Post-load glucose measurements in oral glucose tolerance tests correlate well with 1,5-anhydroglucitol, an indicator of overall glycaemic state, in subjects with impaired glucose tolerance

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ABSTRACT
Using both cross-sectional and longitudinal methods, we investigated the relationship between post-load serum glucose concentration in a 75 g oral glucose tolerance test (OGTT) and overall glycaemic state in subjects with impaired glucose tolerance (IGT). Glycaemic state was assessed by measuring glycated haemoglobin (HbA1c) and the serum concentration of 1,5-anhydroglucitol (1,5-AG). In the cross-sectional study, the concentration of 1,5-AG, while remaining within a normal range, was reduced to a degree proportional to the post-load glycaemic level. Although the correlation between HbA1c and post-load plasma glucose was relatively weak ($r = 0.281, P < 0.001$), a significant inverse correlation ($r = -0.824, P < 0.0001$) was found between 1,5-AG and mean post-load plasma glucose concentration in 211 subjects with IGT. Fasting plasma glucose ($r = -0.539, P < 0.0001$) and 2 h plasma glucose ($r = -0.621, P < 0.0001$) were correlated with 1,5-AG less strongly than was post-load glycaemia. Both 1,5-AG and HbA1c were correlated weakly but significantly with the fasting insulin concentration. In the longitudinal study we measured 1,5-AG and mean post-load plasma glucose with an OGTT once yearly for 10 years in 15 subjects with IGT. Strong inverse correlations were seen between 1,5-AG and mean post-load plasma glucose in each subject (range of $r$ values among subjects of $-0.584$ to $-0.978$). These findings suggest a close relationship between post-load plasma glucose concentration measured by OGTT and overall glycaemic state in subjects with IGT.

INTRODUCTION
The overall glycaemic state preceding the onset of disease has received recent attention in terms of preventing the development of clinical diabetes. Impaired glucose tolerance (IGT), as proposed by the World Health Organization (WHO) in 1980 [1], is a condition intermediate between normal blood glucose tolerance and diabetes, that is defined according to an oral glucose tolerance test (OGTT). IGT is considered to be a major risk factor for future diabetes mellitus and cardiovascular disease [2]. Thus a better understanding of the glycaemic state in IGT is important for improved understanding of the development of Type II diabetes. In 1997, a committee of...
the American Diabetes Association [3] defined IGT not as 'hyperglycaemia', but rather as a metabolic stage intermediate between normal glucose homeostasis and diabetes. The committee report emphasized that many individuals with IGT typically are euglycaemic in their daily lives [3,4], and may have normal or nearly normal levels of glycated haemoglobin (HbA1c) [3,5], concluding that 'individuals with IGT often manifest hyperglycaemia only when challenged with the oral glucose load used in the standardized OGTT' [3].

Few reports have described changes in HbA1c during the course of IGT. Little et al. [5] reported that 70% of their subjects with IGT showed normal levels of HbA1c, but their study was cross-sectional only. In a study assessing the effects of troglitazone on glycaemia in subjects with IGT, neither HbA1c nor fructosamine levels had changed 12 weeks after starting treatment, even though a significant reduction in the glucose response was evident in an OGTT performed 12 weeks after the start of treatment [6].

Developed in Japan as a new marker for glycaemia [7–14], the measurement of 1,5-anhydroglucitol (1,5-AG) in serum can detect slight changes in the glycaemic state, and therefore appears well suited to monitoring glucose homeostasis in patients with near-normoglycaemia [13,15,16] or postprandial hyperglycaemia [13,17,18]. Within its epidemiologically defined normal range, 1,5-AG has an individual normal value for each patient, and falls rapidly from this norm (by as much as hundreds of milligrams of glucose per day) if it is being excreted continuously in the urine [19]. Thus measurement of 1,5-AG may be a valid way to monitor slight changes in glycaemia in subjects with IGT. Tsukui et al. [20] have reported that serum 1,5-AG was lower in non-diabetic subjects with a family history of Type II diabetes mellitus than in those with no family history, suggesting that 1,5-AG better reflects early glycaemic changes in these subjects than does HbA1c.

