Associations between GDF15 levels and pre-diabetes in non-obese subjects

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ABSTRACT

Growth differentiation factor 15 (GDF15) is a stress-response cytokine which belongs to the transforming growth factor β superfamily. Although GDF15 was initially found to have a role in metabolic diseases, the association between GDF15 and dysglycemic status remains inconclusive. Thus, the aim of this study was to examine the relationships between GDF15 and different glycemic statuses in non-obese subjects. We enrolled 502 non-obese subjects, among individuals who had normal glucose tolerance (NGT; n=125), isolated impaired fasting glucose (IFG; n=116), isolated impaired glucose tolerance (IGT; n=106), IFG plus IGT (n=27), and newly diagnosed diabetes (NDD; n=128). A multivariate linear regression analysis of GDF15 levels was used to find independent predictors. The median (IQR) GDF15 levels were 1641.0 (1187.0–1985.5) pg/mL, 1656.1 (1226.8–2379.7) pg/mL, 1487.8 (1145.9–1987.2) pg/mL, 1722.2 (1172.9–1939.0) pg/mL, and 2204.5 (1767.4–2919.1) pg/mL in NGT, IFG, IGT, IFG plus IGT, and NDD groups, respectively. The NDD group had significantly higher GDF15 levels than those with NGT, IFG, IGT, and IFG plus IGT. The IFG group had a significantly higher GDF15 value than the NGT group. In multivariate linear regression analysis, IFG (beta=0.145, 95% CI 192.487 to 740.937, p=0.001), NDD (beta=0.227, 95% CI 390.459 to 888.145, p<0.001), and high-sensitivity C reactive protein (beta=0.105, 95% CI 3.276 to 27.768, p=0.013) were independently associated with GDF15 levels. Non-obese subjects with isolated IFG and NDD had significantly higher GDF15 levels than those with NGT. In addition, A1C was independently associated with GDF15 levels. IFG and NDD, but not isolated IGT or IFG plus IGT, were positively associated with GDF15 levels.

Significance of this study

What is already known about this subject?
- Growth differentiation factor 15 (GDF15) is a stress-response cytokine which belongs to the transforming growth factor β superfamily.
- GDF15 was initially found to have a role in metabolic diseases.
- The association between GDF15 and dysglycemic status remains inconclusive.

What are the new findings?
- Non-obese subjects with isolated impaired fasting glucose (IFG) and newly diagnosed diabetes (NDD) had significantly higher GDF15 levels than those with normal glucose tolerance.
- A1C was independently associated with GDF15 levels.
- IFG and NDD, but not isolated impaired glucose tolerance (IGT) or IFG plus IGT, were positively associated with GDF15 levels.

How might these results change the focus of research or clinical practice?
- GDF15 level had a potential to be a biomarker for the diagnosis of IFG and type 2 diabetes in non-obese subjects.

INTRODUCTION

Growth differentiation factor 15 (GDF15), also known as macrophage-inhibiting cytokine 1, is a stress-response cytokine which belongs to the transforming growth factor β superfamily. GDF15 was found to be expressed in most tissues, and it can be upregulated in most cell types in response to various stresses in vitro.[1] It was known that glial cell-derived neurotrophic factor (GDNF) family receptor α-like (GFRAL), a GDF15 receptor, can be detected in the neurons of the area postrema and nucleus of the solitary tract, which are important hindbrain centers involved in appetite regulation. In addition, studies elucidated physiological pathways in which GDF15 regulates energy homeostasis and body weight via appetite suppression.[1] Although the functions of GDF15 in the regulation of appetite and body weight were well illustrated,[1] the primary physiological role of GDF15 is still unclear. Nevertheless, elevated circulating GDF15 levels are associated with aging, cancer, cachexia, cardiovascular diseases (CVDs), kidney diseases, appetite regulation, and metabolic diseases.[2 3]

Pre-diabetes is defined as a heterogeneous metabolic state of glucose dysregulation, which includes impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and intermediate hemoglobin A1C (HbA1C) levels. Subjects with pre-diabetes are at high risk of diabetes

and CVDs. Pathophysiologically, IFG is characterized by a combination of hepatic insulin resistance (IR) and defective first-phase insulin secretion, while IGT primarily consists of peripheral IR and impaired first-phase and second-phase insulin secretion.[4]

Previous studies suggested a link between GDF15 and glucose metabolism. In a middle-aged urban population, circulating levels of GDF15 were positively associated with risk of incident diabetes over a 19-year follow-up period.[5] In subjects with severe obesity, GDF15 was higher in those with pre-diabetes and diabetes, but was not associated with the grade of impairment of glucose metabolism.[6] However, the association between GDF15 and dysglycemic status, especially in non-obese subjects, remains inconclusive.

