Transcriptional Activation of Apolipoprotein CIII Expression by Glucose May Contribute to Diabetic Dyslipidemia


Objective—Hypertriglyceridemia and fatty liver are common in patients with type 2 diabetes, but the factors connecting alterations in glucose metabolism with plasma and liver lipid metabolism remain unclear. Apolipoprotein CIII (apoCIII), a regulator of hepatic and plasma triglyceride metabolism, is elevated in type 2 diabetes. In this study, we analyzed whether apoCIII is affected by altered glucose metabolism.

Methods and Results—Liver-specific insulin receptor–deficient mice display lower hepatic apoCIII mRNA levels than controls, suggesting that factors other than insulin regulate apoCIII in vivo. Glucose induces apoCIII transcription in primary rat hepatocytes and immortalized human hepatocytes via a mechanism involving the transcription factors carbohydrate response element–binding protein and hepatocyte nuclear factor-4α. ApoCIII induction by glucose is blunted by treatment with agonists of farnesoid X receptor and peroxisome proliferator-activated receptor-α but not liver X receptor, ie, nuclear receptors controlling triglyceride metabolism. Moreover, in obese humans, plasma apoCIII protein correlates more closely with plasma fasting glucose and glucose excursion after oral glucose load than with insulin.

Conclusion—Glucose induces apoCIII transcription, which may represent a mechanism linking hyperglycemia, hypertriglyceridemia, and cardiovascular disease in type 2 diabetes. (Arterioscler Thromb Vasc Biol. 2011;31:513-519.)

Key Words: apolipoproteins ■ lipids ■ metabolism ■ nuclear receptors ■ type II diabetes

Type 2 diabetes is a progressive disease that is due to increased insulin resistance and progressive pancreatic failure. Type 2 diabetic patients often display lipid metabolism abnormalities (namely hypertriglyceridemia; low high-density lipoprotein–cholesterol levels; and increased small, dense low-density lipoprotein particles) that result in increased cardiovascular disease risk. Epidemiological studies identified hypertriglyceridemia as an independent risk factor for atherosclerosis. The hypertriglyceridemia is due to hepatic overproduction of triglyceride-rich very-low-density lipoprotein particles, as well as impaired intravascular catabolism as a result of decreased lipoprotein lipase activity.

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Apolipoprotein CIII (apoCIII) is a 79-amino-acid glycoprotein synthesized in the liver and the intestine that controls triglyceride metabolism in humans and mice. ApoCIII is a component of triglyceride-rich lipoproteins, low-density lipoprotein, and high-density lipoprotein. Plasma triglyceride and apoCIII concentrations positively correlate in normo- and hypertriglyceridemic subjects. ApoCIII gene deficiency results in a hypotriglyceridemia because of an accelerated catabolism of triglyceride-rich lipoproteins, whereas apo-CIII overexpression in mice leads to hypertriglyceridemia. In vitro and in vivo studies have established that apoCIII delays the catabolism of triglyceride-rich lipoproteins by inhibiting lipoprotein lipase and inhibits the hepatic uptake of triglyceride-rich remnants. Moreover, the rate of hepatic apoCIII production correlates with the level of very-low-density lipoprotein–triglyceride production. Recently, 2 variant alleles of the apoCIII gene have been
associated with the development of nonalcoholic fatty liver disease and insulin resistance, suggesting a role in intrahepatic fat metabolism. ApoCIII is also an independent predictor of cardiovascular disease and longevity. ApoCIII levels in diabetic patients correlate with atherosclerosis, and apoCIII exerts direct proatherogenic activities in vascular cells. In line with this, a null mutation in human apoCIII gene correlates with lower coronary artery calcification scores. Plasma apoCIII levels are pharmacologically modulated by fibrates and statins, which lower triglycerides in type 2 diabetics. Plasma free fatty acids stimulate apoCIII production in humans, whereas insulin inhibits apoCIII expression in diabetic mice. However, the effects of glucose signaling on apoCIII have not yet been assessed.

Glucose modulates the transcription of genes involved in glycolysis and lipogenesis in the liver, including liver pyruvate kinase (LPK) and fatty acid synthase (FAS), key enzymes of the glycolytic and lipogenic pathways. Promoter analysis has led to the identification of glucose-response DNA-regulatory sequences, called carbohydrate response elements, composed of 2 consensus 6-base-pair E-box motifs separated by 5 nucleotides. Different transcription factors bind to carbohydrate response elements, but glucose responsiveness appears mediated by the carbohydrate response element–binding protein (ChREBP). However, other transcription factors also participate in this response, including the nuclear receptors hepatocyte nuclear factor-4α (HNF-4α) and liver X receptors (LXRs).