In the present study, we examined the relationship between post-load glycaemia during an OGTT and overall glycaemic state as reflected by 1,5-AG in subjects with IGT, using cross-sectional and longitudinal observations.

METHODS

Subjects and protocol

Subjects for this study consisted of two groups. The first group included 137 men and 74 women selected from among individuals who had undergone a 75 g OGTT as part of a routine medical check-up at our hospital. All exhibited a pattern of IGT (see below) in this test. In this group, a single measurement of 1,5-AG was used to assess overall glycaemic status for cross-sectional analysis. The second group consisted of 15 subjects (eight male; seven female) who consistently met criteria for IGT in a 75 g OGTT administered once a year for 10 years at our hospital. This group was used to evaluate successive annual measurements of 1,5-AG as a predictor of glycaemic change in IGT subjects (longitudinal study). At the start of the longitudinal study, 26 subjects with IGT were registered. Over the course of 10 years, four of the 26 subjects developed diabetes and were excluded from the study. The mean 1,5-AG level of these four subjects was $5.7 \pm 1.2 \mu g/ml$ (range $3.7–7.1 \mu g/ml$) at the onset of diabetes. Five of the 26 subjects returned to normal glucose tolerance and were also excluded from the study. The 1,5-AG levels of all five were $>24.0 \mu g/ml$ at the time that they returned to normal tolerance. Two subjects dropped out (one due to lung cancer and another due to change of residence).

IGT and diabetes mellitus were diagnosed based on a single OGTT, according to revised American Diabetes Association (ADA) criteria [3]. All subjects who had an average post-load plasma glucose value of $\geq 11.1 \text{mmol/l}$ showed a 2 h glucose concentration of $<11.1 \text{mmol/l}$. Subjects who had a fasting plasma glucose concentration of $\geq 7.0 \text{mmol/l}$ were excluded from the study. Also excluded from evaluation were patients with nephropathy (serum creatinine $\geq 120 \text{\mu mol/l}$) or previously diagnosed diabetes mellitus.

All subjects underwent a 75 g OGTT and were tested for plasma glucose and serum insulin levels after a 12 h overnight fast. In all subjects, 1,5-AG and HbA1c levels were measured on the day that the OGTT was administered. The blood samples for 1,5-AG and HbA1c measurements were collected in the fasting state. The mean age of the first group (cross-sectional study) was 51.4±11.1 years (range 23–79 years), and their mean body mass index was $23.7 \pm 3.1 \text{kg/m}^2$ (range 14.0–33.2 kg/m$^2$). The subjects in the second group (longitudinal study) ranged in age from 23 to 70 years (mean 47.3 years). Clinical profiles of the 15 subjects in the second group are described in Table 2 (see below). All subjects in the second group received advice regarding diet and exercise during the 10-year monitoring period. All subjects gave their informed consent for participation. The study protocol was approved by the ethics committee of our institution.

Measurements

Glucose concentration was measured from a venous blood sample by the glucose oxidase method. The interassay coefficient of variation (CV) was 2.6% and 2.2% for the low- and high-quality control pools respectively. HbA1c (normal range 4.9–5.9%) was assayed by HPLC (Auto A1c; Kyoto Daichi Kagaku, Japan), with a CV of 2.4%. In the cross-sectional study, the serum concentration of 1,5-AG (normal range
14.0–39.0 µg/ml) was measured with an autoanalyser system (Automatic Clinical Analyzer, Model 7150; Hitachi, Tokyo, Japan) [21] using a modified column enzymic test [22]; the interassay CV was 4.5% and the intra-assay CV was 1.0%. In the longitudinal study, 1,5-AG levels were determined by GLC according to a modified method reported previously [23]; the interassay CV was 2.1% and the intra-assay CV was 1.1%. The reason that GLC was used to assay 1,5-AG in the longitudinal study is that the autoanalyser was not available at the beginning of the study. Plasma insulin was measured by RIA using dextran-charcoal separation [24]; the interassay CV was 11.5%. Antibody and tracer for the insulin RIA were purchased from Novo Nordisk (Bagsvaerd, Denmark).

