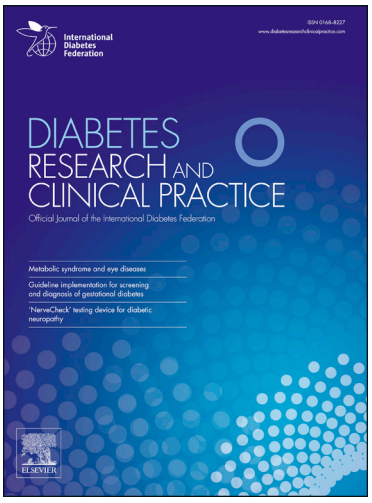

Serum Adiponectin/Ferritin Ratio in Relation to the Risk of Type 2 diabetes and Insulin Sensitivity

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Original contribution

Title

Serum Adiponectin/Ferritin Ratio in Relation to the Risk of Type 2 diabetes and Insulin Sensitivity

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ABSTRACT

Aims: Body iron inhibits the metabolism of adiponectin, an insulin sensitizing adipokine. We investigated the relationships of baseline and average of 4-year change in values of serum adiponectin (sA), serum ferritin (sF) and sA/sF ratio on type 2 diabetes (T2D) risk and insulin sensitivity (Matsuda ISI) and secretion (disposition index; DI_{30}).

Methods: Prospective analyses were conducted in participants with impaired glucose tolerance of the Finnish Diabetes Prevention Study (n=516) recruited in 1993-1998. Cox and linear regression analyses were used to investigate the associations of sA, sF and sA/sF ratio, as continuous variables, with incident T2D, Matsuda ISI, and DI_{30}.

Results: During the mean follow-up of 8.2 years, 157 incident T2D cases occurred (intervention group, n=65 and control group, n=92). In adjusted models, baseline sA and sA/sF ratio were inversely associated with T2D risk (HR=0.49, 95% CI 0.31-0.76, P=0.002 and HR=0.83, 95% CI 0.70-0.99, P=0.044, respectively). Furthermore, a direct association was observed with Matsuda ISI (β=0.13, 95% CI 0.03-0.22, P=0.009, for sA and β=0.04, 95% CI 0.01-0.07, P=0.035, for sA/sF ratio) during the average 4-year follow-up. The changes in sA and sA/sF ratio were also inversely associated with T2D risk (HR=0.36, 95% CI 0.20-0.63, P<0.001 and HR=0.76, 95% CI 0.62-0.92, P=0.006, respectively), and directly with Matsuda ISI (β=0.27, 95% CI 0.17-0.38, P<0.001, for sA and β=0.07, 95% CI 0.03-0.11, P<0.001, for sA/sF ratio). No consistent associations were found with DI_{30}

Conclusions: Baseline levels and changes during the follow-up in sA and sA/sF ratio are related to T2D risk and insulin sensitivity.

Keywords: Type 2 Diabetes, Impaired Glucose Tolerance, Diabetes Prevention Study, Serum Adiponectin, Serum Adiponectin/Ferritin Ratio, Prospective Study

1. Introduction

Excess body iron is a known independent risk factor of type 2 diabetes (T2D) [1]. Iron potentiates oxidative stress in the pancreatic beta cells [2] and facilitates insulin resistance in the liver and peripheral tissues [3]. The role of body iron in adipocyte insulin resistance and adiponectin metabolism [4] has been suggested as one of the mechanistic pathways by which elevated body iron stores may increase the risk of T2D [5]. A high body mass index (BMI) in an overweight or obese individual is a well-established predictor of T2D risk [6]. Furthermore, adiponectin; an insulin sensitizing adipokine, which is produced by the adipose tissue, is low in obese individuals [7]. A few studies have demonstrated low concentrations of serum adiponectin (sA) in people with T2D [8]. Further, a genetic variation in the locus of genes coding for the human adiponectin has been shown to contribute to sA concentrations, which in turn could modify the risk of T2D [9].

Body iron influences fat metabolism via its interaction with adipocytes and adiponectin [4, 10]. Thus, serum ferritin (sF), a known marker of body iron stores may interact with sA in modulating the risk of T2D. The influence of body iron stores on sA concentrations in the development of T2D is sparsely studied. Molecular studies have shown that iron represses the transcription of adiponectin mRNA [11], which leads to a reduction in adiponectin production and subsequent disturbance in insulin sensitivity. An inverse association has also been demonstrated between sF and sA in both epidemiological [12, 13] and molecular [11] studies. Some authors have suggested that a simultaneous analysis of sF and sA to predict insulin sensitivity and T2D risk may be more accurate than using either of the two biomarkers alone [12]. A few lifestyle intervention studies have assessed the effect of changes in sF concentration on insulin sensitivity [14] and changes in sA concentration on insulin sensitivity [15] and the risk of T2D [16]. No single lifestyle intervention study has simultaneously analyzed the effect of a change in both sF and sA concentrations on the risk of T2D and insulin sensitivity and secretion. Thus, we investigated the relationships of baseline and average 4-year change in values of sA, sF and sA/sF ratio on T2D risk and insulin sensitivity and secretion in subjects with impaired glucose tolerance (IGT).