Here, we studied the regulation of apoCIII gene expression by glucose using liver-specific insulin receptor–deficient (LIR-KO) mice, rat primary hepatocytes, and immortalized human hepatocytes (IHH). In addition, studies were performed in obese humans to assess the correlation between plasma apoCIII protein levels and glucose homeostasis.

Methods

Animal Experiment

Three- to 5-month-old male LIR-KO mice and their littermates were euthanized either nonfasted, after 24 hours fasting, or after 24 hours fasting and 6 hours refeeding a high-carbohydrate diet (Harlan Teklad 88122) at 2:30 pm. Livers were snap frozen in liquid nitrogen.

Primary Rat Hepatocytes and IHH Experiments

Primary rat hepatocyte isolation and cell culture is described in the Supplemental Information, available online at http://atvb.ahajournals.org. IHH were incubated in low glucose medium supplemented with 10 mmol/L lactate or glucose with or without GW4064 (5 μmol/L), ciprofibrate (100 μmol/L), or T0901317 (1 μmol/L) for 24 hours. Each experiment was performed at least 3 times, each time with independent triplicates. Results from a representative experiment are shown.

ApoCIII Protein Measurement

Ninety-six-well plates were coated with rabbit anti-serum, the wells were saturated with bovine serum albumin, and caloribators or supernatants were incubated in these antibody-coated wells. Goat anti-rat apoCIII IgGs (provided by PJ Dolphin, Halifax, Nova Scotia, Canada) were incubated overnight at 4 °C, and then horseradish peroxidase–conjugated rabbit anti-goat immunoglobulins were incubated for 2 hours at 37 °C. The plates were read at 450 nm using the SpectraMax 384plate reader (Molecular Devices). The total protein amount was determined by the Peterson method.

Transient Transfection Assays

Primary rat hepatocytes were transfected using JetPEI (Qiogene, Illkirch, France) in glucose-free Dulbecco’s modified Eagle’s medium for 16 hours with the indicated pGL3-luciferase constructs and the Renilla reporter plasmid pRL-Null (Promega, Madison, WI). After transfection, cells were treated for 24 hours in glucose-free Dulbecco’s modified Eagle’s medium containing the indicated concentration of glucose and insulin.

Hepatic ApoCIII mRNA Is Lower in LIR-KO Mice

Because hepatic apoCIII mRNA is inhibited by insulin, we analyzed the impact of impaired liver insulin signaling on apoCIII expression in vivo using LIR-KO mice. Surprisingly, apoCIII mRNA levels were lower in LIR-KO compared with wild-type mice under all dietary conditions (Figure 1A). mRNA levels of the glycolytic enzyme LPK, which is controlled by glucose, increased to a lesser extent in LIR-KO, indicating that LIR-KO mice have a blunted glucose response, likely because of a lack of insulin-induced glucokinase activity (Figure 1B). These data indicate that factors other than hepatic insulin signaling also contribute to apoCIII regulation in vivo.

Glucose Induces ApoCIII Transcription in Primary Rat Hepatocytes

To test the hypothesis that glucose regulates apoCIII gene expression, primary rat hepatocytes were incubated with high or low glucose concentrations. Glucose increased apoCIII protein secretion (Figure 2A), as well as apoCIII mRNA, in a time- (Figure 2B) and concentration-dependent manner (Figure 2C). Pretreatment of primary rat hepatocytes with actinomycin D, a RNA polymerase II inhibitor, abolished the glucose-induced increase of apoCIII mRNA levels (Figure 2D), suggesting a transcriptional mechanism.

Transient transfection experiments in primary rat hepatocytes using the −1200/+24 human apoCIII promoter, containing the hepatic enhancers driving apoCIII expression in human and rodent livers, revealed an increase of apoCIII promoter activity in response to glucose (Figure 2E). Because glucose activates transcription via E-boxes, computer-
assisted analysis (Matinspector, Genomatix) was used, and E-box sequences conserved in the rat and human promoters were found in the distal −1050 and proximal C3P regions (Supplemental Figure IA and IB). Interestingly, glucose induced the binding of a complex only to the C3P probe (Supplemental Figure IC). Transient transfection experiments using mutated C3P site constructs showed that the C3P region confers glucose responsiveness (Figure 2F). Thus, glucose induces apoCIII transcription in primary rat hepatocytes via the C3P region of the apoCIII gene promoter.

