Atherosclerosis 210 (2010) 536–541

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Changes in Cytokine Levels During Acute Hyperinsulinemia in Offspring of Type 2 Diabetic Subjects

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ARTICLE INFO

Article history:
Received 14 May 2009
Received in revised form
20 November 2009
Accepted 23 November 2009
Available online 2 December 2009

Keywords:
Offspring of type 2 diabetic patients
type 2 diabetes
cytokines
hyperinsulinemia

ABSTRACT

Objective: To investigate the changes in the levels of cytokines and adhesion molecules in response to acute hyperinsulinemia in the offspring of type 2 diabetic subjects.
Methods: Forty healthy offspring of type 2 diabetic subjects and 19 control offspring of healthy parents were included in the study. Twenty offspring had normal glucose tolerance (NGT) and twenty offspring impaired glucose tolerance (IGT). Insulin sensitivity was determined by the hyperinsulinemic euglycemic clamp and insulin secretion with the intravenous glucose tolerance test. The levels of cytokines and adhesion molecules were measured before and at the end of the clamp.
Results: Acute hyperinsulinemia induced by the euglycemic hyperinsulinemic clamp reduced the levels of TNF-α, IL-8, IL-10 and IL-18 in healthy controls but not in the offspring of type 2 diabetic subjects having NGT or IGT. In response to insulin, levels of hs-CRP decreased and levels of IL-6 increased significantly in all study groups. The levels of adhesion molecules (ICAM-1, VCAM-1, E-Selectin) remained unchanged in response to hyperinsulinemia.
Conclusions: The suppression of cytokine levels (particularly proinflammatory cytokines) during acute hyperinsulinemia observed in healthy controls was not present in offspring of type 2 diabetic patients. This emphasizes the crucial role of low-grade inflammation in insulin resistance in subjects with high risk of developing diabetes.

1. Introduction

Insulin resistance or hyperinsulinemia predicts the development of type 2 diabetes [1] and cardiovascular disease [2], independently of other risk factors. We have previously shown that offspring of type 2 diabetic patients are insulin resistant and characterized by increased levels of markers of low-grade inflammation [3]. In contrast, the levels of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and E-Selectin were not increased in the offspring of type 2 diabetic subjects [4].

Previous studies suggest that markers of low-grade inflammation are associated with insulin resistance [3,5]. However, these studies are based on samples drawn in the fasting state. To our knowledge, there is only one previous study reporting the effects of acute hyperinsulinemia on the levels of interleukin 8 (IL-8) [6], whereas the information on other cytokines remains unclear, although previous studies have addressed the changes in expression of genes regulating cytokines [7]. Furthermore, it is not clear whether hyperinsulinemia modulates the levels of adhesion molecules during acute hyperinsulinemia in the offspring of subjects with type 2 diabetes. Therefore, we performed detailed metabolic studies in healthy nondiabetic offspring of type 2 diabetic subjects and measured the levels of proinflammatory cytokines and adhesion molecules in the fasting state and during acute hyperinsulinemia induced by the hyperinsulinemic euglycemic clamp.

2. Methods

Forty healthy nondiabetic offspring of patients with type 2 diabetes (men/women 19/21, age 36.6 ± 6.6 years, body mass index [BMI] 28.1 ± 6.1 kg/m^2) were included in the study. The control group consisted of 19 healthy normoglycemic subjects with no family history of type 2 diabetes (men / women 8/11, age 34 ± 4.5 years,

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doi:10.1016/j.atherosclerosis.2009.11.036

BMI 24.6 ± 2.6 kg/m^2). Probands (men or women) were randomly selected from type 2 diabetic subjects living in the region of Kuopio. The spouses of the patients with type 2 diabetes had NGT in an OGTT. The exclusion criteria for the selection of the offspring were: 1) diabetes mellitus or any other disease or drug treatment that could potentially disturb carbohydrate metabolism; 2) diabetes mellitus in both parents; 3) pregnancy; 4) age less than 25 or more than 50 years. The Ethics Committee of the University of Kuopio approved the study protocol. All study subjects gave informed consent.

Blood pressure was measured in the sitting position with a mercury sphygmomanometer after a 5 min rest. The average of 3 measurements was used to calculate systolic and diastolic blood pressure. Height and weight were measured to the nearest 0.5 cm and 0.1 kg, respectively. BMI was calculated as weight in kilograms divided by height in meters squared. Waist (at the midpoint between the lateral iliac crest and the lowest rib) and hip (at the level of the trochanter major) circumferences were measured to the nearest 0.5 cm. Initial blood samples were drawn after 12 hours of fasting, and an oral glucose tolerance test (OGTT) using 75 g of glucose was performed**.** On the second day, after a 12-hour fast, an intravenous glucose tolerance test (IVGTT) and the hyperinsulinemic euglycemic clamp were performed.

An IVGTT was performed to determine the first-phase insulin release after an overnight fast. After baseline blood collection, a bolus of glucose (300 mg/kg in a 50% solution) was infused over 30 seconds into the antecubital vein. Samples for the measurement of blood glucose and plasma insulin (arterialized venous blood) were drawn at –5, 0, 2, 4, 6, 8 and 10 min. Immediately after the IVGTT, the euglycemic hyperinsulinemic clamp (insulin infusion rate of 240 pmol/ min / m^2 body surface area) was started to determine the degree of insulin sensitivity, as previously described [8]. Blood glucose was clamped at 5.0 mmol/l for the next 120 minutes using an infusion of 20% glucose, with rate adjusted according to blood glucose measurements performed at 5-minute intervals. The mean amount of glucose infused during the final hour was used to calculate the rate of whole-body glucose uptake (WBGU). Blood samples for cytokine measurements were drawn before the clamp and at the end of the clamp.

