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Responsiveness of the innate immune system and glucose concentrations in the oldest old

Carolien A. Wijsman · Simon P. Mooijaart · Rudi G. J. Westendorp · Andrea B. Maier

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Abstract Patients with diabetes mellitus show increased risk of infectious disease as well as disturbances in innate immunity. In critical care settings, hyperglycemia is associated with increased risk of sepsis. It is unclear whether elevated glucose concentrations and innate immunity are associated in a non-clinical setting. We aimed to assess the association between glucose concentrations and innate immune response in the oldest old, who are at increased risk of both disturbed glucose metabolism as well as infectious disease. This study was part of the Leiden 85-plus Study. In 562 subjects aged 85 years old of the general population, venous blood samples were taken for measurement of morning glucose, C-reactive protein (CRP) and glycated hemoglobin (HbA1c). The innate immune response was assessed by performing ex vivo whole blood lipopolysaccharide (LPS) stimulation for production capacity of tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), interleukin 1-beta (IL1-β), interleukin 10 (IL-10) and interleukin 1 receptor antagonist (IL-1Ra). Using linear regression analysis, cross-sectional analysis between glucose and cytokine production capacity was performed. We found a significant negative association between glucose concentrations, but not HbA1c, and cytokine response capacity in four out of five measured cytokines (all $p<0.05$). Both glucose and HbA1c were positively associated with circulating levels of CRP. Higher glucose concentrations in non-diabetic elderly are associated with lower innate immune response. As elderly show increased vulnerability for disturbances in glucose metabolism as well as infectious disease, this relation could be of clinical significance.

Keywords Aging · Glucose · Cytokine response · Innate immunity

C. A. Wijsman (✉) · S. P. Mooijaart · R. G. J. Westendorp · A. B. Maier
Department of Gerontology and Geriatrics,
Leiden University Medical Center,
P.O. Box 9600, 2300 RC Leiden, The Netherlands
e-mail: c.a.wijsman@lumc.nl

R. G. J. Westendorp · A. B. Maier
Netherlands Consortium of Healthy Ageing,
Leiden University Medical Center,
P.O. Box 9600, 2300 RC Leiden, The Netherlands

Introduction

Patients with diabetes mellitus have a higher vulnerability for infectious diseases, which contributes to increased hospitalization and mortality (Shah and Hux 2003). It has been hypothesized that elevated glucose concentrations negatively influence cytokine production and neutrophil function (Andreasen et al. 2010; Delamaire et al. 1997). In vitro studies testing the effect of different glucose concentrations on innate immunity have yielded conflicting results (reviewed by Geerlings and Hoepelman 1999). In healthy young subjects, induction of hyperglycemia and lipopoly-

saccharide (LPS) stimulation in vivo did not attenuate the cytokine response (Krogh-Madsen et al. 2004). In the critical care setting, detrimental effects of hyperglycemia have been shown in non-diabetic patients, where stress-induced hyperglycemia is related to higher risk of sepsis and increased morbidity and mortality (Van den Berghe et al. 2001)

In the oldest old both infectious disease and disturbances of glucose homeostasis are highly prevalent. In the present study we tested the relationship between innate immunity and glucose concentrations in non-acutely diseased community-dwelling 85-year-olds without diabetes mellitus.

Methods

The Leiden 85-plus Study is a prospective, observational, population-based follow-up study of all inhabitants aged 85 years in Leiden, The Netherlands. Subjects were enrolled between 1997 and 1999. All participants were aged 85 years. There were no exclusion criteria. Of the 705 eligible subjects, 599 subjects were willing to participate (87%). At baseline, non-fasted venous blood samples were obtained in the morning (before 11:00 AM) of all participants. Ex vivo whole blood LPS stimulation was performed in 562 subjects. Levels of tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), interleukin 1-beta (IL1-β), interleukin 10 (IL-10) and interleukin 1 receptor antagonist (IL-1Ra) were determined 24 h after incubation with LPS, as more extensively described earlier (Van der Linden et al. 1998). Non-fasted plasma glucose concentrations, glycated hemoglobin (HbA1c) and C-reactive protein (CRP) levels were measured in 559 subjects. Subjects with known diabetes (n=52) or hyperglycemia (non-fasted glucose >11 mmol/l, n=39) were excluded from analysis. Analyses were done using linear regression with adjustment for gender using SPSS version 16.0.

Results

Figure 1 shows the relation between quartiles of glucose and cytokine levels in response to LPS-stimulation in 468 participants. After adjustment for gender, concentrations of non-fasted glucose correlated negatively with TNF-α, IL-6, IL-1β, and IL-10 (all p for trend <0.05), but not with IL-1Ra (p for trend= 0.73). Additionally excluding the subjects with glycated hemoglobin >6.5% (n=20) did not alter results

