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Serum high–molecular weight adiponectin decreases abruptly after an oral glucose load in subjects with normal glucose tolerance or impaired fasting glucose, but not those with impaired glucose tolerance or diabetes mellitus

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Abstract

Adiponectin exists in the blood as 3 forms, which are a trimer, a hexamer, and a high–molecular weight (HMW) form. We investigated whether circulating HMW adiponectin levels were altered by oral glucose or fat ingestion. Forty male subjects underwent a 75-g oral glucose loading test (OGTT), and 11 healthy subjects (5 women and 6 men) received a fat loading test. Serum levels of HMW and total adiponectin were measured during the OGTT and the fat loading test. The fat loading test was performed for at least 8 hours. Among the 40 male subjects, 11 had normal glucose tolerance (NGT), 9 had impaired fasting glucose (IFG), 11 had impaired glucose tolerance, and 9 had diabetes mellitus (DM). In all 40 subjects, the serum total adiponectin level did not change significantly, whereas serum HMW adiponectin decreased significantly after a glucose load and reached 92.2% of the basal level at 120 minutes after the OGTT ($P < .01$). The HMW to total adiponectin ratio decreased significantly from 0.47 ± 0.15 at baseline to 0.43 ± 0.13 at 120 minutes after a glucose load ($P < .05$). Serum HMW adiponectin measured at 120 minutes after the OGTT decreased significantly to 86.0% and 85.6% of the basal level in subjects with NGT or IFG, respectively (both $P < .01$). In subjects with impaired glucose tolerance or DM, however, serum HMW adiponectin did not change. The area under the curve for insulin at 30 minutes after a glucose load during the OGTT was significantly larger in subjects with NGT or IFG than in those with DM ($P < .05$). In addition, the insulinogenic index ($\Delta I_{0\text{-}30}/\Delta G_{0\text{-}30}$) was significantly higher in subjects with NGT or IFG than in those with DM ($P < .001$). Percentage changes in serum HMW adiponectin of the baseline at 120 minutes correlated negatively with those in serum insulin ($r = -0.468$, $P = .0023$), but not plasma glucose, of the baseline at 30 minutes in 40 subjects. On the other hand, serum triglycerides increased significantly after an oral fat load in 11 healthy subjects; but neither serum total nor HMW adiponectin changed. In conclusion, serum HMW adiponectin (but not total adiponectin) decreased rapidly after glucose loading in subjects with NGT or IFG; and the decrease of HMW adiponectin may be associated with an increase of serum insulin at 30 minutes.

1. Introduction

Adiponectin is an important adipocyte-specific protein among the various adipokines, which has a beneficial role in both glucose and lipid metabolism [1,2]. Interestingly, low serum total adiponectin levels are found in patients with type 2 diabetes mellitus (DM) and cardiovascular disease [3,4]. Adiponectin undergoes posttranslational modification within adipocytes to yield various multimeric forms: a trimer (low–molecular weight [LMW] adiponectin), a hexamer (trimer-dimer) of medium molecular weight, and a larger multimeric high–molecular weight (HMW) form [5,6]. Previous studies have suggested that HMW adiponectin may be the active form of this protein because changes of the HMW to total adiponectin ratio after treatment with a thiazolidinedione, but not those of total adiponectin, were associated with improvement of hepatic insulin sensitivity

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[7] or glycemic control [8]. However, it remains unclear how circulating adiponectin (including HMW adiponectin) is regulated in vivo.

Adiponectin has a relatively high serum concentration and a much longer half-life than other hormones [9,10]. Several clinical studies have failed to find any significant acute effect of glucose or fat intake on the serum total adiponectin concentration after an oral glucose or fat load [11-13]. Thus, the serum concentrations of adiponectin are believed to be fairly stable in humans. However, most of the previous studies examined the effect of a glucose load on total adiponectin in healthy subjects; and although Peake et al [10] investigated the acute effects of glucose or fat loading on the different oligomers of adiponectin in humans, few authors have assessed the acute effects of glucose or fat on circulating HMW adiponectin levels by the oral glucose tolerance test (OGTT) or fatty meal test.

We investigated whether circulating total or HMW adiponectin levels were influenced acutely in humans by an oral glucose or fat load using a novel enzyme-linked immunosorbent assay (ELISA) for detection of HMW adiponectin.

