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The effect of glucose ingestion on inflammation and oxidative stress in obese individuals

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Abstract

Ingestion of 75 g glucose during an oral glucose tolerance test (OGTT) increases systemic inflammation and oxidative stress in healthy subjects and patients with type 2 diabetes mellitus, but the effect in overweight/obese nondiabetic individuals is uncertain. The aim of the present study was to determine the effect of an OGTT on plasma concentrations of inflammatory cytokines and peroxides in 33 subjects with body mass index >27 kg/m^2. After an overnight fast, blood samples were taken from participants immediately before and at 30, 60, 90, and 120 minutes after ingestion of 75 g glucose. Plasma glucose, insulin, free fatty acid, interleukin (IL)-6, tumor necrosis factor α, and peroxides were measured during the tests. Plasma IL-6 concentrations decreased (13%) significantly ($P < .001$) at 30 and 60 minutes, whereas plasma peroxide concentrations decreased slightly (3%, $P = .003$) at 30 minutes during the tests. The 30-minute decrease in plasma IL-6 was correlated significantly and inversely with the concomitant increase in plasma insulin ($r = −0.410$, $P = .02$) and with the ratio of insulin to glucose at 30 minutes during the OGTT ($r = −0.366$, $P = .04$). These data suggest that plasma concentrations of IL-6 are acutely decreased possibly because of the predominance of the anti-inflammatory effect of hyperinsulinemia over the proinflammatory effect of hyperglycemia after ingestion of a large quantity of glucose in obese individuals.

1. Introduction

Obesity is a risk factor for the development of insulin resistance, glucose intolerance, type 2 diabetes mellitus, and coronary heart disease. Markers of inflammation including C-reactive protein (CRP), interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)–α are increased [1], indicating chronic low-grade inflammation in obese individuals. Several cytokines and chemokines are synthesized in adipose tissue and associated macrophages [2]. In obesity, the number of macrophages in adipose tissue is increased [3] and may lead to excessive proinflammatory cytokine production [4] that probably contributes to increased systemic inflammation. There is evidence that increased oxidative stress also accompanies obesity in animals and humans [5,6] and dysregulates adipose tissue cytokines production [5]. Both inflammation and increased oxidative stress are thought to play a role in the development of tissue insulin resistance [5,7].

There is evidence that acute hyperglycemia increases inflammation and oxidative stress. Ingestion of glucose (75 g) increases monocyte nuclear factor (NF)–κB that is the main cellular signal of inflammation and induces transcription of proinflammatory cytokines and enzymes that generate reactive oxygen species (ROS) [8]. Infusion of glucose with concomitant inhibition of endogenous insulin secretion acutely increases proinflammatory cytokines TNF-α, IL-18, and IL-6 in humans [9]. Plasma concentrations of inflammatory markers CRP, IL-6, and intracellular adhesion molecule also increase during an oral glucose tolerance test (OGTT) in patients with type 2 diabetes mellitus [10]. Oxidative stress as indicated by plasma nitrotyrosine concentrations, and monocyte ROS generation increase during an OGTT in healthy subjects and patients with type 2 diabetes mellitus [9-12]. However, few studies have investigated the effect of ingesting a large quantity of glucose on inflammation and oxidative stress in obese

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individuals. Thus, the aim of the present study was to test the effect of an OGTT on markers of inflammation and oxidative stress in overweight/obese individuals.

2. Subjects and methods

2.1. Subjects

Thirty-three healthy subjects aged 31 to 65 years with a body mass index (BMI) >27 kg/m^2 were recruited from respondents to a newspaper advertisement. Exclusion criteria were known diabetes, cigarette smoking, current treatment with anti-inflammatory or other medications, serious illness, and clinical or biochemical evidence of acute or chronic infection. Subjects received a clinical examination; and anthropometric, health, and lifestyle information was collected. Participants gave informed and written consent. The study was approved by the Otago Ethics Committee.

2.2. Oral glucose tolerance test

After an overnight fast, subjects ingested 75 g glucose in 300 mL water (Glucaid, Histo-Labs, Riverstone, Australia); and blood samples were taken at 0, 30, 60, 90, and 120 minutes. Plasma glucose, insulin, free fatty acids (FFAs), IL-6, TNF-α, and peroxides were measured in these blood samples.