2. Materials and Methods

2.1 Subjects and study design

The Finnish Diabetes Prevention Study (DPS) involved 522 overweight or obese individuals with IGT recruited from 1993 to 1998 in five centers in Finland, i.e. Helsinki, Kuopio, Turku, Tampere, and Oulu, who participated in a randomized controlled trial (NCT00518167, Clinical Trials. gov)

[17]. The inclusion criteria at screening were, age, 40-64 years, BMI>25kg/m^2 and IGT based on the mean value of two oral glucose tolerance tests (OGTTs) as defined by World Health Organization (WHO) 1985 criteria [18].

Excluded from DPS were people with the previous history of diabetes or any chronic conditions, which may disturb glucose homeostasis. Subjects finally included in the DPS were randomly allocated into two groups. The intervention group, n=265, which received intensive personalized counselling on weight loss of 5% at the minimum, physical activity on a moderate level (4-h/week), fat intake and saturated fat intake of less than 30 energy % and 10 energy %, respectively of the total energy consumed, and increase in dietary fiber intake to 15g/1000 kcal or more. The subjects in the control group, n=257, were advised on general information about healthy diet and the importance of physical exercise at the beginning of the study [17, 19]. The study annual follow-up visits (2001-2009) were discontinued once the diagnosis of T2D was established in any of the two groups, which formed part of the original protocol of the DPS.

A total of 516 subjects (intervention, n=263; control, n=253) served as the baseline for the current analysis. Active lifestyle intervention period lasted 1-6 years, but the follow-up analyses on the markers of insulin sensitivity and secretion were restricted to 1-4 years due to an approximate 60% loss of data on sF beyond this period as compared to the available baseline sF data (n=396). Furthermore, subjects with sF value above 1000 µg/L at baseline or at any of the follow-up visits were excluded (n=6). Hence, baseline and average 4-year change in values of sF, sA and the sA/sF ratio (n=390) were used to perform the final analysis with incident T2D and markers of insulin sensitivity and secretions during 6-year and average 4-year follow-up periods, respectively (Figure 1, supplementary data).

DPS was approved by the Ethics Committee of the National Public Health Institute of Finland, Helsinki. All study participants gave a written informed consent.

2.2 Data collection

Individuals completed a questionnaire on their medical history and physical activity. Data on BMI and other anthropometric measurements, dietary intake of foods using a 3-day food recording were obtained at baseline and during the subsequent annual follow-up visits [20]. A smoker in this study was defined as an individual smoking at least once a week at any time during the study period and the data were available for the entire study period. Data on alcohol and dietary intakes, such as, carbohydrates, fiber, and energy intake, were available from baseline until the third year follow-up

visit. Detailed descriptions of assessment of dietary intakes and physical activity have been published elsewhere [19]. Diagnosis of T2D was based on repeated OGTT applying WHO 1985 criteria for diabetes (fasting plasma glucose ≥7.8 mmol/L or 2-h glucose measurement≥11.1 mmol/L) [18], and confirmed by a physician.

2.3 Biochemical measurements

After an overnight fast, subjects visited the study sites to give fasting blood samples at baseline and during the annual follow-up study visits. Biochemical measurements of sF, sA and high sensitivity C-reactive protein (HsCRP) were carried out and processed at TETHYS Bioscience Inc., CA, USA, in 2010, from stored samples (-80 ^{o}C). sF was assayed using IMMULITE 2000 Ferritin; HsCRP was assayed by IMMULITE 2000 HsCRP, while sA was measured using enzyme linked immunosorbent assay. Standard guidelines that were used for glucose measurements were locally determined and standardized by the central laboratory in Helsinki [17]. Baseline 2-h OGTT was performed with 75g of glucose load during 1993 to 1996. In addition to OGTT performed at follow-up visits, which started during the mid-year of 1996, measurements of 30-minutes insulin and glucose and 60-minutes glucose were also performed. Serum insulin was assayed using radioimmunoassay (RIA Phadaseph Insulin RIA 100, Pharmacia Diagnostica, Uppsala, Sweden).

2.4 Calculations

Surrogate markers of insulin sensitivity and secretion were arithmetically computed. Matsuda index of insulin sensitivity (Matsuda ISI: 10000/√ [fasting glucose × fasting insulin] × [arithmetic mean of glucose × arithmetic mean of insulin during OGTT at 0, 30, and 120 minutes]) was calculated according to equations published earlier [21]. The ratio of total insulin area under the curve and glucose area under the curve during OGTT at 0 and 30 minutes ($AIGR_{0\text{-}30}$) was used as a surrogate of early-phase insulin secretion [22]. Disposition index (DI_{30}) was derived by multiplying $AIGR_{0\text{-}30}$ and Matsuda ISI [18]. The ratio of sA/sF was also arithmetically computed by dividing the value of sA concentration (µg/L) by sF concentration (µg/L).

2.5 Statistical analysis

Test of linear trend across tertiles of baseline sA/sF ratio was conducted for baseline characteristics of DPS participants (Table 1). Skewed variables (sA, sF, sA/sF ratio, Matsuda ISI, DI_{30}) were all normalized by logarithmic transformation (log10) and were treated as continuous variables in the models. The changes in the concentrations of sA, sF and sA/sF ratio between and within allocation groups of DPS was assessed using independent sample t-test and paired sample t-test, respectively.

