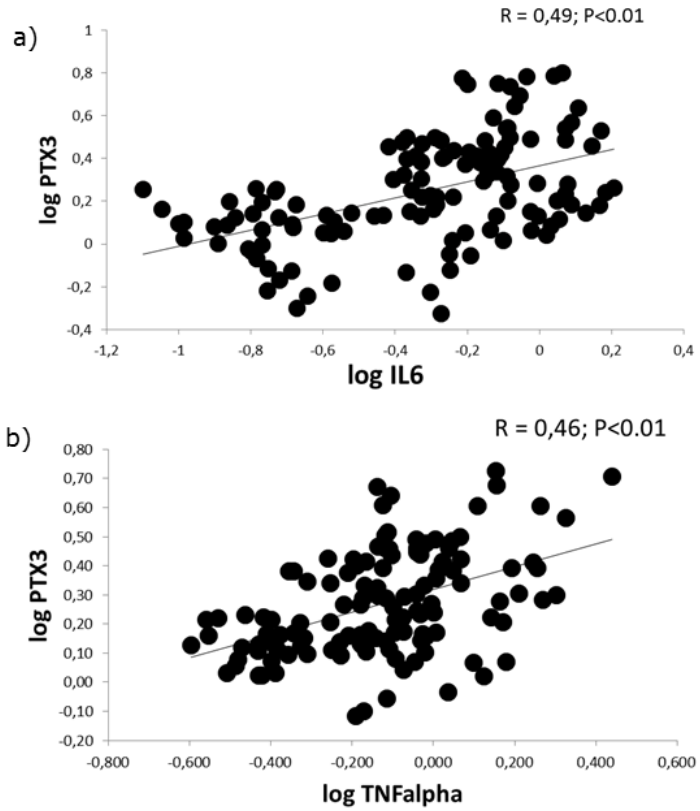

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Fig 4

Figure 4

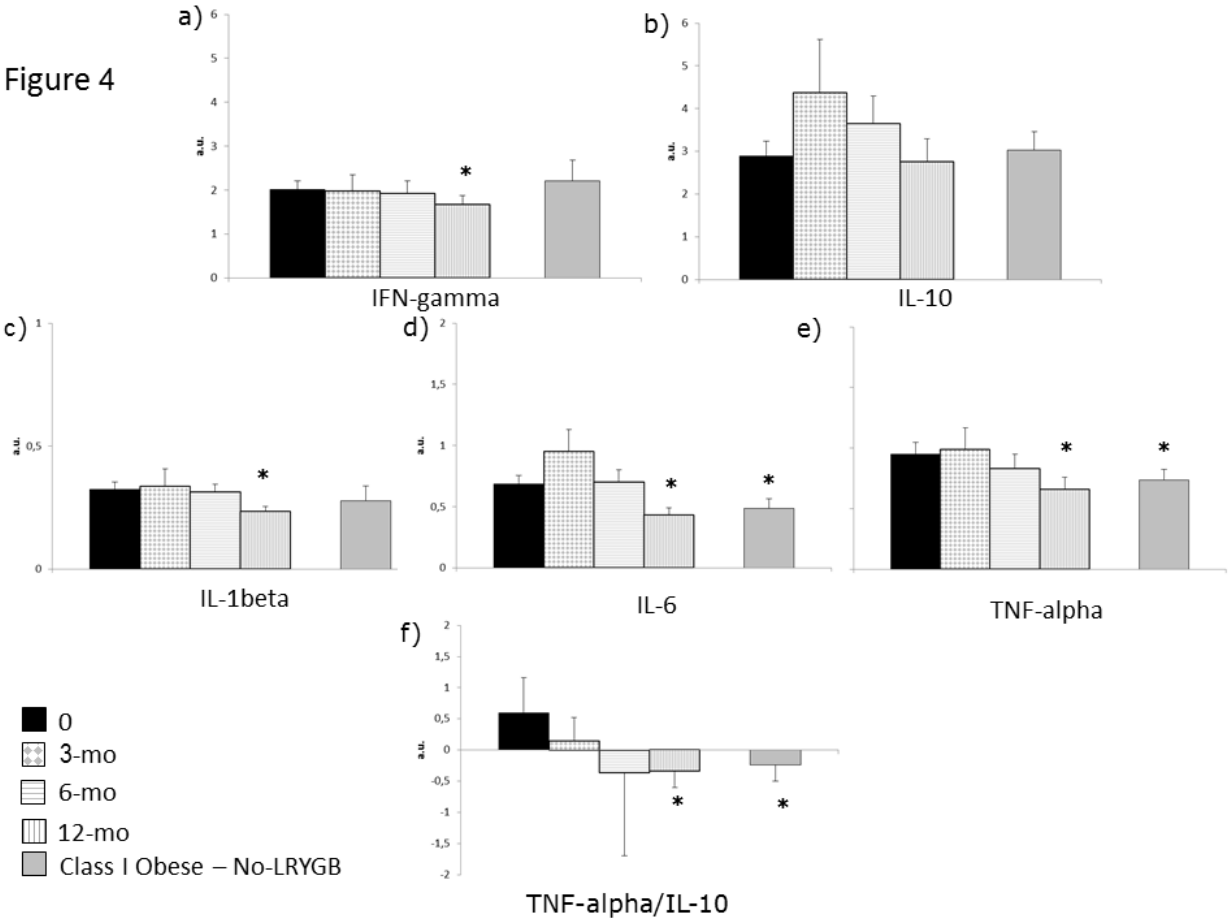

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Fig 5

Figure 5

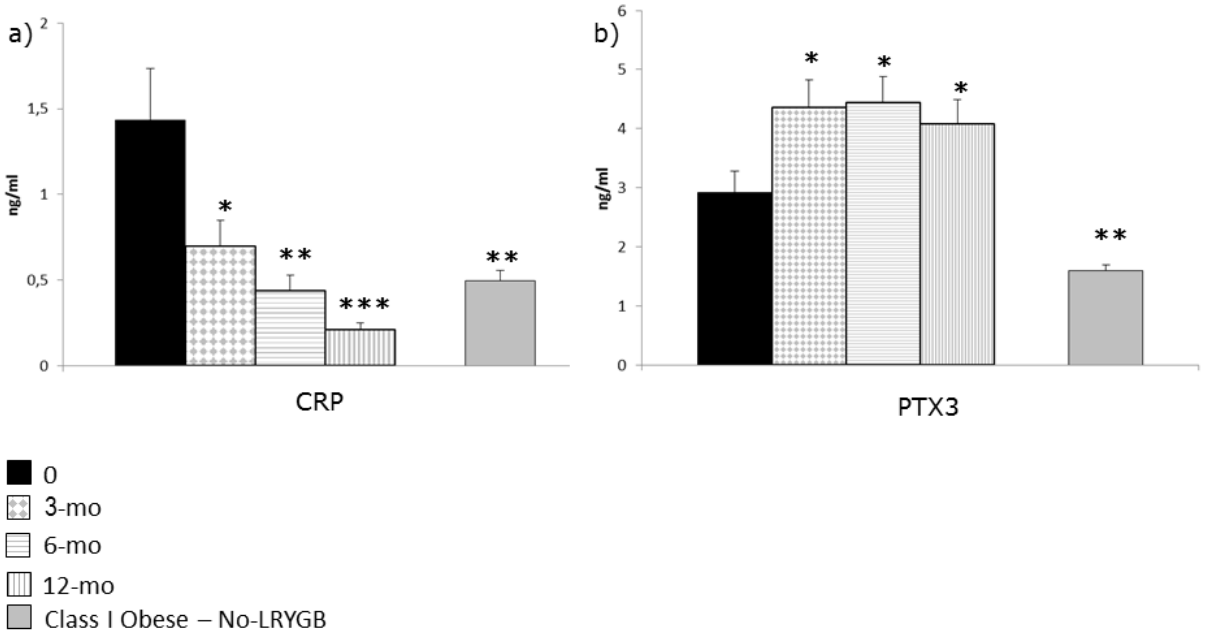

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Table 1: Gender, age, body mass index (BMI), waist circumference (WC), plasma total and HDL-cholesterol (Chol), plasma triglycerides, systolic (SBP) and diastolic (DBP) blood pressure, plasma glucose and insulin, homeostasis model assessment of insulin resistance (HOMA-IR) in the Normal weight (Normal), Class I Obese (Obese) and Class III Obese patient groups. In each line, *: P<0.05 vs Normal; **: P<0.05 vs Normal and Obese by ANOVA and post-hoc tests.