2.3. Laboratory methods

Venous blood was taken from the subjects into tubes containing EDTA and a plain tube. Tubes were centrifuged at 1500 *g* for 15 minutes at 4°C; and plasma and serum were harvested. Aliquots of serum and plasma were stored at −80°C. Plasma glucose was measured by routine automated methods in the laboratories of HealthOtago, Dunedin Hospital. Plasma insulin and CRP were measured on a Hitachi 911 autoanalyzer using commercial kits and calibrators (Roche Diagnostics, Mannheim, Germany). Plasma IL-6 and TNF-α concentrations were measured in duplicate by high-sensitivity enzyme-linked immunosorbent assay methods using commercial kits (R&D Systems, Minneapolis, MN). The intraassay coefficient of variation was 7% for IL-6 and 9% for TNF-α. The concentration of peroxides in plasma was measured as described previously [13] with an incubation time of 45 minutes. This method is based on cleavage of peroxides by horseradish peroxidase leading to oxidation of tetramethylbenzidine to a colored compound that can be measured spectrophotometrically. The intraassay coefficient of variation was 3% for plasma peroxides. Plasma FFA was measured using a commercial kit (Roche Diagnostics). Samples from an individual were measured in the same assay to reduce interassay variation.

2.4. Statistics

Values are given as mean ± SD unless stated otherwise. Data were log-transformed and then were analyzed by repeated-measures analysis of variance with time after ingestion of glucose as a within-subjects factor. Within-subject contrasts were used to compare values during the OGTT with zero-time values. Sex and the metabolic syndrome were included as between-subject factors in some repeated-measures analysis of variance models. Spearman rank correlation coefficients were used to test for relationships between changes in plasma IL-6 and insulin concentrations. Two-sided tests of significance were used, and a P value of less than .05 was considered to be statistically significant.

3. Results

Table 1 shows the characteristics of the subjects. On average, the participants were obese, with a mean BMI >30 kg/m^2, and had higher baseline levels of systolic and diastolic blood pressure and of plasma concentrations of IL-6, CRP, TNF-α, and peroxides compared with the corresponding values (120 ± 12 mm Hg, 75 ± 8 mm Hg, 1.4 ± 0.6 ng/L, 0.9 ± 0.6 mg/L, 0.58 ± 0.4 ng/L, 84 ± 34 μmol/L, respectively) in a group of 14 lean subjects of comparable ages (53 ± 10 years) determined recently in our laboratory. Twelve of the obese subjects had the metabolic syndrome as defined by the Adult Treatment Panel III criteria [14].

Fig. 1 shows plasma glucose, insulin, FFA, IL-6, TNF-α, and peroxide concentrations during the OGTTs. As expected, there were significant increases in plasma glucose and insulin and a decrease in plasma FFA concentrations. Plasma IL-6 concentrations decreased significantly (P < .001) from baseline (3.0 ± 2.1 ng/L) at 30 minutes (2.6 ± 2.0 ng/L) and 60 minutes (2.6 ± 2.0 ng/L) after ingestion of 75 g glucose. There was a small, significant (P = .01) decrease (−15 ± 26 μmol/L) in plasma peroxide concentra-

Table 1
Characteristics of the participants at baseline (N = 33)

Variable	
Age (y)	54 ± 8
Sex (male/female)	13/20
BMI (kg/m^2)	33.4 ± 4.6
Waist circumference (cm)	106 ± 11
Systolic BP (mm Hg)	134 ± 15
Diastolic BP (mm Hg)	84 ± 8
Fasting glucose (mmol/L)	5.31 ± 0.49
Fasting insulin (pmol/L)	78 ± 39
HbA_{1c} (%)	5.6 ± 0.3
Cholesterol (mmol/L)	5.72 ± 1.02
HDL cholesterol (mmol/L)	1.43 ± 0.38
Triglycerides (mmol/L)	1.46 ± 0.74
CRP (mg/L)	3.7 ± 3.1
IL-6 (ng/L)	3.0 ± 2.1
TNF-α (ng/L)	1.2 ± 0.8
FFAs (mmol/L)	0.28 ± 0.13
Peroxides (μmol/L)	433 ± 169

Values are mean ± SD or numbers of subjects. BP indicates blood pressure; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein.