Bivariate and multivariable regression analyses were used to examine the associations between sA and sF with metabolic parameters at baseline (Table 2)

Associations of baseline and average 4-year change in values of sA, sF and sA/sF ratio with incident T2D and Matsuda ISI and DI_{30} were examined in Cox and linear regression analyses, respectively (Table 3 to Table 5). The influence of baseline and average 4-year change in values of these biomarkers on the association between DPS randomization groups and incident T2D was examined in multivariate Cox regression models using interaction terms (Table 6). The models were adjusted for age (years), sex, DPS randomization groups (intervention and control), BMI (kg/m^2), alcohol intake (g/week), HsCRP (mg/L), baseline Matsuda ISI and adiponectin single nucleotide polymorphism (SNP, rs6773957) [9]. All statistical analyses were conducted with SPSS version 21 for Windows (Armonk, NY: IBM corporation) and tests of statistical significance ($P \leq 0.05$) were two sided.

3. Results

3.1 Characteristics of DPS participants

The characteristics of DPS participants according to tertiles of baseline sA/sF ratio are presented in Table 1. The mean age of the participants was 55.2±7.2 years and the mean sA, sF and sA/sF ratio were 8.05±3.45 µg/mL, 134.4 µg/L, and 164.40±317.17, respectively. The difference in the mean values of sA, sF and sA/sF ratio between DPS randomization groups did not reach statistical significance between baseline and 1-year or during the average 4-year follow-up visit. However, within the intervention group, the difference in the mean values between baseline and 1-year follow-up showed a statistically significant increase in sA (P=0.050), and a decrease in sF (P=0.017) and sA/sF ratio (P=0.004). In the control group, there was a decrease in the mean value of sF (P=0.023), whereas no significant change was found in sA (P=0.506). A marginal increase was, however, observed in the mean values of sA/sF ratio (P=0.065). During the average 4-year follow-up, the changes in the mean values of these biomarkers were similar to that observed during 1-year follow-up. Participants in the lowest sA/sF ratio were likely to have higher energy intake and lower sessions of leisure time physical activity (Table 1).

3.2 Associations between sA and sF with metabolic parameters at baseline

The associations between sA and sF with metabolic parameters at baseline are presented in Table 2. While an inverse association was observed between sA and fasting plasma glucose and insulin at baseline, a direct association was observed between sF and these parameters.

3.3 Association of baseline and average 4-year change in values of sA, sF and sA/sF ratio with the risk of T2D

During the mean follow-up of 8.2 years, 157 incident T2D cases occurred (intervention group, n= 65 [41.4% of the cases] and control group, n=92 [58.6%]). In multivariate-adjusted Cox regression models, baseline sA and sA/sF ratio were inversely associated with the risk of T2D. Similar inverse associations were observed between average 4-year change in the values of sA and sA/sF ratio and the risk of T2D (Table 3). As expected, these associations became weaker after adjustment for baseline Matsuda ISI and adiponectin SNP rs6773957 that modifies adiponectin concentration [9]. No statistically significant associations were observed with baseline sF. Baseline and the average 4-year change in values of sA showed an approximate 50% greater reduction in T2D risk when compared with the corresponding reduction observed with sA/sF ratio (Table 3).

3.4 Association of baseline sA, sF and sA/sF ratio with Matsuda ISI and DI_{30} during the average 4-year follow-up.

In the multivariable linear regression models, baseline sA and sA/sF ratio were directly associated with Matsuda ISI during the average 4-year follow-up (Table 4). In Model 3, sA showed an approximately threefold increase in the strength of the association observed with Matsuda ISI when compared with the association observed with sA/sF ratio. No associations were observed with $DI_{30.}$

3.5 Association of average 4-year change in values of sA, sF and sA/sF ratio with Matsuda ISI and DI_{30} during the average 4-year follow-up.

The average 4-year change in values of sA and sA/sF ratio was directly associated with Matsuda ISI during the average 4-year follow-up (Table 5). However, the change in the value of sF was inversely associated with Matsuda ISI. The strength of the associations observed with the average 4-year change in values of the markers was higher than the associations observed when baseline values of these markers were used (Table 4). No consistent associations were observed with $DI_{30.}$

3.6 Effect of sA and sA/sF ratio on the association between DPS randomization groups and the risk of T2D.

The influence of baseline sA and average 4-year change in values of sA and sA/sF ratio on the association between DPS randomization groups and T2D risk are presented in Table 6. The interaction term between DPS randomization groups and baseline sA was statistically significant with T2D risk (P=0.018). While the average 4-year change in values of sA showed a similar

influence on the association between DPS randomization groups and T2D risk, no statistically significant influence (P=0.065) was observed with the average 4-year change in sA/sF ratio.

4. Discussion

We investigated the association of baseline and average 4-change in values of sA, sF and sA/sF ratio with T2D risk and insulin sensitivity and secretion in a lifestyle intervention study. Baseline and average 4-year change in values of sA and sA/sF ratio predicted a decreased risk of T2D. The inverse associations were attenuated with further adjustment for baseline Matsuda ISI and adiponectin SNP rs6773957, a determinant of sA concentration [9]. Baseline and average 4-year change in values of sA showed a stronger inverse association with the incidence of T2D when compared to the corresponding associations observed with sA/sF ratio. The strength of the associations observed using both baseline and average 4-year change in values of sA and sA/sF ratio with T2D risk was similar. Our findings are in agreement with the results in the Diabetes Prevention Program by Mather et al. [16], which investigated the association of baseline and treatment-related change in adiponectin with incident T2D. They found an inverse relationship between baseline and treatment-related-change in adiponectin and T2D risk during a relatively short follow-up period when compared with our study.