Statistical analysis
Data are presented as the means ± S.D. The Shapiro–Wilks test was used to assess the distribution of 1,5-AG, and the W value was 0.9471 (P < 0.05). Thus we regarded 1,5-AG as following a normal distribution in the range observed in this study. Correlation coefficients were determined by linear regression. Statistical analyses were performed using JMP 2.0 statistical software (SAS, Tokyo, Japan). A P value of < 0.05 was considered statistically significant.

RESULTS
Cross-sectional study
In the cross-sectional study, serum 1,5-AG concentrations were distributed over a wide range (8.0–35.0 µg/ml), with a mean value of 17.3 ± 5.7 µg/ml. In 148 of the 211 subjects with IGT, 1,5-AG remained within the epidemiologically defined normal range (14.0–39.0 µg/ml), even though it decreased as mean glucose levels increased [9]. A significant negative correlation (r = −0.824, P < 0.0001; Figure 1) was found between 1,5-AG and plasma glucose concentrations in 211 subjects with IGT.

**Figure 1** Correlations between 1,5-AG and plasma concentrations of glucose in 211 subjects with IGT
Upper panel, relationship between 1,5-AG and mean post-load plasma glucose concentration in a 75 g OGTT (r = −0.824, P < 0.0001). Lower panel, relationship between 1,5-AG and fasting plasma glucose (r = −0.539, P < 0.0001).

**Figure 2** Correlations between HbA1c and plasma concentrations of glucose in 211 subjects with IGT
Upper panel, relationship between HbA1c and mean post-load plasma glucose concentration in a 75 g OGTT (r = 0.281, P < 0.001). Lower panel, relationship between HbA1c and fasting plasma glucose (r = 0.184, P < 0.01).
between 1,5-AG and mean post-load plasma glucose values (mean values at 30, 60, 90 and 120 min after 75 g of oral glucose) in the 211 IGT subjects. A strong inverse correlation ($r = -0.621$) was also found between 1,5-AG and 2 h plasma glucose concentrations in the 75 g OGTT. However, 1,5-AG was correlated more strongly with post-load glycaemic levels than with fasting glycaemia ($r = -0.539$, $P < 0.0001$; Figure 1). In contrast, only weak correlations were observed between HbA1c and plasma glucose parameters in IGT subjects (Figure 2; Table 1).

Statistically significant inverse correlations were found between levels of 1,5-AG and HbA1c ($r = -0.329$, $P < 0.0001$), and between fasting plasma glucose and mean post-load plasma glucose ($r = 0.510$, $P < 0.0001$). Both 1,5-AG and HbA1c showed weak but significant correlations with the summated serum immunoreactive insulin concentration (representing the summation of immunoreactive insulin concentrations at 0, 30, 60, 90 and 120 min after oral ingestion of 75 g of glucose; Table 1). No significant correlation was found between 1,5-AG and age ($r = -0.088$, $P = 0.202$) or body mass index ($r = 0.084$, $P = 0.226$).

### Longitudinal study

In the longitudinal study, we monitored 1,5-AG and mean post-load plasma glucose with an OGTT once a year for 10 years in 15 subjects with IGT. During this period, none of the 15 subjects was diagnosed with diabetes, as defined by the revised American Diabetes Association criteria [3]. For each subject, a total of 10 values were obtained for each parameter: annual measurements over the 10-year period. A marked inverse correlation was found between the level of 1,5-AG and mean post-load plasma glucose ($r = -0.584$ to $-0.978$; mean $r$ value $= -0.826$) in each subject (Table 2, Figure 3). A less marked correlation was seen between 1,5-AG and fasting plasma glucose (mean $r = -0.460$) (Table 2).
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Figure 3 Longitudinal relationship between mean post-load plasma glucose concentration in a 75 g OGTT and serum 1,5-AG in 15 subjects with IGT

Subjects are identified by number, as listed for the clinical profiles in Table 2. Data are based on the results for subjects who underwent a 75 g OGTT and 1,5-AG measurements once a year for 10 years after an initial diagnosis of IGT.

did not estimate the relationship between HbA1c and plasma glucose in the longitudinal study, because the method for measuring HbA1c was changed during the study period (from one that included unstable HbA1c to one that excluded this fraction), and the correlation between the old and new methods was not complete ($r = 0.917$).