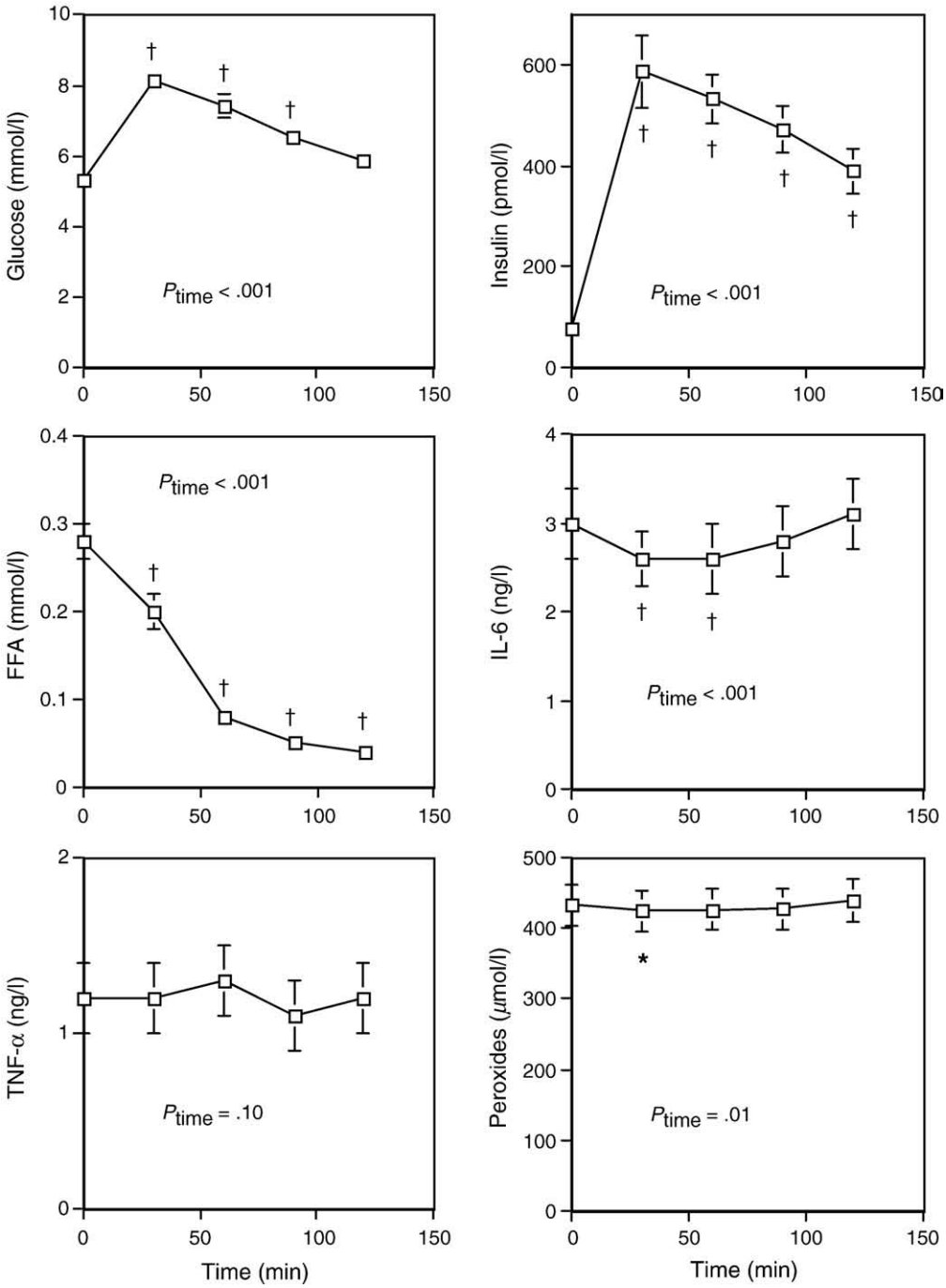

Fig. 1. The response of plasma glucose, insulin, FFA, IL-6, and peroxide concentrations during OGTTs in obese subjects (N = 33). Values are mean ± SEM. *$P < .01$, †$P < .001$, compared with baseline.