2. Materials and methods

2.1. Oral glucose tolerance test

We studied 40 consecutive male subjects who were suspected of having impaired glucose tolerance (IGT) or type 2 DM. None of the subjects received hypoglycemic agents. An OGTT was performed with 75 g of glucose at 9:00 AM after an overnight fast for 12 hours. Blood samples were obtained at 0, 30, 60, 120, and 180 minutes after the intake of glucose. Afterward, the subjects were divided into the following 4 categories based on their fasting plasma glucose (FPG) and 2-hour PG levels during the 75-g OGTT: *normal glucose tolerance* (NGT) was defined as FPG less than 126 and 2-hour PG less than 140; *impaired fasting glucose* (IFG) was defined as an FPG of at least 100 but less than 126 mg/dL and 2-hour PG less than 140; *IGT* meant a 2-hour PG of at least 140 but less than 200 mg/dL; and *diabetes* was indicated by an FPG of at least 126 and/or 2-hour PPG of at least 200. Eleven subjects had NGT, 9 subjects had IFG, 11 subjects had IGT, and 9 subjects had diabetes.

2.2. Oral fat loading test

We also studied a group of 11 healthy subjects (5 women and 6 men). None of them had diabetes, their age was 31.9 ± 7.3 years, and their body mass index was 24.3 ± 3.3 (mean ± SD). At baseline, serum total and HMW adiponectin levels were 9.6 (6.7, 12.2) and 6.8 (3.5, 9.1) μg/mL, respectively. Their triglyceride (TG) level was 76.0 (66.5, 113.0) mg/dL. The OFTT cream (JOMO Food, Takasaki, Japan) containing 35% fat without added sugar was ingested after a 12-hour overnight fast. This cream contained 57% water, 35% lipid, 2.5% protein, and 7.4% carbohydrate [14]. The fat distribution in the cream is 64.3% saturated, 29.3% monounsaturated, and 3.5% polyunsaturated. Each subject took a dose of 30 g of fat per square meter of body surface area within 3 minutes. The subjects were only allowed to drink a small amount of water during the study period to prevent dehydration. Blood was collected before and 1, 2, 3, 4, 6, and 8 hours after the fat load, followed by immediate separation of serum.

All subjects gave informed consent to this study.

2.3. Measurements

The total serum adiponectin concentration was measured by a sandwich ELISA (Otsuka Pharmaceuticals, Tokyo, Japan), as described previously [15]. In brief, after boiling serum samples in sodium dodecyl sulfate buffer for 5 minutes to convert adiponectin to its monomeric form, samples were analyzed with an ELISA to determine the total adiponectin level. The intraassay and interassay coefficients of variation

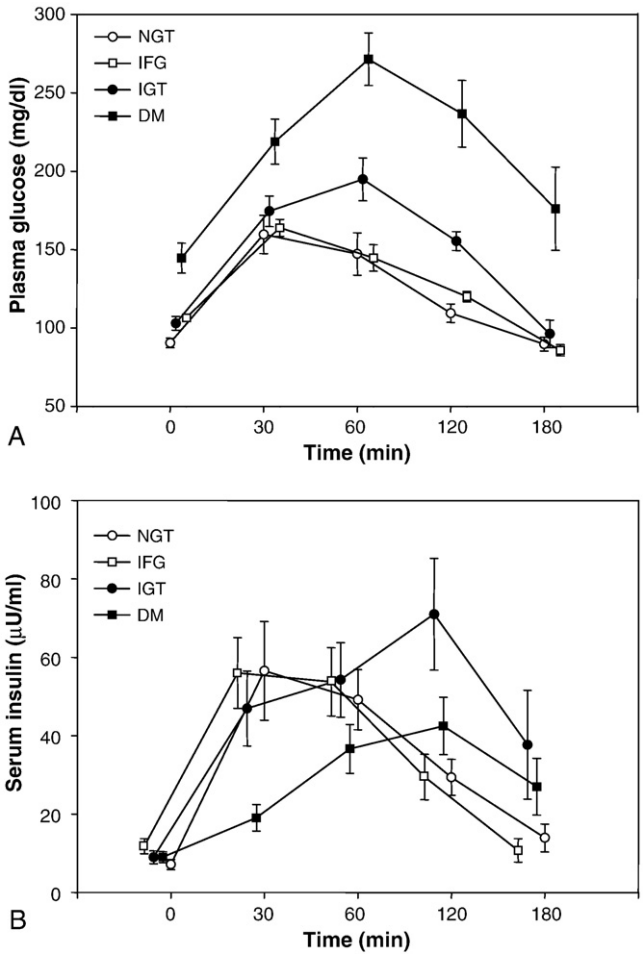

Fig. 1. Plasma glucose (A) and serum insulin (B) concentrations during a 75-g glucose tolerance test in the 4 groups: NGT (○), IFG (□), IGT (●), and DM (■). The points are offset with respect to the time axis to avoid overlap.