To investigate whether GDF15 is associated with impaired glucose metabolism in non-obese subjects, this study examined the relationship between GDF15 levels and different glycemic statuses, including normal glucose tolerance (NGT), isolated IFG, isolated IGT, IFG plus IGT, and newly diagnosed diabetes (NDD).

METHODS

All study subjects provided written informed consent. From June 2007 to July 2009, subjects aged 20–80 years who had been admitted for a health check-up at the Prevention Health Center of National Cheng Kung University Hospital were screened. All subjects underwent 12-hour overnight fasting and blood sampling for biochemical examination and complete blood count. Subjects who did not have a history of diabetes mellitus received a 75 g oral glucose tolerance test (OGTT).

Each subject's body height and weight in light indoor clothing were measured. Body mass index (BMI) (in kg/m^2) was calculated as the weight (in kilograms) divided by the height (in meters squared). Obesity was defined according to the recommendations of the Health Promotion Administration in Taiwan as BMI ≥ 27 kg/m^2. For blood pressure measurements, each subject rested 10 min in a supine position in a quiet environment after a 12-hour fast. Two blood pressure readings, separated by an interval of at least 5 min, were taken with an appropriate-sized cuff wrapped around the right upper arm using a DINAMAP vital signs monitor (Model 1846SX; Critikon, Irvine, California, USA). Subjects with systolic blood pressure (SBP) of ≥140 mm Hg, or diastolic blood pressure (DBP) of ≥90 mm Hg, or a history of hypertension were defined as having hypertension.

Individuals who had the following diseases or conditions were excluded from the study: obesity, pregnancy, history of diabetes mellitus, history of malignancy, history of CVD, any acute or chronic inflammatory disease as determined by a leukocyte count of ≥10^4per microliter or clinical signs of infection, elevated AST/ALT of more than two times the normal upper limit, or creatinine of >1.5 mg/dL.

Blood glucose was measured by a hexokinase method. Isolated IFG was defined as a fasting plasma glucose (FPG) of 100–125 mg/dL and a 2-hour postload glucose of <140 mg/dL. Isolated IGT was defined as an FPG of <100 mg/dL and a 2-hour postload glucose level of 140–199 mg/dL. NDD was diagnosed by an FPG of >126 mg/dL or a 2-hour postload glucose of >200 mg/dL. Serum insulin was measured by ELISA (Mercodia, Uppsala, Sweden). IR was defined by the homeostasis model assessment-insulin resistance (HOMA-IR) index as the following: fasting insulin (μU/mL) × FPG (mM)/22.5. A1C was measured with a high-performance liquid chromatographic method (Tosoh Automated Glycohemoglobin Analyzer HLC-723GHbVA1c 2.2; intra-assay coefficient of variation (CV) of 0.5% and interassay CV of 2.0%; Tokyo, Japan). High-sensitivity C reactive protein (hsCRP) was measured using a highly sensitive ELISA kit (intra-assay CV of 2.9% and interassay CV of 4.7%; Immunology Consultants Laboratory, Lake Oswego, Oregon, USA). Serum total cholesterol, triglyceride (TG), high-density lipoprotein (HDL) cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and creatinine levels were determined at the central laboratory of National Cheng Kung University Hospital. Estimated glomerular filtration rate (eGFR, mL/min/1.73 m^2) was calculated using the Modification of Diet in Renal Disease equation. Chronic kidney disease was defined as an eGFR of <60 mL/min/1.73 m^2. GDF15 levels were measured using a commercial human GDF15 ELISA Kit (R&D Systems, Minneapolis, Minnesota, USA) with intra-assay and interassay CVs of <6% and 2.8%, respectively.