**Glucose Induces ApoCIII in IHH**

To investigate the regulation of apoCIII by glucose in human cells, IHH were studied.36 Interestingly and unlike other human hepatocyte cell lines, such as the HepG2 hepatoma cell line, IHH cells respond to glucose, as illustrated by the induction of LPK gene expression in a glucose-dose-dependent manner (data not shown). Incubation of IHH cells with glucose induced apoCIII mRNA (Figure 3A) in a time- (Figure 3B) and dose-dependent (Figure 3C) manner. This increase of apoCIII mRNA level correlated with an elevated very-low-density lipoprotein secretion as shown by the increase of triglyceride (Supplemental Figure IIA) and apolipoprotein B secretion (Supplemental Figure IIB). The glucose-mediated apoCIII induction was abolished by actinomycin D preincubation (Figure 3A). Transient transfection experiments showed that the activity of the −1200/+24 apoCIII human promoter increased after glucose stimulation in IHH cells (Figure 3D). Moreover, the activity of the wild-type, but not the mutated C3P footprint, was also induced by glucose (Figure 3E).

**HNF-4α and ChREBP But Not LXRs Mediate Glucose Induction of ApoCIII Expression**

The C3P locus is a complex site that contains a direct repeat spaced by 1 nucleotide, a direct repeat spaced by 2 nucleotides, and 2 E-boxes (E-box) (Supplemental Figure IB). The 2 direct repeat sites bind transcription factors of the nuclear
receptor family, HNF-4α, which binds to the direct repeat spaced by 1 nucleotide site of the C3P footprint, is involved in the glucose-regulated expression of genes such as those for LPK and FAS. To investigate whether HNF-4α is implicated in the induction of apoCIII gene expression by glucose, siRNA experiments were performed in IHH cells, and the expression of LPK and apoCIII was subsequently analyzed in conditions of low and high glucose concentrations. HNF-4α siRNA transfection reduced its basal and glucose-induced expression (Supplemental Figure IIIA). Interestingly, LPK and apoCIII mRNA induction by glucose was lower on HNF-4α knockdown, suggesting that HNF-4α is at least partially implicated in the induction of apoCIII gene expression by glucose (Figure 4A).

SiRNA knockdown of ChREBP, a glucose-responsive transcription factor binding to E-boxes (Supplemental Figure IIIB) attenuated glucose induction of the LPK and apoCIII genes (Figure 4B).

By contrast, siRNA knockdown of LXRα and LXRβ (Supplemental Figure IIIC), also implicated in the response to glucose, did not influence apoCIII induction by glucose, whereas the induction of a classical LXR target gene, ABCG8, by the LXR agonist T0901317 was diminished (Supplemental Figure IIID).

ApocIII Induction by Glucose Can Be Pharmacologically Modulated

Because apoCIII expression is regulated by nuclear receptor agonists, we investigated whether their activation inhibits glucose induction of apoCIII expression. IHH cells were incubated in medium containing low or high glucose concentrations in presence of either dimethyl sulfoxide (vehicle), the farnesoid X receptor (FXR) agonist GW4064, the peroxisome proliferator-activated receptor-α (PPARα) agonist ciprofibrate, or the LXR agonist T0901317. Glucose induction of apoCIII gene expression was partially abolished by GW4064 (Figure 5A) and ciprofibrate (Figure 5B) treatment, whereas LXR agonist treatment did not have an effect (Figure 5C).

ApoCIII Protein Correlates Positively With Fasting Plasma Glucose and Plasma Glucose Excursion After a Glucose Bolus in Overweight Subjects, Independently of Gender, Age, and Body Mass Index