	Normal	**Obese**	**Class III Obese**
Gender (M/F)	38/18	30/14	18/6
Age (years)	43±1	44±1	43±1
BMI (kg/m^2)	22±0.2	31.2±0.3 *	43.1±0.1 **
WC (cm)	80±1	99±1 *	139±2 **
Total-Chol (mg/dl)	190±5	212±6 *	214±5 *
HDL-Chol (mg/dl)	52±1	47±1 *	48±1 *
Triglycerides (mg/dl)	76±3	134±8 *	164±8 **
SBP (mmHg)	126±2	128±2	134±2*
DBP (mmHg)	78±1	85±1*	90±1**
Glucose (mMol)	87±1	92±1*	103±3**
Insulin (µU/ml)	5.1±0.2	6.6±0.3 *	20.5±1.4 **
HOMA-IR	1.01±0.04	1.47±0.07 *	5.41±0.44 **

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Table 2 – Linear and multiple regression analyses

a) Linear regression analysis between PTX3 as dependent variable and age, Body Mass Index (BMI), waist circumference (WC), plasma Total and HDL-cholesterol (Chol), triglycerides, systolic (SBP) and diastolic (DBP) blood pressure, plasma glucose, insulin, HOMA index, Interferon gamma (IFNgamma), Interleukin (IL)10, IL1beta, TNFalpha to IL10 ratio, and plasma C-reactive protein (CRP) in all individuals (n=124).

b) Multiple regression analyses (t-value) between PTX3 (dependent variable) and each inflammatory marker in a model also including BMI and HOMA index, in all individuals (n=124). Similar results were observed when plasma insulin was used instead of HOMA index (not shown).

*: P<0.05; **: P<0.01.

a)	**PTX3**
	r
Age (years)	0.109
BMI (kg/m^2)	0.342*
WC (cm)	-0.168
Total-Chol (mg/dl)	-0.158
HDL-Chol (mg/dl)	0.075
Triglycerides (mg/dl)	-0.128
SBP (mmHg)	-0.181
DBP (mmHg)	-0.138
Glucose (mg/dl)	0.087
Insulin (µU/ml)	0.373**
HOMA	0.315*
IFNgamma (pg/ml)	0.160
IL10 (pg/ml)	0.111
IL1beta (pg/ml)	0.147
TNFalpha-IL10 ratio	0.150
CRP (mg/L)	0.352*

b)	**PTX3**
	t
IL6 (pg/ml)	2.161*
TNFalpha (pg/ml)	1.893*
CRP (mg/L)	0.051

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Table 3: Body mass index (BMI), excess BMI loss (EBMIL), total weight body loss (TWBL), excess weight loss (EWL), waist circumference (WC), plasma glucose and insulin, homeostasis model assessment of insulin resistance (HOMA-IR), plasma total and HDL cholesterol (Chol), triglycerides (Tg) and systolic (SBP) and diastolic (DBP) blood pressure in Class III Obese individuals before LRYGB (0) and during a 12-month follow-up period with evaluation at 3,6,12 months from surgery (3-month, 6-month, 12-month; n=24 at all time points).

$ Denotes a statistically significant decline (P<0.05) for the corresponding variable from 0 to 12 months by ANOVA. *: P<0.05 vs 0; **: P<0.05 vs 0 and 3-month; ***: P<0.05 vs 0, 3-month and 6-month by paired t-test.

	0 month	3-month	6-month	12-month
BMI (kg/m^2)*	43.1±0.1	37.2±1.1 *	33.2±1.2 **	30.9±1.2 ***
EBMIL (kg/m^2)*	0.0±0.0	44.3±2.1 *	64.3±3.1 **	79.0±3.0 ***
TBWL (%)*	0.0±0.0	19.0±0.8 *	27.5±1.0 **	34.6±1.1 ***
EWL (%)*	0.0±0.0	43.5±2.2 *	63.6±3.2 **	79.0±3.1 ***
WC (cm)*	139±2	117±2 *	109±3 **	100±3 ***
Glucose (mMol)*	101±3	89±2 *	85±1**	86±2 **
Insulin (μU/ml)*	20.9±1.5	8.8±1.1 *	7±0.7 **	5.8±0.4 **
HOMA-IR*	5.41±0.44	1.96±0.25 *	1.50±0.16 **	1.26±0.1 ***
Total-Chol (mg/dl)	211±5	185±8 *	184±9 *	192±11 *
HDL-Chol (mg/dl)	48±1	44±1 *	49±1	53±1 ***

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Triglycerides (mg/dl)*	151±7	124±9 *	100±9 **	82±6 ***	
SBP (mmHg)*	134±2	131±3	126±3 **	125±3 **	
DBP (mmHg)*	90±1	88±3	82±2 **	83±2 **	

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