SPSS software (V.25.0) was used for all statistical analyses. Continuous variables are expressed as mean±SD or percentages. GDF15 and TG concentrations are expressed as median (IQR) and were log-transformed before analysis. Study subjects were categorized into one of the following five groups: NGT, isolated IFG, isolated IGT, IFG plus IGT, and NDD. Continuous variables among the five groups were compared using an analysis of variance or a Kruskal-Wallis test for non-normally distributed variables. χ^2 tests were used to analyze differences in categorical variables among groups. A multivariate linear regression analysis was conducted to identify independent factors associated with the GDF15 concentration. Variables included age, sex, A1C, BMI, HOMA-IR, SBP, hsCRP, TG, HDL, and eGFR. A p value of <0.05 was considered statistically significant.

RESULTS

A total of 502 subjects were enrolled in this study: 125 had NGT, 116 had isolated IFG, 106 had isolated IGT, 27 had IFG plus IGT, and 128 had NDD (table 1). There were significant differences in SBP, DBP, FPG, A1C, postload 2-hour plasma glucose, insulin, HOMA-IR, TG, hsCRP, and prevalence of hypertension among the five groups. The median (IQR) GDF15 levels were 1641.0 (1187.0–1985.5) pg/mL, 1656.1 (1226.8–2379.7) pg/mL, 1487.8 (1145.9–1987.2) pg/mL, 1722.2 (1172.9–1939.0) pg/mL, and 2204.5 (1767.4–2919.1) pg/mL in the NGT, IFG, IGT, IFG plus IGT, and NDD groups, respectively (figure 1). Subjects with NDD had significantly higher GDF15 levels than those with NGT (p<0.001), IFG (p<0.001), IGT (p<0.001), and IFG plus IGT (p<0.001). The IFG group had a significantly higher GDF15 values than the NGT group (p=0.048).

The results of the multivariate linear regression analysis of GDF15 and clinical variables are shown in table 2. In model 1, age (beta=0.251, 95% CI 14.961 to 31.922, p<0.001), A1C (beta=0.208, 95% CI 110.370 to 263.827, p<0.001), and hsCRP (beta=0.112, 95% CI 4.103 to 29.172, p=0.009) were positively associated, while BMI (beta=−0.119, 95% CI −92.871 to −14.140, p=0.008)

Table 1 Comparison of clinical characteristics among subjects with NGT, IFG, IGT, IFG +IGT, and NDD

	NGT	IFG	IGT	IFG+IGT	NDD	P value*
n	125	116	106	27	128	–
Age (years)	61.4±11.0	60.0±11.3	60.7±12.5	64.3±9.3	63.0±11.2	NS
Female (%)	42.4	37.9	36.8	44.4	43.0	NS
BMI (kg/m^2)	23.2±2.4	23.9±2.2	23.4±2.4	24.1±2.0	23.5±2.5	NS
SBP (mm Hg)	122.1±15.4	127.6±15.8	125.5±16.7	134.9±15.8	132.9±19.3	<0.001
DBP (mm Hg)	71.7±9.7	74.7±9.7	73.2±10.4	78.2±10.2	76.8±11.0	<0.001
Hypertension (%)	13.6	24.3	18.1	22.2	30.7	0.017
FPG (mg/dL)	85.7±7.2	105.0±5.4	85.6±7.4	107.9±6.3	136.6±57.0	<0.001
PPG (mg/dL)	99.8±22.7	108.6±20.7	159.6±15.3	167.5±14.8	255.2±80.0	<0.001
HbA1C (%)	5.7±0.3	5.9±0.4	5.8±0.3	6.0±0.3	7.3±1.8	<0.001
Insulin (mU/L)	2.9±2.5	3.6±3.3	3.1±4.7	4.9±3.5	4.0±3.7	0.020
HOMA-IR	0.61±0.52	0.87±0.55	0.57±0.51	1.32±0.97	1.42±1.75	<0.001
Total cholesterol (mg/dL)	205.1±31.6	210.6±35.1	201.8±37.1	201.0±34.4	214.5±47.7	NS
Triglyceride (mg/dL)	99.2 (77.1–133.90)	112.0 (81.0–160.8)	102.0 (72.7–145.7)	115.0 (84.3–194.0)	118.5 (83.7–184.0)	0.028
HDL cholesterol (mg/dL)	53.8±15.8	52.1±14.0	52.2±13.8	53.4±15.3	51.0±13.1	NS
eGFR (mL/min/1.73 m^2)	94.2±18.5	89.1±16.3	92.2±18.0	92.7±15.9	89.2±19.3	NS
AST (U/L)	26.0±7.1	26.1±7.9	26.1±8.0	27.3±12.2	31.2±37.3	NS
ALT (U/L)	25.4±11.9	28.3±17.2	26.5±15.8	28.1±19.3	34.3±50.7	NS
hsCRP (mg/L)	3.2±6.2	3.0±4.6	2.8±4.5	3.3±5.3	5.9±10.8	0.003