To determine whether apoCIII is also modulated by parameters of glucose homeostasis in humans, plasma apoCIII protein levels were measured in drug-naive overweight subjects and correlated with different metabolic parameters related to glucose metabolism. After controlling for gender, age, and body mass index, plasma apoCIII protein levels correlated positively with fasting plasma glucose (r=0.28; P=0.007) and glucose excursion after a glucose bolus.
Moreover, impaired LPK induction after high-carbohydrate refeeding. and show decreased glucose signaling, as illustrated by an lower in livers of LIR-KO mice that do not respond to insulin transcription factors ChREBP and HNF-4

expression is induced in rat and apoCIII-deficient mice exhibit an aggravated diet-induced insulin resistance. Moreover, apoCIII expression is downregulated by insulin. However, a direct link between apoCIII and glucose had not been demonstrated until now. Our results showing that apoCIII gene expression is regulated by glucose via a transcriptional mechanism provide a mechanistic explanation for the recently raised hypothesis that a low-fat, high-carbohydrate diet could lead to an overproduction of apoCIII protein in humans. This regulation occurs via the C3P footprint in the apoCIII promoter, which is different from the insulin response site, with a structural organization closely resembling the organization of the LPK promoter. Moreover, the mechanism by which glucose affects apoCIII transcription implies the activation of the same transcription factors as LPK, eg, ChREBP and HNF-4α. ChREBP mediates glucose induction of key glycolytic and lipogenic enzymes. The nuclear receptor HNF-4α is a partner of the glucose response complex on the LPK promoter, necessary for an optimal glucose responsiveness of the FAS gene. Because of its interaction with SREBP-1c and ChREBP, HNF-4α functions as a scaffold protein to assemble the transcriptional machinery required for insulin and glucose responsiveness.

Intriguingly, apoCIII gene expression is regulated in an opposite manner by insulin (negatively) and glucose (positively). Most genes in the glycolytic (LPK) and lipogenic (fatty acid synthase, acetyl-coenzyme A carboxylase) pathways are regulated positively both by insulin and glucose. It is tempting to speculate that glucose induction of apoCIII expression may contribute to a lower lipolysis, and resulting in a lower fatty acid uptake by peripheral tissues and thus contributing to an increase in peripheral glucose handling as alternative substrate for energy metabolism. The observation that apoCIII-deficient mice exhibit an aggravated insulin resistance is in line with this hypothesis. However, chronic elevation of apoCIII will increase plasma lipids and consequently the risk of atherosclerosis development. We speculate that in insulin resistance associated with hyperglycemia, as in type 2 diabetes, insulin no longer represses apoCIII expression, whereas chronic glucose elevation enhances its expression, leading to an overproduction of apoCIII, resulting in an increased risk for atherosclerosis, via either hypertriglyceridermia or direct vascular effects of apoCIII.

Table. Correlation of Plasma ApoCIII Protein Levels With Metabolic Parameters of Glucose Homeostasis in Drug-Naive Overweight Patients

<table>
<thead>
<tr>
<th>Metabolic Parameter</th>
<th>Correlation Coefficient (Spearman Rank)</th>
<th>P Value</th>
<th>Sample No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma fasting glucose (mg/dL)</td>
<td>0.280**</td>
<td>0.007</td>
<td>91</td>
</tr>
<tr>
<td>Plasma glucose excursion (area under the curve) after a glucose bolus</td>
<td>0.461**</td>
<td>0.000</td>
<td>81</td>
</tr>
<tr>
<td>Plasma fasting insulin (μU/mL)</td>
<td>0.159</td>
<td>0.136</td>
<td>90</td>
</tr>
<tr>
<td>Plasma insulin excursion (area under the curve) after a glucose bolus</td>
<td>0.158</td>
<td>0.165</td>
<td>79</td>
</tr>
<tr>
<td>Plasma fasting C-peptide (nmol/L)</td>
<td>0.193</td>
<td>0.067</td>
<td>91</td>
</tr>
</tbody>
</table>

Shown are correlations between plasma apoCIII protein level and the indicated metabolic parameters in overweight patients (n=91) using the Spearman statistical test.

**P<0.01.
**ApoCIII** gene expression is downregulated at the transcriptional level by several nuclear receptors. FXR, PPARα, and Rev-erbα inhibit apoCIII gene expression. Interest-ingly, the induction of apoCIII gene expression by glucose can be inhibited pharmacologically by activators of the nuclear receptors PPARα and FXR but not by LXR agonists. FXR represses basal apoCIII expression by displacing HNF-4α from its site upstream of the proximal region in the enhancer region. PPARα binds to the C3P footprint and inhibits apoCIII transcription by displacing HNF-4α, as well as by inhibiting forkhead box O 1 activation of apoCIII transcription via the insulin-responsive element. PPARα-mediated inhibition of glucose-induced apoCIII expression could thus be a result of a combination of molecular mechanisms.