In our study, we also observed a direct association between baseline sA and sA/sF ratio with Matsuda ISI during the average 4-year follow-up. In addition, during the average 4-year change in the values of these markers, although, the direction of the associations observed with Matsuda ISI remained the same, the strength of the associations increased. Interestingly, sF showed an inverse association with Matsuda ISI during the average 4-year follow-up, supporting the view that sF may modify insulin sensitivity [3]. In line with the decreased T2D risk, the baseline and average 4-year change in values of sA showed a stronger association with Matsuda ISI than the corresponding sA/sF ratio.

Kelly et al. [15] investigated the effect of lifestyle modification on adipokines. They found a significant increase in high molecular weight adiponectin and adiponectin/leptin ratio after 12 weeks of intervention within each allocation group. Their findings support our results in which we found a statistically significant increase in sA within DPS intervention group, and after a longer follow-up. Our results provide additional evidence for the positive effect of lifestyle intervention on sA concentrations. Further, the change in high molecular weight adiponectin was inversely

correlated with insulin resistance (r=−0.48) in Kelly et al. study [15], which supports the direct association observed in our study between changes in sA and Matsuda ISI.

Previous epidemiological studies [23, 24] have shown that sA and sF are predictors of insulin sensitivity and T2D risk. Li et al. [23] in a meta-analysis of thirteen prospective studies found an inverse association between sA and T2D risk. They observed a relative risk of 0.72 for T2D per 1 μg/mL increase in sA. Likewise, Kunutsor et al. [24], in a meta-analysis of twelve studies showed a relative risk of 1.73 for T2D in subjects in the highest sF group compared to the lowest group. However, to the best of our knowledge, no report has been published so far in long-term intervention trials on the effect of simultaneous changes in both sF and sA on the risk of T2D and insulin sensitivity and secretion.

Gabrielsen et al. [11] demonstrated the inhibitory effect of iron on adiponectin production. In addition, Ku et al. [12] in their cross-sectional study showed an inverse relationship between sF and sA. They further suggested that a simultaneous analysis of both biomarkers may help to identify risk individuals in predicting the development of T2D. We tested this idea by using sA, sF and sA/sF ratio to predict the risk of T2D in the same cohort. We found that the strongest association with the risk of T2D was observed with sA as compared with the associations observed using other studied markers. A similar idea was tested in a Danish study designed to assess 5-year risk of developing T2D. They found a model, which incorporated six circulating biomarkers, which included sF and sA as a better predictor of T2D risk than the individual marker [25].

An intervention study by Roumen et al. [14] found no statistically significant difference in sF concentrations between the intervention and control group after 1-year follow-up. This is in keeping with our results in which the difference in the mean values of sF between the DPS randomization groups did not reach statistical significance. However, we observed a significant decrease in the mean values of sF within both intervention and control groups. Contrary to Roumen et al. study [14], which reported no association between changes in sF and insulin sensitivity, we observed a statistically significant inverse association between changes in sF and Matsuda ISI.

Since both sA and sA/sF ratio were inversely related to the risk of T2D, we examined the contribution of these two markers to the association observed between DPS randomization groups and T2D risk. While both markers contributed to the association between the DPS randomization groups and T2D risk, sA, however, showed a stronger contribution to the risk. In addition to providing further support for the previous studies, which found an inverse relationship between

single measurement of sA and T2D risk [23], our study showed the effect of changes in repeated sA measurements on T2D risk and insulin sensitivity.

The anti-inflammatory and insulin-sensitizing properties of adiponectin have been suggested in the pathophysiological mechanisms through which adiponectin reduces the risk of T2D. Adiponectin downregulates inflammation through the activation of its receptors (AdipoR1, AdipoR2, and T-cadherin) [26]. It inhibits inflammatory cells (tumor necrosis factor-α, and interleukin-6), which are associated with insulin resistance. It improves insulin sensitivity via cellular glucose uptake and decreases hepatic gluconeogenesis through the activation of adenosine monophosphate –activated protein kinase [27].

Further, the negative influence of sF on sA is supported by a biological link between iron and adiponectin. Iron decreases the acetylation of Foxo1, adiponectin-transcription stimulators [28, 29], thus, repressing the transcription of adiponectin [11]. In addition, adipocytes, contains ferroportin; an iron-export peptide that regulates adipocyte iron load and as such, adiponectin secretion is regulated by the iron load.

The strengths of our study include the use of both baseline and long-term changes in values of sA, sF and sA/sF ratio to investigate the risk of T2D in people with IGT. Furthermore, we examined insulin sensitivity by calculating Matsuda ISI and DI_{30} reflecting insulin secretion. The use of sA/sF ratio and the simultaneous analysis of sA and sF allowed us to test the strength of the association of these markers with insulin sensitivity and T2D risk. A limitation of our study is the use of a cohort that comprised of participants with IGT who were advised on the importance of physical activity and quality of diet, such as, reduced red meat intake to reduce the diabetes risk. These lifestyle changes could have had a direct effect on body iron stores. The mean sF concentrations at baseline (134.4 µg/L) and during the average 4-year follow-up (127.3 µg/L) in our study were well below the concentrations at which body iron has been shown to cause an increase in the risk of T2D [30]. This may be one reason why sF predicted only insulin sensitivity but not the risk of T2D.