Data are expressed as mean±SD, median (IQR), or %.
*By analysis of variance, χ^2, or Kruskal-Wallis test.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HbA1C, hemoglobin A1C; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; hsCRP, high-sensitivity C reactive protein; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NDD, newly diagnosed diabetes; NGT, normal glucose tolerance; PPG, postload 2-hour plasma glucose; SBP, systolic blood pressure.

was negatively associated with GDF15 after adjusting for sex, SBP, and insulin. These associations remained statistically significant after further adjusting for eGFR, TG, and HDL, as shown in model 2. In model 3, age (beta=0.175, 95% CI 7.613 to 25.021, p<0.001), IFG (beta=0.145, 95% CI 192.487 to 740.937, p=0.001), NDD (beta=0.227, 95% CI 390.459 to 888.145, p<0.001), hsCRP (beta=0.105, 95% CI 3.276 to 27.768, p=0.013), and eGFR (beta=−0.180, 95% CI −15.698 to −5.469, p<0.001) were independently associated with GDF15 levels. These associations remained statistically significant after substituting insulin with HOMA-IR (beta=0.099, 95% CI 10.017 to 187.491, p=0.029), as shown in model 4.

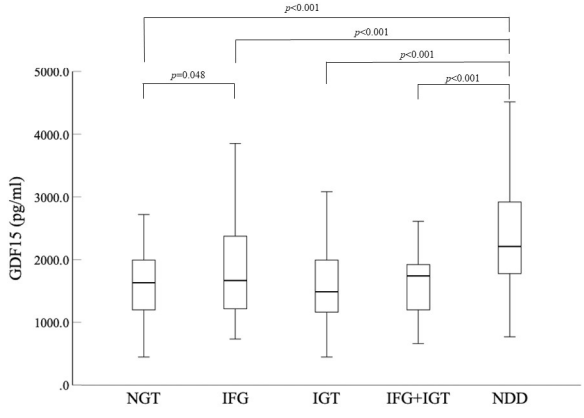

Figure 1 Comparison of growth differentiation factor 15 (GDF15) levels among subjects with normal glucose tolerance (NGT), isolated impaired fasting glucose (IFG), isolated impaired glucose tolerance (IGT), IFG plus IGT, and newly diagnosed diabetes (NDD).

DISCUSSION

Our study showed that non-obese subjects with isolated IFG (1656.1 pg/mL, 1226.8–2379.7) and those with NDD (2204.5 pg/mL, 1767.4–2919.1) had significantly higher GDF15 levels than those with NGT (1641.0 pg/mL, 1187.0–1985.5). In addition, A1C was independently associated with GDF15 levels. IFG and NDD, but not isolated IGT or IFG plus IGT, were positively associated with GDF15 levels.

The association between GDF15 and different glycemic statuses was similar to the results of previous studies. Hong *et al*[7] found that fasting GDF15 level was higher in non-obese subjects with IFG (n=29, BMI=25.44 kg/m^2) and type 2 diabetes (n=75, BMI=26.42 kg/m^2). Among them, GDF15 and HOMA-IR significantly discriminated IFG from NGT. However, a 75 g OGTT was not performed in that study, and it is unknown whether subjects with IGT were included. Furthermore, A1C levels were also not measured, which might have led to misdiagnoses of IFG and diabetes. Vila *et al*[8] found that obese (n=120, BMI=47 kg/m^2) subjects with diabetes had significantly higher GDF15 concentrations compared with those with NGT, but obese subjects with IGT had similar GDF15 concentrations to obese subjects with NGT. In addition, the average fasting glucose level was 110 mg/dL; thus, subjects with IFG might have been included. Yalcin *et al*[9] found that serum