tions at 30 minutes after ingestion of glucose. Plasma TNF-α concentration did not vary significantly during the OGTTs. There were no significant interactions between metabolic syndrome and variation in plasma IL-6 ($P = .60$) and peroxide ($P = .54$) concentrations and between sex and plasma IL-6 ($P = .33$) and peroxides ($P = .63$) during the OGTTs.

Fig. 2 shows the relationship between the changes in plasma IL-6 and insulin concentrations during the OGTTs. The 30-minute decrease in plasma IL-6 was significantly and inversely correlated with the corresponding increase in plasma insulin.

4. Discussion

These data indicate that plasma concentrations of the inflammatory marker IL-6 decrease acutely during an OGTT

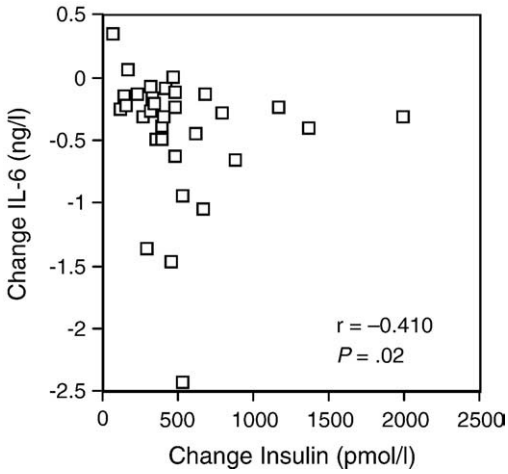

Fig. 2. Relationship between 30-minute changes in IL-6 and insulin during OGTTs in obese subjects (N = 33).

in obese individuals. The early increase in plasma insulin may contribute to this decrease in plasma IL-6 after ingestion of glucose.

In the present study, the 30-minute decrease in plasma IL-6 was correlated inversely with the 30-minute increase in plasma insulin after ingestion of glucose. Thus, it is possible that a large acute increase in insulin concentration decreases inflammation and plasma IL-6 concentrations during OGTTs in obese individuals. There is evidence that insulin has potent anti-inflammatory activity. Dandona and coworkers [14] have reported that infusion of insulin into obese subjects reduces monocyte intranuclear levels of NF-κB that is a major mediator of cellular inflammatory activity. Insulin may also reduce inflammatory activity and IL-6 production in adipose tissue. Adipose tissue is an important source of circulating IL-6 [15] especially in obesity [16]. Insulin decreases lipolysis of adipose tissue triglycerides and plasma levels of FFAs including saturated fatty acids. In cocultures of hypertrophied adipocytes and macrophages, saturated fatty acids and adipocyte-derived FFAs increase secretion of TNF-α from macrophages; and TNF-α increases IL-6 and FFA release from adipocytes, forming a vicious inflammatory cycle [4]. An insulin-induced decrease in FFA release from cultured adipocytes decreases proinflammatory cytokine release from cocultured macrophages [4]. In the present study, plasma FFA concentrations decreased markedly; and this may have also contributed to the concomitant decrease in plasma IL-6 during OGTTs. However, plasma IL-6 levels returned to fasting values despite very low plasma FFA concentrations at the end of the OGTTs, suggesting that other factors may be more important in regulating IL-6 at that time. In contrast with plasma IL-6 concentrations, plasma levels of TNF-α did not vary appreciably during OGTTs. However, circulating concentrations of TNF-α may not be a sensitive marker of tissue or systemic inflammation. Adipose tissue, for example, does not appear to release appreciable amounts of TNF-α to the circulation [15].