DISCUSSION

This is, to our knowledge, the first report confirming a close correlation between the post-load plasma glucose concentration (as measured by OGTT) and overall glycaemic state in subjects with IGT, as evaluated by 1,5-AG. 1,5-AG is more useful than HbA1c for detecting glycaemic change in the near-normoglycaemic range [13,15,16]. While the general diagnostic concept of IGT is that of a hyperglycaemic stage representing a transition from the normal state to diabetes mellitus, diagnosis of IGT is based mainly on OGTT results [1]. Previous studies have indicated that results from OGTTs are frequently not in agreement with averaged daily blood glucose measurements, especially in patients with diabetes [25,26]. Few previous studies have examined the relationship between post-load glucose concentration and overall glycaemic state in IGT subjects.

Several previous studies [6,27] assessing glycaemic state have suggested that the HbA1c level does not correlate well with post-OGTT glucose levels. We similarly obtained a relatively poor correlation between HbA1c and post-load glucose. Because the plasma glucose concentration obtained with a single OGTT can be influenced by intercurrent illnesses or stresses that would not be reflected in the HbA1c concentration, we strongly question whether HbA1c can reliably reflect slight changes in overall glycaemic state in subjects with IGT [27]. As shown in the present study, 1,5-AG is a better indicator than HbA1c of postprandial glucose levels in subjects with IGT.

We found that the serum 1,5-AG concentration was decreased in IGT subjects, although it remained within normal limits. The magnitude of this reduction was related to the degree of impairment of glucose tolerance. While 1,5-AG, like HbA1c, is considered relatively insensitive for detecting subjects with IGT [9,28], the
normal serum 1,5-AG concentration of each subject is specific to that individual and does not necessarily correspond to an epidemiologically defined normal range [9,29]. Detection of slight changes in glucose homeostasis by measurement of 1,5-AG can be important, even within such a normal range [19]. 1,5-AG, a 1-deoxyglucopyranose, is one of the major polyols in the human body. The substance originates in the diet and exists in a large pool in the body, being minimally degraded and metabolized [23]. Its renal reabsorption is competitively inhibited by the glucosuria induced by hyperglycaemia. Upon onset of glucosuria, 1,5-AG promptly falls below its normal value in that individual [8,30,31].

We found a strong correlation between the percentage decrease in plasma 1,5-AG and the total amount of urinary glucose excreted in 1 day. The time required for a significant change ordinarily can be up to several days [19]. Although individual IGT cases may not be detected by conventional cut-off values for 1,5-AG, we found it useful to monitor 1,5-AG at intervals to check the progression of hyperglycaemia. Studies have revealed a skewed distribution of 1,5-AG values, relative to glucose levels [8,31]. Within its normal range, 1,5-AG decreases readily in an exponential manner in response to a small increase in urinary glucose [8]. This means that the higher the level of 1,5-AG, the larger the change in this substance. Thus the slope of the linear regression between 1,5-AG and glucose becomes steeper at higher levels of 1,5-AG, as shown in Figure 3. Although there is little individual variance in the relationship between 1,5-AG and glucose levels, it is worth noting the skewed correlation curve that results when we compare the absolute values of 1,5-AG between individuals. For a given subject, any change in the renal threshold for glucosuria should have only a relative effect on 1,5-AG levels. Individual variance in the renal threshold for glucosuria does not appear to seriously influence the clinical utility of 1,5-AG measurement, judging from our experience of its commercial use for 9 years in Japan. However, a large population study is needed to confirm this.

Isolated post-challenge hyperglycaemia also carries a significantly increased risk of cardiovascular disease [32,33] and overall mortality [34]. In contrast, no significant risk is associated with isolated fasting hyperglycaemia. Our data suggest that many individuals with IGT develop abnormal glucose concentrations. They further suggest that, apart from hyperlipidaemia and other factors, a high blood glucose concentration at 2 h after loading (indicating a general state of hyperglycaemia throughout the day) is associated with an increased risk of death that is independent of fasting blood glucose. Even in a Japanese population, with presumably less tendency toward hyperinsulinaemia than Caucasians, IGT (but not impaired fasting glucose) may be a risk factor for cardiovascular disease. Thus measurement of postprandial glucose concentration is important, even in subjects with IGT.

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