Table 2 Multivariate linear regression analysis of GDF15 and clinical characteristics among patients with NGT, IFG, IGT, IFG+IGT , and NDD

	Model 1		Model 2		Model 3		Model 4	
Variable	**Beta (95% CI)**	**P value**	**Beta (95% CI)**	**P value**	**Beta (95% CI)**	**P value**	**Beta (95% CI)**	**P value**
Age (years)	0.251 (14.961 to 31.922)	<0.001	0.180 (7.966 to 25.594)	<0.001	0.175 (7.613 to 25.021)	<0.001	0.173 (7.488 to 24.864)	<0.001
Sex (male vs female)	0.010 (–161.135 to 203.476)	NS	–0.001 (–194.114 to 189.736)	NS	–0.007 (–205.352 to 173.052)	NS	0.004 (–182.088 to 197.406)	NS
A1C (%)	0.208 (110.370 to 263.827)	<0.001	0.228 (127.697 to 281.485)	<0.001				
IFG vs NGT					0.145 (192.487 to 740.937)	0.001	0.152 (214.351 to 765.572)	0.001
IGT vs NGT					–0.062 (–423.228 to 75.543)	NS	–0.048 (–385.737 to 119.287)	NS
IFG+IGT vs NGT					–0.028 (–524.596 to 265.950)	NS	–0.035 (–560.617 to 228.984)	NS
NDD vs NGT					0.227 (390.459 to 888.145)	<0.001	0.230 (400.764 to 895.284)	<0.001
BMI (kg/m^2)	–0.119 (–92.871 to –14.140)	0.008	–0.095 (–83.599 to –1.643)	0.042	–0.050 (–64.217 to 19.156)	NS	–0.065 (–70.508 to 12.572)	NS
SBP (mm Hg)	0.007 (–5.220 to 6.046)	NS	–0.004 (–5.806 to 5.307)	NS	0.017 (–4.396 to 6.530)	NS	0.005 (–5.182 to 5.791)	NS
Insulin (µU/mL)	–0.003 (–26.308 to 24.698)	NS	0.007 (–23.376 to 27.333)	NS	0.017 (–20.143 to 30.345)	NS		
HOMA-IR							0.099 (10.017 to 187.491)	0.029
hsCRP (mg/L)	0.112 (4.103 to 29.172)	0.009	0.106 (3.347 to 28.130)	0.013	0.105 (3.276 to 27.768)	0.013	0.094 (1.688 to 26.209)	0.026
eGFR (mL/min/1.73 m^2)			–0.203 (–17.041 to –6.733)	<0.001	–0.180 (–15.698 to –5.469)	<0.001	–0.178 (–15.524 to –5.318)	<0.001
Triglycerides (mg/dL)*			–0.090 (–899.322 to 34.861)	NS	–0.037 (–633.685 to 280.596)	NS	–0.053 (–713.894 to 206.919)	NS
HDL cholesterol (mg/dL)			0.024 (–5.730 to 9.298)	NS	0.025 (–5.568 to 9.279)	NS	0.021 (–5.786 to 9.030)	NS

Dependent variable: GDF15 level.
*Log-transformed before analysis.
BMI, body mass index; eGFR, estimated glomerular filtration rate; GDF15, growth differentiation factor 15; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; hsCRP, high-sensitivity C reactive protein; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; NDD, newly diagnosed diabetes; NGT, normal glucose tolerance; SBP, systolic blood pressure.

GDF15 levels were significantly higher in obese (n=37, BMI=33 kg/m^2) subjects with IGT compared with those with NGT, and GDF15 levels were independently associated with age and the area under the curve for glucose during the 75 g OGTT. In their study, the median fasting glucose level was 106.0 mg/dL in the IGT group; thus, there might have been some subjects with both IFG and IGT included. Schernthaner-Reiter *et al*[6] found that in obese (n=160, BMI=45 kg/m^2) subjects, serum GDF15 levels were higher in the pre-diabetes (IFG plus IGT) and diabetes groups, but the grade of impairment of glucose metabolism was not significantly associated with GDF15 levels. Our study in non-obese subjects categorized subjects into NGT, isolated IFG, isolated IGT, IFG plus IGT, and NDD groups. We found that only isolated IFG and NDD were positively associated with GDF15 levels after adjusting for age, gender, BMI, insulin/HOMA-IR, hsCRP, TG, HDL, and eGFR. Inconsistent with our results, a genome-wide association study (GWAS) found that five single nucleotide polymorphisms (SNP) of GDF15 were not related to type 2 diabetes risk, HbA1C, fasting glucose levels, or BMI.[10] The study was limited by the validity of the study depending on the chosen SNP,[10] and that the differences in GDF15 are modest compared with the alterations seen in different diseases.[2] Thus, further studies are still needed when more SNPs of GDF15 have been identified in a larger GWAS.[10]