In conclusion, our results suggest that baseline and follow-up changes in sA and sA/sF ratio are related to T2D risk and insulin sensitivity with stronger associations observed with sA. No consistent associations were observed with insulin secretions as assessed by DI_{30}. While sA and sA/sF ratio influenced to some extent the effect of DPS intervention on the risk of T2D, a stronger effect was observed with sA.

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Conflict of interests

None declared

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Table 1 Baseline characteristics of the participants in the DPS according to tertiles of baseline serum adiponectin/serum ferritin ratio

	sA/sF ratio			
Variables	1 (3.7 to 51.8)	2 (51.9 to 118.7)	3 (118.8 to 4031.7)	P-trend
Age (years)	56.3±6.9	55.5±6.7	54.1±7.7	0.014
Male gender (%)	64.6	40.7	40.7	<0.001
DPS intervention group (%)	48.5	53.1	49.2	0.902
BMI (kg/m^2)	30.9 ±4.1	31.2±4.8	31.0±5.1	0.897
Carbohydrate intake (E %)	42.3	43.8	44.4	0.018
Total fat intake (E %)	36.5	36.3	37.4	0.265
Fiber intake (g/1000kcal)	11.0±3.7	12.5±4.2	12.0±3.9	0.051
Alcohol intake (g/week)	10.2±19.8	4.8±9.1	3.0±5.9	<0.001
Smoker (%)	12.3	10	12.2	0.954
Energy intake (kcal/day)	1881±53	1706±496	1735±522	0.024
Leisure time physical activity (minutes/week)	7.21±5.43	7.49±642	7.39±6.45	0.815
Serum ferritin (µg/L)	253.8±129.1	107.3±38.7	42.1±29.2	<0.001
Serum adiponectin (µg/mL)	6.7±2.2	8.1±2.4	9.4±4.7	<0.001
Serum C-reactive protein (mg/L)	3.11±3.17	3.98±5.12	4.14±4.99	0.068
Fasting plasma glucose (mmol/L)	5.99±0.62	5.98±0.54	5.84±0.59	0.033
Two-hour plasma glucose (mmol/L)	8.47±1.29	8.63±1.19	8.51±1.24	0.795
Fasting plasma insulin (mU/L)	15.36±7.23	14.11±7.43	12.63±5.80	0.003
Two-hour plasma insulin (mU/L)	91.36±58.94	99.01±78.92	80.99±56.57	0.232
Matsuda ISI	2.74±1.55	2.85±1.64	3.20±1.67	0.031

Values are mean ±SD or percentages.
P value for the trend across tertiles of baseline serum adiponectin/serum ferritin ratio (sA/sF ratio)
DPS=Finnish Diabetes Prevention Study; Matsuda ISI=Matsuda insulin sensitivity index at 0, 30 and 120 minutes

Table 2 Associations between serum adiponectin and serum ferritin with metabolic parameters at baseline

	Serum adiponectin (µg/mL)	Serum ferritin (µg/L)
Bivariate analysis		
Age (years)	β= 0.19, 95% CI 1.85 to 5.80, P<0.001	β= 0.20, 95% CI 1.70 to 4.99, P<0.001
BMI (kg/m^2)	β= 0.27, 95% CI −1.03 to 1.58, P=0.680	β= 0.05, 95% CI −1.04 to 1.14, P=0.929
Energy intake (kcal/day)	β= −0.16, 95% CI −0.21 to −0.05, P=0.002	β= 0.08, 95% CI −0.01 to 0.12, P=0.120
FPG (mmol/L)	β= −0.11, 95% CI −0.06 to −0.01, P=0.034	β= 0.12, 95% CI 0.01 to 0.15, P=0.021
FPI (mU/L)	β= −0.27, 95% CI −0.48 to −0.22, P<0.001	β= 0.11, 95% CI 0.01 to 0.23, P=0.039
Matsuda ISI	β= 0.27, 95% CI 0.25 to 0.55, P<0.001	β= −0.08, 95% CI −0.23 to 0.04, P= 0.159
Serum CRP (mg/L)	β= 0.06, 95% CI −0.12 to 0.46, P=0.241	β= −0.04, 95% CI −0.35 to 0.13, P=0.387
Multivariable analysis		
Energy intake (Kcal/day)		
Model 1	β= −0.02, 95% CI −0.10 to 0.06, P=0.687	β= −0.04, 95% CI −0.10 to 0.04, P=0.474
Model 2	β= −0.01, 95% CI −0.09 to 0.07, P=0.824	β= −0.07, 95% CI −0.12 to 0.02, P=0.156
Model 3	β= −0.01, 95% CI −0.08 to 0.10, P=0.846	β= −0.04, 95% CI −0.11 to 0.05, P=0.507
FPG (mmol/L)		
Model 1	β= −0.10, 95% CI −0.06 to 0.01, P=0.058	β= 0.12, 95% CI 0.01 to 0.05, P=0.041
Model 2	β= −0.10, 95% CI −0.06 to 0.01, P=0.062	β= 0.09, 95% CI −0.01 to 0.05, P=0.129
Model 3	β= −0.04, 95% CI −0.08 to 0.04, P=0.527	β= 0.07, 95% CI −0.01 to 0.04, P=0.250
FPI (mU/L)		
Model 1	β= −0.28, 95% CI −0.49 to −0.22, P<0.001	β= 0.17, 95% CI 0.06 to 0.31, P=0.005
Model 2	β= −0.27, 95% CI −0.47 to −0.23, P<0.001	β= 0.12, 95% CI 0.01 to 0.25, P=0.031
Model 3	β= −0.01, 95% CI −0.09 to 0.06, P=0.613	β= 0.04, 95% CI −0.02 to 0.11, P=0.169