The decrease in plasma IL-6 during OGTTs in our data is in contrast with previous studies that have reported increases in plasma IL-6 or monocyte NF-κB in healthy subjects and/or patients with type 2 diabetes mellitus during an OGTT [8,10]. These divergent findings may be due to differences in the subjects studied. In the obese subjects we studied, it is possible that plasma insulin concentrations during OGTTs were abnormally high and enhanced the anti-inflammatory effect of hyperinsulinemia to an extent that counteracted the proinflammatory effect of hyperglycemia, leading to a decrease in plasma IL-6. Gastaldelli and coworkers [17] have reported that plasma insulin concentrations during an OGTT were approximately 2-fold higher in obese compared with lean nondiabetic subjects. In diabetic patients, however, secretion of insulin during an OGTT may be reduced and may favor the proinflammatory effect of hyperglycemia. There is evidence that hyperglycemia increases systemic inflammation when concomitant hyperinsulinemia is prevented. Infusion of glucose to give a circulating glucose concentration of 15 mmol/L while endogenous insulin secretion is inhibited increases plasma IL-6, IL-18, and TNF-α concentrations in healthy subjects and individuals with impaired glucose tolerance [18].

Systemic oxidative stress as indicated by plasma peroxide concentrations did not change appreciably during OGTT in the present study. Although plasma peroxides decreased significantly at 30 minutes during the OGTT, the magnitude of this decrease was small (3%) and was comparable with the intraassay coefficient of variation for the assay of plasma peroxides (2.4%). Previous studies have reported an increase in oxidative stress markers including plasma nitrotyrosine concentration and production of ROS by isolated blood monocytes in healthy subjects and/or patients with type 2 diabetes mellitus during an OGTT [8,10]. Differences in subjects and the markers of oxidative stress may contribute to these divergent findings between the studies. We cannot exclude the possibility that the marker of oxidative stress we used may have failed to detect an increase in oxidative stress during OGTTs that may have been revealed by other markers. On the other hand, previous data from our laboratory suggest that plasma peroxides respond to changes in oxidative stress. We have reported previously that plasma peroxides are correlated inversely with plasma concentrations of the antioxidant vitamin E and decrease appreciably during supplementation with vitamin E in obese individuals [19]. Furthermore, this decrease was paralleled by an unrelated decrease in plasma 8-isoprostane concentrations [20]. In addition, plasma peroxide concentrations were markedly higher in obese compared with lean women (Manning, unpublished data), in keeping with the reported increase in oxidative stress in obesity [5,6]. Whether abnormally high insulin concentrations prevent an increase in oxidative stress in obese individuals during OGTTs remains to be determined. A previous study in obese subjects has reported that insulin infusion decreases the generation of ROS by mononuclear cells [14].

This study has limitations. The number of subjects studied was relatively small. Thus, care must be exercised in the extrapolation of our findings to larger populations of obese individuals. In addition, the study was observational and the conclusions are limited. The study design did not include ingestion of water as a control intervention and a lean control group. However, the magnitude of the decrease in plasma IL-6 was approximately 2-fold larger than the intraassay coefficient of variation for the IL-6 assay; and all samples from an individual were measured in the same analytical run. Furthermore, in an earlier study from our laboratory, we have reported unchanged plasma IL-6 concentrations 1 hour after ingestion of a dilute cordial drink that did not appreciably increase plasma glucose concentrations in men who were on average overweight [21]. Thus, it is unlikely that the present decrease in plasma IL-6 after ingestion of glucose was mainly due to analytical variation or factors other than glucose ingestion. In the absence of a lean control group, we cannot determine the impact of obesity on our data. The subjects we studied were characteristic of obese individuals, with higher fasting concentrations of insulin, glucose, IL-6, CRP, TNF-α, and peroxides compared with mean values for lean subjects that were recently determined in our laboratory. These higher values are in keeping with increased insulin resistance, inflammation, and oxidative stress in obese individuals reported previously [1,5,6].

In conclusion, our data indicate that ingestion of a large amount of glucose is associated with a temporary decrease in plasma IL-6 concentrations in obese individuals, possibly due to the anti-inflammatory effect of hyperinsulinemia. Further studies are needed to clarify the influence of obesity on response of markers of inflammation and oxidative stress to an OGTT.

Acknowledgment

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