Studies found that GDF15 is a biomarker for the use of metformin in subjects with dysglycemia,[11] and metformin increased serum GDF15 concentrations and is associated with a reduction in body mass in patients with type 2 diabetes.[12] In addition, metformin increased serum GDF15 levels to activate GFRAL, and further reduced food intake, body mass, and glucose intolerance in experimental animals.[12 13] Thus, these studies suggested that the therapeutic benefits of metformin on appetite, body mass, and serum insulin depend on GDF15.[12] In addition, GDF15 is associated with several conditions associated with weight loss, such as cancer anorexia-cachexia[14] and pregnancy-associated vomiting.[15]

The mechanism underlying the association between GDF15 and impaired glucose metabolism remains to be determined. Karczewska-Kupczewska *et al*[16] found that hyperinsulinemia resulted in an increase in GDF15 levels and an inverse correlation between insulin sensitivity and change in GDF15 levels during the hyperinsulinemic clamp study. They hypothesized that fasting and postprandial GDF15 levels might be differentially regulated, where postprandial hyperinsulinemia might upregulate GDF15 and the increased GDF15 levels might act as a satiety signal. On the other hand, although IFG and IGT were classified as pre-diabetes, the extent of IR and β-cell dysfunction was substantially different.[4] Individuals with isolated IFG typically have greater hepatic IR, whereas those with isolated IGT typically have greater β-cell dysfunction and greater peripheral IR, and those with IGT plus IFG have both β-cell dysfunction and whole-body IR. Using post-hoc analysis, we found no differences in insulin levels of the five dysglycemic groups. NDD had significantly higher HOMA-IR values than those in the other four groups, and the IFG group had similar HOMA-IR values, as compared with the NGT, IGT, or IFG plus IGT groups. The discrepancy is possibly due to the relatively small sample size of our study. In addition, we are unable to calculate the hepatic and peripheral IR. Thus, there is not enough evidence to deduce the pathway of GDF15 among the different glycemic statuses in non-obese subjects in this study. Further studies are required to clarify the relationship among GDF15, IR, and β-cell dysfunction.

The association between GDF15 and dysglycemia might be confounded by the presence of obesity. Schernthaner-Reiter *et al*[17] found that GDF15 levels at baseline and the response to 75 g OGTT did not differ between lean and obese subjects. Subjects in most previous studies of the association between GDF15 and dysglycemia were obese.[6 8 9] Hong *et al*[7] found no association between GDF15 and BMI in non-obese subjects with IFG. Karczewska-Kupczewska *et al*[16] found that during a 2-hour euglycemic hyperinsulinemic clamp study of normal-weight and obese subjects who had NGT, hyperinsulinemia resulted in a significant increase in GDF15 concentrations in both groups. No difference in GDF15 levels between normal-weight and obese women was noted at baseline or in postclamp conditions. In addition, both baseline and postclamp GDF15 levels were inversely related to BMI. Patel *et al*[3] found that GDF15 levels were unchanged in non-obese humans with an 8-week overfeeding intervention despite increased insulin and glucose levels. In our study, the results of the multivariate linear regression analysis (table 2) showed that BMI was negatively associated with GDF15 levels in models 1 and 2, but this association was attenuated in models 3 and 4. Further studies are needed to clarify the association between GDF15 and impaired glucose metabolism in subjects with and without obesity.

Our results showed that hsCRP was positively associated with GDF15 in non-obese subjects. However, Hong *et al*[7] found no significant correlation between GDF15 and CRP in non-obese subjects. The different results may be due to differences in sample size and the different methodology, especially the measurement of CRP rather than hsCRP, which allows detection of low-grade inflammation. On the other hand, several studies found no association between hsCRP and GDF15 in obesity. Vila *et al*[8] and Yalcin *et al*[9] found that hsCRP was not correlated with GDF15 levels in obese subjects. As obesity is associated with chronic systemic low-grade inflammation, obesity-associated inflammation might offset the association between dysglycemia and GDF15 in individuals with obesity.