were 4.06% and 4.69%, respectively. Serum HMW adiponectin levels were measured with a novel sandwich ELISA using a monoclonal antibody for human HMW adiponectin, as described previously [16,17]. This sandwich HMW ELISA kit used the same monoclonal antibody (IH7) as the solid-phase capturing antibody and the horseradish peroxidase–conjugated detection antibody (Fujirebio, Tokyo, Japan). In brief, 96 wells of a microtiter plate were coated with the IH7 anti-HMW adiponectin monoclonal antibody. By using the same antibody for both capture and detection, the sandwich ELISA could specifically measure HMW adiponectin in serum [16]. The intraassay and interassay coefficients of variation were 2.4% to 3.0% and 4.2% to 5.1%, respectively.

Plasma insulin concentrations were determined by radioimmunoassay. The *area under the curve for insulin at 30 minutes* (AUC [30 minutes]) was defined as the area under the insulin concentration curve for a 30-minute period after intake of the glucose load in the OGTT. The insulinogenic index, an indicator of early insulin secretion, was calculated as the change of insulin divided by that of glucose during the initial 30 minutes of the OGTT ($\Delta I_{0\text{-}30}/\Delta G_{0\text{-}30}$). Insulin resistance was evaluated by homeostasis model assessment (HOMA-IR), calculated as fasting plasma insulin (in microunits per milliliter) × FPG/405.

2.4. Statistical analysis

Data are presented as the mean ± SD or as the median and interquartile range unless otherwise indicated. Differences between 2 groups were analyzed by Student paired *t* test or an unpaired *t* test. Differences between 2 groups concerning nonparametric data were analyzed by Wilcoxon matched-pairs test or the Mann-Whitney *U* test. Differences of normally distributed data were assessed by 1-way analysis of variance using the Newman-Keuls multiple-comparison test. For data that did not show a normal distribution, differences between groups were analyzed by the Kruskal-Wallis test and Dunn multiple-comparison test. Correlations were determined by linear regression analysis or multivariate analysis. Logarithmic transformation of the insulin AUC (30 minutes) data and insulinogenic index data was done to normalize the distribution for parametric tests. A *P* value of less than .05 was accepted as indicating statistical significance.

3. Results

The plasma glucose and serum insulin profiles of the 4 groups during the OGTT are presented in Fig. 1A, B. In all 40 male subjects, there were no significant changes of serum total adiponectin during the OGTT (6.5 [5.4, 8.7] at baseline, 6.8 [5.3, 8.3] at 30 minutes, 7.0 [5.3, 8.0] at 60 minutes, 6.7 [5.3, 8.0] at 120 minutes, and 6.3 [5.5, 7.3] μg/mL at 180 minutes after the OGTT). In contrast, serum HMW adiponectin decreased significantly after the oral glucose load to reach 92.2% of the baseline level at 120 minutes after the start of the OGTT ($P = .0016$). Similarly, the absolute values for HMW adiponectin decreased significantly from 3.1 (2.2, 4.3) μg/mL at baseline to 2.6 (1.8, 4.0) μg/mL at 120 minutes ($P = .0025$). The HMW to total adiponectin ratio also decreased significantly from 0.47 ± 0.15 at baseline to 0.43 ± 0.13 at 120 minutes ($P = .0223$).