Model 1 is adjusted for age, sex and DPS randomization group
Model 2 is adjusted for Model 1 plus BMI, alcohol intake and serum CRP
Model 3 is adjusted for Model 2 plus baseline Matsuda ISI and adiponectin SNP (rs6773957)
β=regression coefficient; CI=confidence interval
Serum adiponectin and serum ferritin were log-transformed and treated as continuous variables.
DPS=Finnish Diabetes Prevention Study; BMI=body mass index; CRP=C-reactive protein; FPG= baseline fasting plasma glucose; FPI=baseline fasting plasma insulin

Table 3 Association of baseline and average 4-year change in values of serum adiponectin, serum ferritin and serum adiponectin/serum ferritin ratio with the risk of type 2 diabetes in DPS participants

		Serum adiponectin (µg/mL)	Serum ferritin (µg/L)	Serum adiponectin/serum ferritin ratio
Baseline				
	Model 1	HR=0.55, 95% CI 0.36 to 0.83, P =0.005	HR=1.41, 95% CI 0.92 to 2.17, P =0.112	HR=0.81, 95% CI 0.68 to 0.96, P=0.015
	Model 2	HR=0.49, 95% CI 0.31 to 0.76, P =0.002	HR=1.26, 95% CI 0.82 to 1.94, P =0.302	HR=0.83, 95% CI 0.70 to 0.99, P=0.044
	Model 3	HR=0.42, 95% CI 0.37 to 1.02, P=0.060	HR=1.18, 95% CI 0.74 to 1.90, P=0.498	HR=0.80, 95% CI 0.72 to 1.07, P=0.195
Average 4-year change				
	Model 1	HR=0.41, 95% CI 0.24 to 0.71, P=0.001	HR=1.76, 95% CI 1.09 to 2.84, P=0.021	HR=0.73, 95% CI 0.61 to 0.89, P=0.002
	Model 2	HR=0.36, 95% CI 0.20 to 0.63, P<0.001	HR=1.54, 95% CI 0.94 to 2.50, P=0.084	HR=0.76, 95% CI 0.62 to 0.92, P=0.006
	Model 3	HR=0.40, 95% CI 0.25 to 0.87, P=0.017	HR=1.46, 95% CI 0.86 to 2.46, P=0.153	HR=0.80, 95% CI 0.65 to 0.99, P=0.038

Model 1 is adjusted for age, sex and DPS randomization group
Model 2 is adjusted for Model 1 plus body mass index, alcohol intake and serum C-reactive protein
Model 3 is adjusted for Model 2 plus baseline Matsuda ISI and adiponectin SNP (rs6773957)
HR=hazards ratio; CI=confidence interval
Serum adiponectin in (µg/mL) was converted to (µg/L) in serum adiponectin/serum ferritin ratio
Serum adiponectin, serum ferritin and serum adiponectin-serum ferritin ratio were log-transformed and treated as continuous variables.
DPS=Finnish Diabetes Prevention Study

Table 4 Associations of baseline serum adiponectin, serum ferritin and serum adiponectin/serum ferritin ratio with Matsuda ISI and DI_{30} during the average 4-year follow-up in participants from the DPS

		Serum adiponectin (µg/mL)	Serum ferritin (µg/L)	Serum adiponectin/ferritin ratio
Matsuda ISI	Model 1	β= 0.45, 95% CI 0.30 to 0.59, P <0.001	β= −0.20, 95% CI −0.33 to −0.07, P =0.002	β= 0.13, 95% CI 0.08 to 0.18, P<0.001
	Model 2	β= 0.44, 95% CI 0.31 to 0.57, P <0.001	β= −0.14, 95% CI −0.27 to −0.02, P =0.024	β= 0.11, 95% CI 0.06 to 0.16, P<0.001
	Model 3	β= 0.13, 95% CI 0.03 to 0.22, P=0.009	β= −0.05, 95% CI −0.14 to 0.03, P =0.212	β= 0.04, 95% CI 0.01 to 0.07, P=0.035
DI_{30}	Model 1	β= 0.05, 95% CI −0.06 to 0.16, P=0.381	β= −0.05, 95% CI −0.15 to 0.05, P=0.310	β= 0.03, 95% CI −0.01 to 0.07, P=0.207
	Model 2	β= 0.05, 95% CI −0.06 to 0.15, P=0.390	β= −0.02, 95% CI −0.12 to 0.08, P=0.688	β= 0.01, 95% CI −0.03 to 0.05, P=0.493
	Model 3	β= 0.001, 95% CI −0.12 to 0.12, P=0.981	β= −0.03, 95% CI −0.14 to 0.08, P=0.600	β= 0.01, 95% CI −0.03 to 0.05, P=0.629