Blood and plasma glucose levels were measured by the glucose oxidase method (Glucose & Lactate Analyzer 2300 Stat PLUS, Yellow Springs Instruments Co, Inc)**,** and the levels of plasma insulin by radioimmunoassay (Phadeseph Insulin RIA 100, Pharmacia Biotech, Uppsala, Sweden). Plasma concentrations of cytokines (tumor necrosis factor-α [TNF-α], IL-1β, IL-1 receptor antagonist [IL-1Ra], IL-6, IL-8, IL-10 and IL-18) were measured by solid phase ELISA (Quantikine, R&D Systems) and IL-8 by Ultrasensitive ELISA (BioSource International), as previously described [3]. The levels of C-reactive protein (hs-CRP) were determined by an Immulite 2000 High Sensitivity CRP assay (Diagnostic Products Corp). The levels of soluble adhesion molecules (s-ICAM-1, s-VCAM-1 and sE-Selectin) were measured by high-sensitivity assay kits from R&D Systems.

All data analyses were performed with the SPSS 14.0 for Windows programs. The results for continuous variables are given as means ± SD, if not stated otherwise. Variables with skewed distributions (hs-CRP, TNF-α, IL-1Ra, IL-6, IL-8 and IL-10) were logarithmically transformed for statistical analyses. The differences between the three groups were assessed by one-way ANOVA for continuous variables and by the χ^2 test for noncontinuous variables. A linear mixed model was applied to test the differences between the groups to adjust for confounding factors. Pedigree membership was included into the model as a random factor**,** and BMI and smoking as cofactors. Correlations were calculated by Spearman correlation analysis. Power calculations were performed using Russ Lenth's power analysis software: http://www.stat.uiowa.edu/~rlenth/Power/index.html.

3. Results

Clinical and biochemical characteristics of the offspring of type 2 diabetic patients and control subjects are given in Table 1. Twenty subjects had normal glucose tolerance (NGT) and 20 impaired glucose tolerance (IGT), as assessed by an OGTT. The groups were comparable with respect to gender, but differed significantly with respect to age (Control 34.5 ± 4.5, NGT 34.6 ± 6.1, IGT 38.6 ± 6.6 years, P < 0.05), and tended to differ with respect to BMI (24.6 ± 2.6, 28.2 ± 6.1, 28.0 ± 6.2 kg/m^2, P = 0.064). In addition, the offspring in the NGT and IGT groups were markedly insulin resistant (P = 0.001 between the three groups), with significantly higher plasma insulin levels at 120 min in the OGTT (P = 0.014 between the three groups). The NGT and IGT groups also had a tendency towards higher systolic blood pressure levels than did the control group (Control 124 ± 10, NGT 133 ± 13, IGT 133 ± 18 mmHg, P = 0.06). No difference in the first-phase insulin release between the study groups was found.

Spearman correlations among the cytokine levels in the fasting state and during hyperinsulinemia are shown in Table 2. The NGT and IGT groups were pooled together in statistical analysis. Fasting cytokine levels correlated positively with the levels of cytokines during hyperinsulinemia. Furthermore, hs-CRP levels showed a positive correlation with the levels of IL-6 and IL-1Ra, both in the fasting state and during hyperinsulinemia**.** Similarly, a positive correlation was found between the levels of IL-1Ra and IL-18, both in the fasting state (r = 0.330, P < 0.05) and during hyperinsulinemia (r = 0.421, P < 0.01). TNF-α levels were associated with IL-8 (r = 0.351, P < 0.05) levels only during hyperinsulinemia.

Table 1
Characteristics of the study groups.

Offspring of patients with type 2 diabetes	Control n = 19	NGT n = 20	IGT n = 20	P
Gender (M/F)	8/11	11/9	8/12	0.603
Age (years)	34.5 ± 4.5	34.6 ± 6.1	38.6 ± 6.6*	0.048
Body mass index (kg/m2)	24.6 ± 2.6	28.2 ± 6.1	28.0 ± 6.2	0.064
Waist to Hip ratio	0.84 ± 0.1	0.90 ± 0.10	0.88 ± 0.08	0.062
Systolic blood pressure (mmHg)	124 ± 10	133 ± 13	133 ± 18	0.060
Diastolic blood pressure (mmHg)	82 ± 10	87 ± 9	90 ± 14	0.070
Fasting plasma glucose (mmol/L)	5.1 ± 0.6	5.3 ± 0.4	5.2 ± 0.5	0.486
120 min plasma glucose (mmol/L)	5.6 ± 1.1	6.4 ± 0.7**	8.7 ± 0.8***	< 0.001
Fasting plasma insulin (pmol/L)	46.9 ± 23.0	52.4 ± 24.5	57.9 ± 34.4	0.474
120 min plasma insulin (pmol/l)	106.98 ± 24.6	238.92 ± 53.4*	261.72 ± 58.5**	0.014
WBGU (μmol/kg/min)	70.1 ± 27.9	50.1 ± 15.6*	45.9 ± 11.1**	0.001
First-phase insulin release (pmol/lxmin)	1897.0 ± 1207.4	2636.6 ± 2339.0	1830.5 ± 1273.2	0.338

Data are mean ± SD. NGT/IGT vs controls *P < 0.05, **P < 0.01, ***P < 0.001 (P value was calculated if P value for ANOVA was < 0.05). NGT = normal glucose tolerance, IGT = impaired glucose tolerance, WBGU = rates of whole body glucose uptake.