Next, we divided the 40 male subjects into 4 groups (NGT, IFG, IGT, and DM) according to the results of the

Table 1
Baseline clinical characteristics of the subjects grouped according to the results of the OGTT

	NGT	IFG	IGT	DM
n	11	9	11	9
Age (y)	41.0 ± 12.0	49.3 ± 12.3	45.9 ± 7.1	50.1 ± 9.4
BMI (kg/m^2)	23.0 ± 4.5	25.7 ± 3.2	25.9 ± 3.4	24.8 ± 2.8
FPG (mg/dL)	90.4 ± 10.0	106.8 ± 4.2	103.3 ± 14.5	144.8 ± 28.7‡,¶,§
2-h PG (mg/dL)	109.5 ± 19.7	120.2 ± 9.9	155.7 ± 20.1†	236.9 ± 63.9‡,¶,§
HbA_{1c} (%)	5.0 ± 0.4	5.2 ± 0.4	5.6 ± 0.5	6.5 ± 0.7‡,¶,§
Fasting insulin (μU/mL)	7.1 ± 4.3	14.0 ± 5.8	9.1 ± 5.4	9.5 ± 4.4
HOMA-IR	1.64 ± 1.09	3.69 ± 1.52*	2.39 ± 1.47	3.39 ± 1.61*
LDL-C (mg/dL)	107.2 ± 35.6	113.4 ± 27.7	125.7 ± 22.4	136.9 ± 22.6
TG (mg/dL)	132.6 ± 103.9	231.6 ± 108.7	177.6 ± 116.9	124.1 ± 34.4
HDL-C (mg/dL)	56.6 ± 15.1	49.6 ± 9.9	48.7 ± 8.8	53.6 ± 11.2
Total AD (μg/mL)	9.2 ± 4.3	7.1 ± 2.2	6.5 ± 1.5	5.9 ± 2.0
HMW AD (μg/mL)	4.9 ± 3.7	2.9 ± 1.3	3.0 ± 1.5	3.1 ± 1.6
HMW to total AD	0.52 ± 0.15	0.40 ± 0.08	0.46 ± 0.15	0.48 ± 0.18

Data are the mean ± SD. BMI indicates body mass index; Hb, hemoglobin; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AD, adiponectin.

* $P < .05$.

† $P < .01$.

‡ $P < .001$ vs NGT.

¶ $P < .001$ vs IFG.

§ $P < .001$ vs IGT.

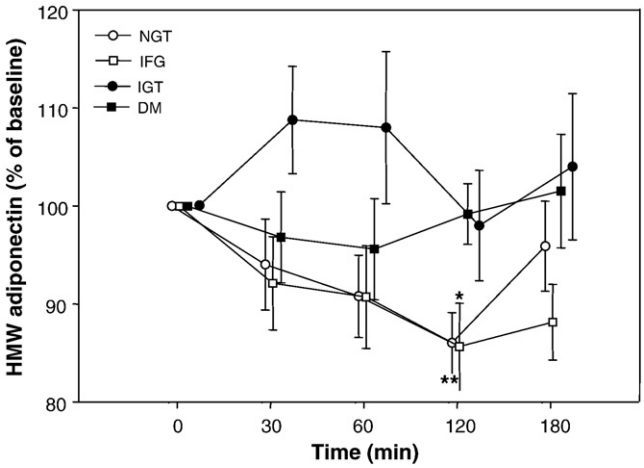

Fig. 2. Serum HMW adiponectin during a 75-g OGTT in the 4 groups: NGT (○), IFG (□), IGT (●), and DM (■). The points are offset with respect to the time axis to avoid overlap.

OGTT. The characteristics of these 4 groups are shown in the Table 1. The HOMA-IR was significantly higher in the IFG or DM groups than in the NGT group (both *P*s < .05). In the NGT group, the serum level of HMW adiponectin decreased significantly to 86.0% of the baseline value ($P = .0011$) at 120 minutes after an oral glucose load (Fig. 2). Similarly, the absolute values for HMW adiponectin decreased significantly from 3.5 (2.9, 5.1) μg/mL at baseline to 3.0 (2.4, 4.9) μg/mL at 120 minutes ($P = .0020$). In the IFG group, serum HMW adiponectin also decreased significantly to 85.7% of the baseline level ($P = .0121$) at 120 minutes (Fig. 2). Like the NGT group, the absolute values for HMW adiponectin decreased significantly from 2.8 (1.9, 3.4) μg/mL at baseline to 2.1 (1.7, 2.5) μg/mL at 120 minutes ($P = .0136$) in the IFG group.