In overweight subjects, plasma apoCIII protein levels were positively correlated with fasting glucose and glucose excursion, but not insulin and insulin excursion. These observations suggest, although they do not prove, that apoCIII levels are predominantly under control of glucose but not insulin.

In summary, our results show that apoCIII gene expression is increased by glucose stimulation. These data may provide a mechanism involved in the development of diabetic hypertriglyceridemia. Interference with glucose-induced apoCIII expression may constitute an additional mechanism to correct atherogenic dyslipidemia.

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**Disclosures**

None.

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SUPPLEMENT MATERIAL

METHODS

Isolation of primary rat hepatocytes and cell culture
Primary hepatocytes were isolated from male Wistar rats using the collagenase perfusion method(1), plated in collagen-coated plates and incubated at 37°C for 4-6h using serum-free William’s E medium (Invitrogen, Cergy-Pontoise, France) supplemented with 2mM gentamicin, 25µg/ml glutamin, 0.1% fatty acid-free BSA (Bovine Serum Albumin) and 100nM dexamethasone. Medium was then changed to glucose-free Dulbecco’s modified Eagle’s medium (DMEM, Invitrogen) supplemented with gentamicin, glutamin, dexamethasone, 10mM lactate and 5.5mM glucose. After 16h of incubation, the medium was replaced for 24h by glucose-free DMEM containing dexamethasone and different concentrations of lactate and glucose. For the actinomycinD experiment, cells were incubated with 5µg/ml of actinomycinD for 90min and then treated with glucose as above.

RNA extraction and analysis
Total RNA from primary rat hepatocytes and IHH cells was extracted with Trizol reagent (Invitrogen). Reverse transcription was performed using MMLV reverse transcriptase (Invitrogen) on 1µg of total RNA. Quantitative PCR were performed on a MX4000 apparatus (Stratagene, Amsterdam, The Netherlands) by using hybridisation probes (TaqMan) in duplex and SYBRGreen.

Plasmid cloning and site directed mutagenesis
The human -1200/+24 apoCIII promoter was previously described (2). Three copies of the C3P site were cloned in the pGL3-TK luciferase vector to obtain the pGL3-TK-C3P-apoCIII. The
mutations were introduced in the C3P region (5’-ATCTCCACTGGTCAGCAGGTGACCTTTGCCCA-3’) by using the QuickChange Site-Directed mutagenesis Kit (Stratagene). The C3P mutant sequences were 5’-ATCTCCACTGGTCAGCAGGTGACCTT-3’ for primary rat hepatocytes and 5’-ATCTCCACTGGTCAGCAGGTGAaggTTTGCagA-3’ for IHH. The LPK promoter plasmid was previously described(3).

Serum triglyceride, apoCIII and apoB protein and total protein measurements

Serum apoCIII protein levels were analyzed by automated immunoturbidimetric assay (K-ASSAY, cat. No. KAI-006, Kamiya Biochemical Compagny, Seattle, WA98168, USA) in a Konelab 601 analyzer (Thermo Electron Oy, Vantaa, Finland). Triglycerides were quantified using the TG PAP 1000 kit (BioMérieux, Marcy l’Etoile, France) and apoB using an ELISA kit (MABTECH AB, Nacka Stand, Sweden).

Nuclear extract preparation and EMSA

Primary rat hepatocytes were cultured and nuclear extracts (NE) were prepared as described and gel shift assays were performed as previously described except that nuclear extracts were used and that incubations and gel electrophoresis were done at 4°C to inhibit nuclear phosphatase activity(4).

Statistical analysis of human study

Statistical calculations were carried out using the statistical package SPSS version 16.0 (SPSS, Chicago, IL, USA). Values are expressed as mean±S.D.. (range). Normality of the variables was verified with a Kolmogorov-Smirnov test. As most of the variables were not normally
distributed, and were not normalized after log or square root transformation, Spearman’s rank correlations were used. Partial correlations were calculated to control for confounding factors.

FIGURE LEGENDS

**Sup. Figure I** : Glucose induces the binding of a protein complex on the C3P region of the apoCIII promoter in primary rat hepatocytes.

(A) Schematic representation of the human apoCIII gene promoter (-1200/+24) indicating the two regions containing E-boxes, the “-1050” region and the “C3P” region. (B) Schematic representation of the C3P footprint of the human apoCIII gene promoter indicating the potential binding sites for transcription factors. (C) Glucose increases the binding of a protein complex to the C3P footprint in gel shift assay. L=low glucose ; H=high glucose.