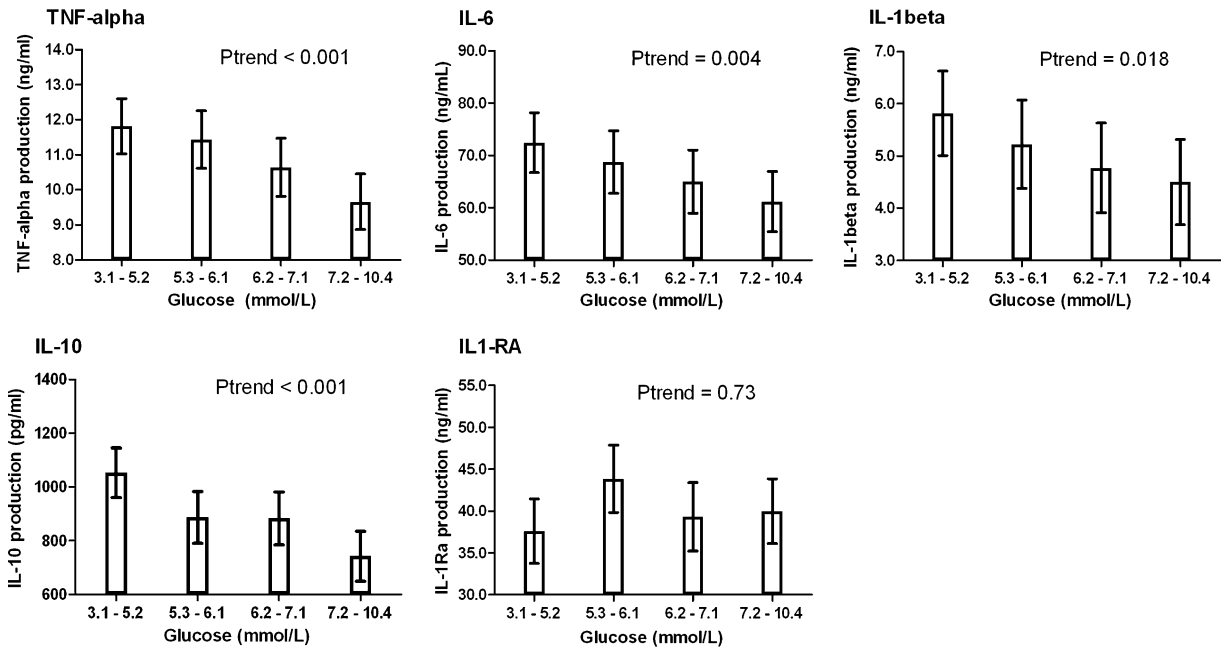

Fig. 1 Cytokine production after LPS-stimulation *ex vivo* by glucose concentration (n=468). Cytokine data represent geometric means ±95% confidence intervals, after linear regression with adjustment for gender

(data not shown). Glucose concentrations were positively associated with CRP (p for trend=0.02).

To assess whether the association between innate immunity and glucose concentrations was affected by acute or long-term regulation of glucose metabolism, we repeated the analyses of stimulated cytokine levels dependent on HbA1c. No consistent linear association was found between HbA1c and cytokines levels in response to LPS-stimulation (data not shown). HbA1c did correlate positively with CRP levels (p=0.001)

Discussion

We found a strong negative association between non-fasted glucose concentrations, but not HbA1c levels, and cytokine production upon LPS stimulation ex vivo in a community-dwelling, non-diabetic population of the oldest old. Both non-fasted glucose concentrations and HbA1c showed a positive correlation with CRP levels.

The observed relationship between non-fasted glucose and cytokine production capacity in non-diabetic subjects suggests that alterations in glucose metabolism, even in non-diabetics, may attenuate innate immune response. The lack of association between HbA1c and cytokine levels suggests that this effect might be driven by temporarily increases of glucose rather than chronic elevations. Earlier, hyperglycemic fluctuations, rather than continuous hyperglycemia, have been associated with negative outcome in a non-diabetic population cohort (Barr et al. 2009).

The relationship between higher glucose concentrations and low cytokine response could be of clinical significance. A low cytokine response has been independently associated with morbidity and mortality in our study population of the oldest old (van den Biggelaar et al. 2004). Furthermore, a low cytokine response has been associated with detrimental outcome in patients with sepsis at the intensive care unit (Muller Kobold et al. 2000; Westendorp et al. 1997). Also in this acutely ill patient cohort, innate immune response related inversely to levels of inflammation, such as CRP (Tschaikowsky et al. 2002).

A limitation of this study is that we did not measure insulin sensitivity. Insulin sensitivity has been associated with higher circulating inflammatory markers and is a prognostic factor for outcome in critically ill patients with myocardial infarction (Lazzeri et al. 2011; Zuliani et al. 2010). We were therefore not able to assess which mechanism of glucose metabolism is associated with innate immune function. Furthermore, as glucose concentrations were assessed at one time point only, we could also not assess glycemic variability accurately. Further studies with sensitive tools, such as continuous glucose monitoring systems, could better clarify this issue.

Importantly, our results were found in a non-diabetic population, suggesting that the blunted cytokine response is not due to specific factors related to diabetes mellitus, such as use of anti-diabetic medication, or other pathophysiological changes due to the disease, but suggest a direct relationship between glucose concentration and innate immune response. This association might be of clinical importance, as also in apparently healthy older individuals, disturbances in glucose and insulin metabolism are found such as impaired beta-cell function and decreased insulin action (Basu et al. 2003; Chang et al. 2006). Moreover, the glycemic response to stress might be increased with aging, which could further aggravate glycemic disturbances in clinical settings (Lazzeri et al. 2010; Odio and Brodish 1988).

Further studies in vivo including non-diabetic subjects are warranted to identify the mechanism behind the association between glucose concentrations and innate immune response and to investigate whether results can be extended to younger populations.

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