In the IGT and DM groups, however, serum HMW adiponectin did not change during the OGTT. The insulin AUC (30 minutes) during the OGTT was significantly larger in the NGT and IFG groups than in the DM group (both *P*s < .05, Fig. 3A). Similarly, the insulinogenic index was significantly higher in the NGT and IFG groups than in the DM group (both *P*s < .001, Fig. 3B). Percentage changes in serum HMW adiponectin of the baseline at 120 minutes correlated negatively with percentage changes in serum insulin ($r = -0.468$, $P = .0023$), but not in plasma glucose, of the baseline at 30 minutes in 40 subjects (Fig. 4A, B).

Serum TGs increased significantly to 250% of the baseline level at 4 hours ($P < .0001$) after an oral fat load in the 11 healthy subjects (Fig. 5). However, we found no

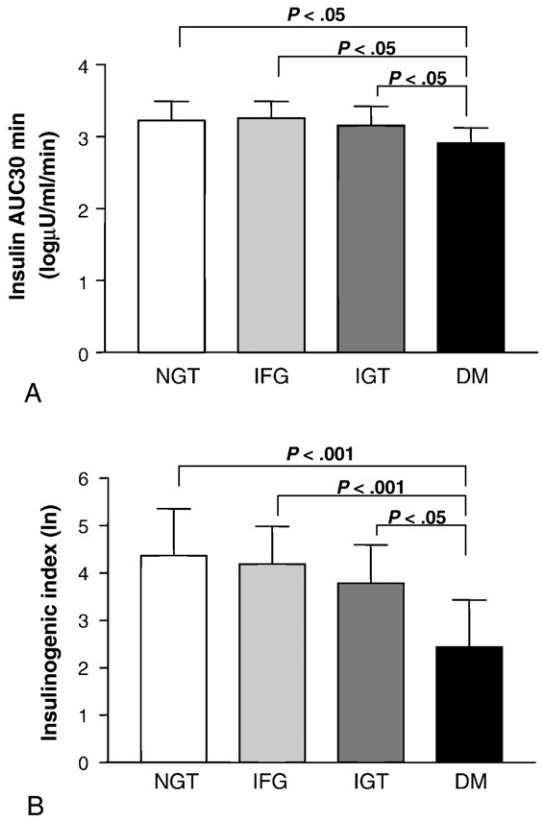

Fig. 3. Area under the curve for insulin at 30 minutes (A) and insulinogenic index (B) in the 4 groups: NGT (○), IFG (□), IGT (●), and DM (■). *P < .05, **P < .01 vs baseline (0 minute).

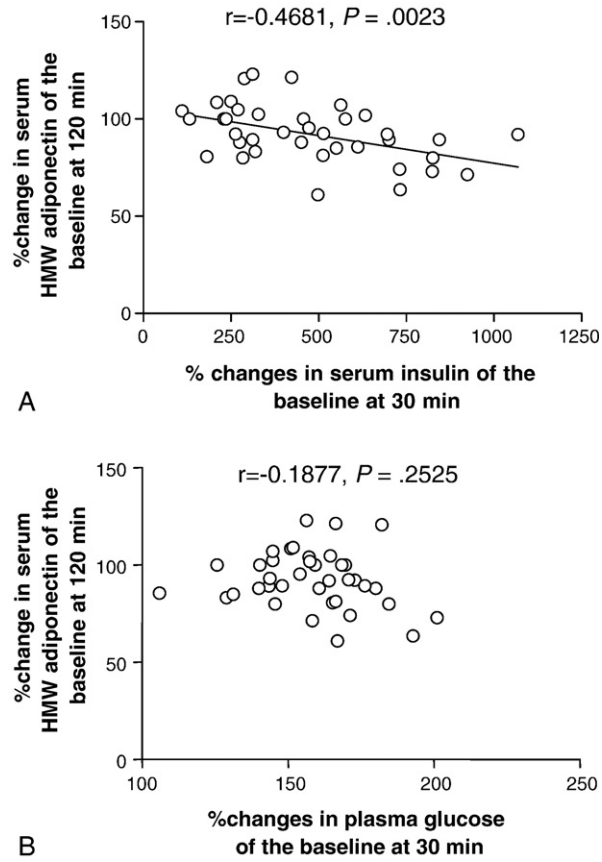

Fig. 4. Correlation between percentage changes in serum HMW adiponectin of the baseline at 120 minutes and percentage changes in serum insulin (A) or plasma glucose (B) of the baseline at 30 minutes in 40 male subjects.