Model 1 is adjusted for age, sex and DPS randomization group
Model 2 is adjusted for Model 1 plus body mass index, alcohol intake and serum C-reactive protein
Model 3 is adjusted for Model 2 plus baseline Matsuda ISI and adiponectin SNP (rs6773957)
Matsuda ISI=Matsuda insulin sensitivity index at 0, 30 and 120 minutes
DI_{30}=Disposition index during 0-30 minutes
β=regression coefficient; CI=confidence interval
Serum adiponectin, serum ferritin and serum adiponectin-serum ferritin ratio were log-transformed and treated as continuous variables.
DPS=Finnish Diabetes Prevention Study

Table 5 Associations of 4-year change in serum adiponectin, serum ferritin and serum adiponectin/serum ferritin ratio with Matsuda ISI and DI_{30} during the average 4-

year follow-up in participants from the DPS

		Serum adiponectin (µg/mL)	Serum ferritin (µg/L)	Serum adiponectin-ferritin ratio
Matsuda ISI	Model 1	β= 0.68, 95% CI 0.52 to 0.83, P <0.001	β= −0.25, 95% CI −0.39 to −0.11, P<0.001	β= 0.17, 95% CI 0.12 to 0.22, P<0.001
	Model 2	β= 0.68, 95% CI 0.54 to 0.82, P<0.001	β= −0.18, 95% CI −0.32 to −0.05, P =0.008	β= 0.15, 95% CI 0.10 to 0. 20, P<0.001
	Model 3	β= 0.27, 95% CI 0.17 to 0.38, P<0.001	β= −0.10, 95% CI −0.19 to −0.01, P =0.040	β= 0.07, 95% CI 0.03 to 0.11, P<0.001
DI_{30}	Model 1	β= 0.10, 95% CI −0.02 to 0.23, P=0.096	β= −0.11, 95% CI −0.22 to −0.01, P=0.037	β= 0.05, 95% CI 0.01 to 0.10, P=0.012
	Model 2	β= 0.10, 95% CI −0.02 to 0.22, P=0.092	β= −0.08, 95% CI −0.19 to −0.03, P=0.136	β= 0.04, 95% CI 0.001 to 0.09, P=0.049
	Model 3	β= 0.06, 95% CI −0.08 to 0.20, P=0.381	β= −0.09, 95% CI −0.21 to 0.03, P=0.137	β= 0.04, 95% CI −0.006 to 0.09, P=0.090

Model 1 is adjusted for age, sex and DPS randomization group
Model 2 is adjusted for Model 1 plus body mass index, alcohol intake and serum C-reactive protein
Model 3 is adjusted for Model 2 plus baseline Matsuda ISI and adiponectin SNP (rs6773957)
Matsuda ISI=Matsuda insulin sensitivity index at 0, 30 and 120 minutes
DI_{30}=Disposition index during 0-30 minutes
β=regression coefficient; CI=confidence interval
Serum adiponectin, serum ferritin and serum adiponectin-serum ferritin ratio were log-transformed and treated as continuous variables.
DPS=Finnish Diabetes Prevention Study

Table 6 Influence of serum adiponectin and serum adiponectin/ferritin ratio on the association between DPS randomization groups and type 2 diabetes risk

Variables	HR	95% CI		P-values
		Low CI	High CI	
Age (years)	0.98	0.95	1.00	0.037
Sex	0.80	0.53	1.21	0.296
Body mass index (kg/m^2)	1.05	1.01	1.09	0.008
Alcohol intake (g/week)	1.01	1.00	1.02	0.042
Serum CRP (mg/L)	1.06	0.88	1.27	0.560
Baseline Matsuda ISI	0.56	0.40	0.78	0.001
Adiponectin SNP	0.95	0.73	1.22	0.676
DPS randomization group	1.84	1.30	2.60	0.001
Influence of baseline serum adiponectin				
Age (years)	0.97	0.95	1.00	0.026
Sex	0.79	0.52	1.20	0.271
Body mass index (kg/m^2)	1.05	1.01	1.09	0.010
Alcohol intake (g/week)	1.01	1.00	1.02	0.034
Serum CRP (mg/L)	1.04	0.86	1.25	0.712
Baseline Matsuda ISI	0.54	0.38	0.75	<0.001
Adiponectin SNP	0.90	0.70	1.16	0.414
DPS randomization group*baseline sA	1.19	1.03	1.38	0.018
Influence of 4-year change in values of serum adiponectin				
Age (years)	0.97	0.95	1.00	0.027
Sex	0.79	0.52	1.21	1.280
Body mass index (kg/m^2)	1.05	1.01	1.09	0.012
Alcohol intake (g/week)	1.01	1.00	1.02	0.032
Serum CRP (mg/L)	1.04	0.86	1.25	0.687
Baseline Matsuda ISI	0.54	0.38	0.75	<0.001
Adiponectin SNP	0.90	0.70	1.16	0.419
DPS randomization group*4-year change in sA values	1.20	1.03	1.39	0.021
Influence of 4-year change in values of serum adiponectin/serum ferritin ratio				
Age (years)	0.98	0.95	1.00	0.064
Sex	0.77	0.50	1.19	0.246
Body mass index (kg/m^2)	1.05	1.01	1.09	0.009
Alcohol intake (g/week)	1.01	1.00	1.03	0.015
Serum CRP (mg/L)	1.03	0.85	1.24	0.761
Baseline Matsuda ISI	0.55	0.39	0.77	<0.001
Adiponectin SNP	0.90	0.70	1.16	0.400
DPS randomization group*4-year change in sA/sF ratio values	1.06	1.00	1.13	0.065