**Sup. Figure II** : Glucose increases the VLDL secretion in Immortalized Human Hepatocytes (IHH). Triglycerides (A) and apolipoprotein B (apoB) (B) secreted by IHH cells after 24h of incubation with low (white) and high (black) glucose concentrations. Secreted triglycerides and apoB were normalized to total intracellular protein.

**Sup. Figure III** : Expression of HNF-4α, ChREBP and LXR after gene silencing.

Effect of HNF-4α (A), ChREBP (B) and LXRα&β (C&D) gene silencing respectively on HNF-4α (A), ChREBP (B), LXRα&β (C) and ABCG8 and ApoCIII (D) gene expression in transfected IHH. IHH cells were incubated in a medium containing low (open bars) or high (solid bars) glucose concentrations for 24h before HNF-4α, ChREBP and LXRα&β gene expression analysis and in medium containing DMSO (open bars) or the LXRs agonist T0901317 (hatched
bars) before analysis of ABCG8 gene expression. mRNA levels were measured by real-time quantitative PCR.

Sup. Figure IV : Plasma ApoCIII protein levels correlate positively with plasma fasting glucose and plasma glucose excursion after a glucose bolus in obese patients.

The scatterplots were made using SPSS software. Each dot represents a patient. The slope of the line illustrates the association between plasma fasting glucose (A) or plasma glucose excursion (AUC) after a glucose bolus (B) and plasma apoCIII protein level.

Sup. Table I : Characteristics of the human patient cohort.

The data are presented as mean±SD for normally distributed variables or as median when the distribution of a variable is skewed.

REFERENCES


Sup. Fig I. Caron S et al.

A

-1200 -1050 +24

tcagCATCTGgaagaCAGGGGccct
E boxes

-1050
atctCCACTG CCACTG E boxes

C3P

C3P

L H

Glucose (mM)

B

DR-1

ATCTCCACTGGTCAGGTGACCTTTTGCCCA
E Box

TAGMCCGTCCAGTOGGCCTGTGAACCGGT

DR-2

C3P
Sup. Fig II. Caron S et al.
Sup. Fig III. Caron S et al.

A. HNF-4 mRNA levels with siRNA treatment.

B. ChREBP mRNA levels with siRNA treatment.

C. LXRα mRNA levels with siRNA treatment.

D. ABCG8 mRNA levels with siRNA treatment.

E. ApoCIII mRNA levels with siRNA treatment.
Sup. Fig IV. Caron S et al.

A

Plasma fasting glucose (mg/dl)

Plasma apoCIII protein level (mg/dl)

B

Plasma glucose excursion (AUC) after a glucose bolus (arbitrary unit)
<table>
<thead>
<tr>
<th>Sample number</th>
<th>Mean±SD / Median</th>
<th>Range</th>
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</thead>
<tbody>
<tr>
<td>Male/female (%)</td>
<td>91</td>
<td>27.5 / 72.5</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>91</td>
<td>46±13</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>91</td>
<td>37.1±6.7</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>90</td>
<td>112.0±13.0</td>
</tr>
<tr>
<td>Visceral adipose tissue (cm²)</td>
<td>87</td>
<td>198±88</td>
</tr>
<tr>
<td>Syst BP (mmHg)</td>
<td>88</td>
<td>133±16</td>
</tr>
<tr>
<td>Diast BP (mmHg)</td>
<td>88</td>
<td>78±12</td>
</tr>
<tr>
<td>HDL-chol (mg/dl)</td>
<td>91</td>
<td>53±16</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>89</td>
<td>152±90</td>
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<tr>
<td>HbA1C (%)</td>
<td>86</td>
<td>5.6</td>
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<tr>
<td>Plasma fasting glucose (mg/dl)</td>
<td>91</td>
<td>83</td>
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<tr>
<td>Plasma glucose excursion (AUC) after a glucose bolus (arbitrary unit)</td>
<td>81</td>
<td>24572±5986</td>
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<td>Plasma fasting insulin (µU/ml)</td>
<td>90</td>
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<td>79</td>
<td>13446±6885</td>
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<tr>
<td>Plasma fasting c-peptide (nmol/l)</td>
<td>91</td>
<td>1.05±0.41</td>
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