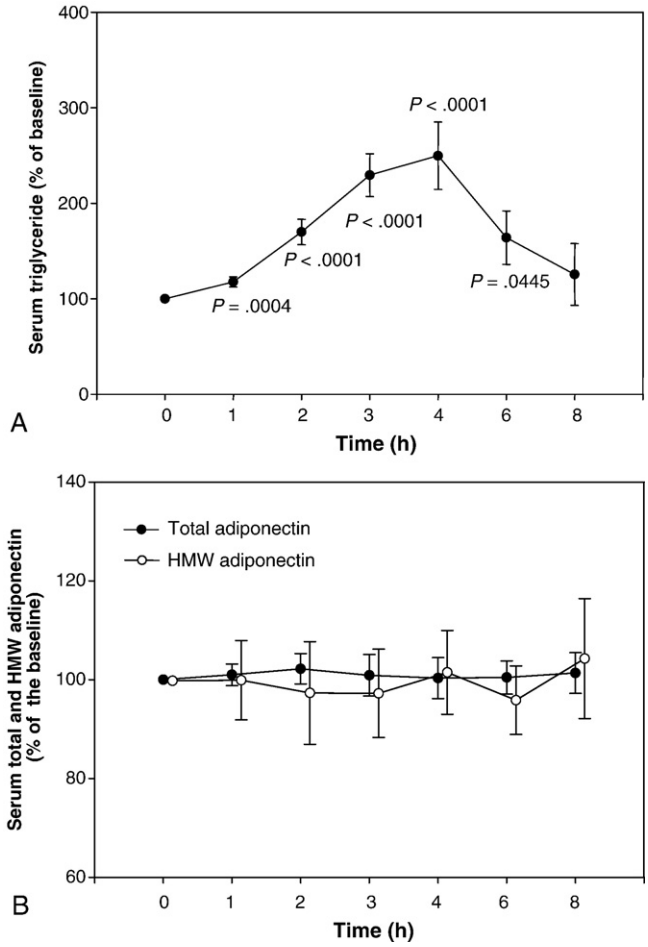

Fig. 5. Serum TGs (A) and HMW adiponectin (B) during a fat loading test in 11 healthy subjects. The points are offset with respect to the time axis to avoid overlap.

significant changes of serum total or HMW adiponectin during 8 hours after fat intake in the fat loading test (Fig. 5).

4. Discussion

The present study demonstrated that serum HMW adiponectin, but not serum total adiponectin, decreased at 120 minutes after an oral glucose load in humans. In addition, the HMW to total adiponectin ratio was significantly decreased at 120 minutes in these subjects. The decrease of HMW adiponectin was more marked in subjects with NGT or IFG than in those with IGT or diabetes. These results suggest that serum HMW adiponectin is acutely influenced by oral glucose intake. Several authors have already reported that oral glucose loading does not acutely alter the serum total adiponectin level in humans. However, they only examined the effect of oral glucose on total adiponectin [11-13], unlike our study. Interestingly, it was reported by Pajvani et al [5] that a glucose load significantly reduced circulating HMW adiponectin, but not LWM adiponectin, after 120 minutes in mice. Recently, Basu et al [18] demonstrated that HMW adiponectin shows a selective decrease under hyperinsulinemic conditions, particularly in insulin-sensitive nondiabetic individuals. Thus, although the serum adiponectin level is generally believed to remain stable in vivo, it seems that HMW adiponectin is affected acutely by glucose intake in persons with NGT or IFG. In contrast, Yamauchi et al [19] recently reported that there was no significant change of HMW adiponectin during the OGTT in healthy subjects. Peake et al [10] also found no significant changes in the mean levels of adiponectin or the proportions of adiponectin in each isoform during the OGTT in healthy subjects. One possible explanation for this discrepancy between our study and the 2 studies is a difference in the sex of the subjects. We only studied male subjects, whereas their subjects were predominantly female; Peake at al especially studied only female subjects. It is well known that serum total adiponectin and HMW adiponectin levels are much higher in women than in men, along with a higher percentage of HMW adiponectin among adiponectin isoforms in women [5,17]. Thus, there appears to be a sex difference in the response of circulating HMW adiponectin to an oral glucose load.