Model 1 is adjusted for age, sex and DPS randomization group
Model 2 is adjusted for Model 1 plus body mass index, alcohol intake and serum C-reactive protein (CRP)
Model 3 is adjusted for Model 2 plus baseline Matsuda ISI and adiponectin SNP (rs6773957)
HR=hazards ratio; CI=confidence interval
Matsuda ISI=Matsuda insulin sensitivity index at 0, 30 and 120 minutes
Serum adiponectin in (µg/mL) was converted to (µg/L) in serum adiponectin/serum ferritin ratio
*=interaction term
DPS=Finnish Diabetes Prevention Study

Serum adiponectin (sA), and serum adiponectin/serum ferritin (sA/sF) ratio were log-transformed and treated as continuous variables.

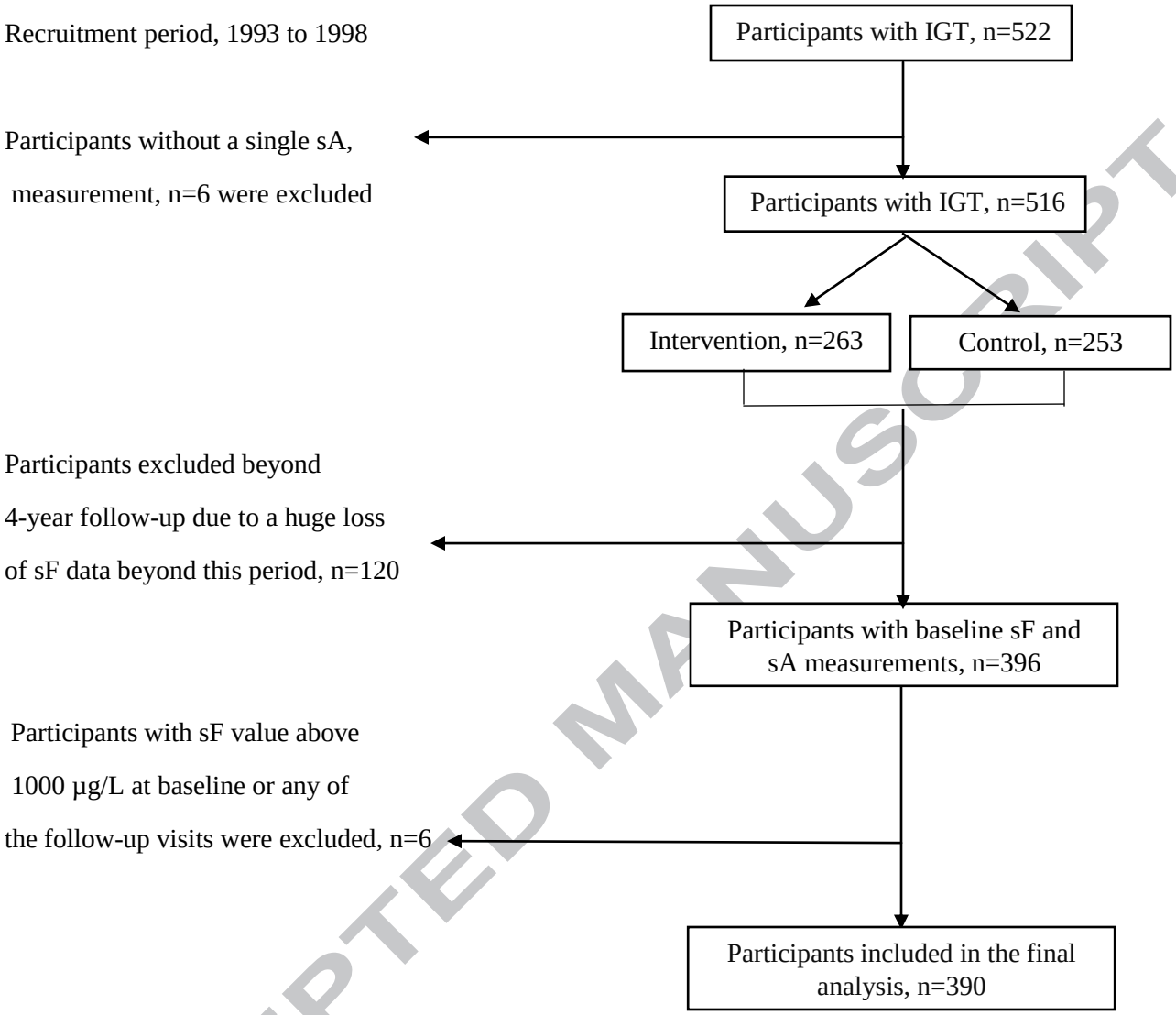

Figure 1. The schema of the total number of participants used in the final analysis.

IGT, impaired glucose tolerance; sF, serum ferritin; sA, serum adiponectin.