The mechanisms responsible for an acute decrease of serum HMW adiponectin after glucose intake remain to be determined. Although glucose loading alters both the blood glucose and insulin concentrations, a previous euglycemic hyperinsulinemic clamp study showed that a short-term supraphysiologic serum insulin level caused a significant decrease of serum total adiponectin, which fell by 10% to 20% in response to hyperinsulinemia during the glucose clamp [20,21]. Thus, one possible explanation is that an elevated serum insulin level may lead to a decrease of HMW adiponectin. An in vitro study has shown that insulin caused a significant reduction of adiponectin messenger RNA expression in 3T3-L1 adipocytes in a dose- and time-dependent fashion [22]. Compared with persons who have NGT or IFG, early-phase insulin secretion is markedly impaired in persons with IGT or DM because the insulin AUC (30 minutes) was significantly larger in our NGT and IFG groups than in the DM group. Similarly, the insulinogenic index, an indicator of early insulin secretion [23], was significantly higher in our NGT and IFG groups than in the DM group. Furthermore, percentage changes in serum HMW adiponectin of the baseline at 120 minutes correlated negatively with percentage changes in serum insulin, but not in plasma glucose, of the baseline at 30 minutes. Thus, a rapid increase of the serum insulin level, that is, an early insulin response, after an oral glucose load may be associated with a decrease of serum HMW adiponectin in subjects with NGT or IFG. However, we could not exclude the possibility that hyperglycemia itself affected serum HMW adiponectin after glucose loading.

Interestingly, the present study showed that serum HMW adiponectin level at 180 minutes returned to the baseline level in subjects with NGT. Pajvani et al [5] also found that,

upon normalization of glucose, the normal level of HMW adiponectin was reconstituted after a glucose load in mice. Thus, this decrease of HMW adiponectin was transient. These results suggest that a rapid increase of serum insulin after the OGTT is involved in a transient decrease of serum HMW adiponectin in subjects with NGT. Thus, chronic hyperinsulinemia, a state of insulin resistance, may be associated with a prolonged down-regulation of HMW adiponectin in adipose tissues, resulting in a decrease of serum HMW adiponectin in subjects with metabolic syndrome or type 2 DM.

Adiponectin is very stable in vivo compared with other hormones, partly because its half-life is thought to be longer (5-14 hours) [10], although another study in humans indicated a relatively short half-life of 2.5 hours for circulating adiponectin [9]. The present study showed that only HMW adiponectin decreased after a glucose load, suggesting that the HMW form of adiponectin may be affected more rapidly than its LMW or medium–molecular weight forms. The reason why only HMW adiponectin decreased during the OGTT remains unclear; but the possible explanations include decreased secretion of HMW adiponectin by adipocytes, increased clearance of HMW adiponectin from the circulation, increased metabolism of HMW adiponectin, or a combination of these. Recently, Wang et al [24]demonstrated that a pair of endoplasmic reticulum chaperones, ERp44 (ER protein 44 kd) and Ero 1 (ER oxidoreductase 1), is critically associated with the assembly pathway of higher-order complexes of adiponectin. It is possible that insulin may affect the function of these chaperones, resulting in a decrease in secretion of HMW adiponectin by adipocytes. On the other hand, a study using rabbits showed that HMW adiponectin was more rapidly metabolized than LMW adiponectin because the fractional catabolic rate of the HMW form was significantly higher than that of the LMW form [10]. Taken together, it is possible that the acute decrease of HMW adiponectin after glucose intake may be associated with increased clearance from the circulation or with redistribution of HMW adiponectin from the intravascular to extravascular compartment [10]. Furthermore, as mentioned above, in subjects with NGT, serum HMW adiponectin level at 180 minutes returned to the baseline level. Thus, the circulating half-life of HMW adiponectin may be in the ranges of only a few hours.

In this study, we found no significant changes of serum total or HMW adiponectin after fat loading. Although serum TGs were significantly increased after an oral fat load, serum total or HMW adiponectin did not change. This result is in agreement with previous findings [12,13]. Several cross-sectional clinical studies have demonstrated that TG and/or high-density lipoprotein cholesterol is an independent determinant of total or HMW adiponectin in nondiabetic and diabetic subjects [25-27]. Because our fatty test meal included no sugar, an insulin response may have been absent during the fat loading test. Thus, it appears that there was no direct effect of an elevated TG level on serum HMW adiponectin in healthy subjects.

In conclusion, serum HMW adiponectin was acutely decreased by an oral glucose load in subjects with NGT or IFG, but IGT and DM interfered with this effect. A sudden decrease of serum HMW adiponectin may be associated with an increase of serum insulin after glucose loading.

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