Previous studies showed that age was an independent predictor of GDF15 in non-obese and obese subjects.[6–9] Consistently, our results showed that age was positively associated with GDF15 in non-obese subjects. Chronological age is associated with GDF15 in adults, which may be due to the involvement of GDF15 in the development of aging-related frailty syndrome.[2] We found that eGFR was negatively associated with GDF15. Similarly, Schernthaner-Reiter *et al*[6] found that GFR was negatively associated with GDF15 in obese patients. Yalcin *et al*[9] found that GFR was negatively correlated with GDF15 levels in obese subjects. A nationwide cohort study in community-dwelling elderly showed that GDF15 was negatively associated with eGFR even adjusting for age.[18] Although GDF15 had renoprotective actions in vitro and in vivo,[2] the mechanisms are still unclear. Further studies are needed to clarify the underlying mechanisms.

There are some limitations to this study. First, this was a cross-sectional study, so causal inferences could not be made. Second, OGTT-derived insulin sensitivity and β-cell function were not measured. Thus, the association between GDF15 and IR or β-cell function was unknown. However, the current definition of pre-diabetes was according to glucose levels, but not IR nor β-cell function, which is not feasible in clinical practice. Additionally, differences in levels of insulin sensitivity and β-cell function among the isolated IFG, isolated IGT, and IFG plus IGT groups are still unclear. Finally, this work was confined to non-obese subjects in Taiwan and might not be generalizable to obese individuals or other ethnicities.

In conclusion, our study showed that non-obese subjects with isolated IFG and NDD had significantly higher GDF15 levels than those with NGT. In addition, isolated IFG and NDD, but not isolated IGT or IFG plus IGT, were positively associated with GDF15 levels.

Contributors H-CH was involved in the conceptualization, design, data acquisition and statistical analysis, investigation, and writing of the manuscript. H-TW was involved in the conceptualization and design of the manuscript. C-HL and H-WC were involved in the data acquisition and statistical analysis. H-YO and C-JC were involved in the interpretation of data, review, and editing of the manuscript.

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Competing interests None declared.

Patient consent for publication Not required.

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REFERENCES

1 Assadi A, Zahabi A, Hart RA. GDF15, an update of the physiological and pathological roles it plays: a review. *Pflugers Arch* 2020;472:1535–46.
2 Lockhart SM, Saudek V, O'Rahilly S. GDF15: a hormone conveying somatic distress to the brain. *Endocr Rev* 2020;41:610–42.
3 Patel S, Alvarez-Guaita A, Melvin A, *et al*. GDF15 provides an endocrine signal of nutritional stress in mice and humans. *Cell Metab* 2019;29:707–18.
4 Campbell MD, Sathish T, Zimmet PZ, *et al*. Benefit of lifestyle-based T2DM prevention is influenced by prediabetes phenotype. *Nat Rev Endocrinol* 2020;16:395–400.
5 Bao X, Borné Y, Muhammad IF, *et al*. Growth differentiation factor 15 is positively associated with incidence of diabetes mellitus: the Malmö diet and Cancer–Cardiovascular cohort. *Diabetologia* 2019;62:78–86.
6 Schernthaner-Reiter MH, Itariu BK, Krebs M, *et al*. GDF15 reflects beta cell function in obese patients independently of the grade of impairment of glucose metabolism. *Nutrition Metabolism Cardiovasc Dis* 2019;29:334–42.
7 Hong JH, Chung HK, Park HY, *et al*. GDF15 is a novel biomarker for impaired fasting glucose. *Diabetes Metab J* 2014;38:472–9.
8 Vila G, Riedl M, Anderwald C, *et al*. The relationship between insulin resistance and the cardiovascular biomarker growth differentiation factor-15 in obese patients. *Clin Chem* 2011;57:309–16.
9 Yalcin MM, Altinova AE, Akturk M, *et al*. GDF-15 and hepcidin levels in nonanemic patients with impaired glucose tolerance. *J Diabetes Res* 2016;2016:1–5.
10 Au Yeung SL, Luo S, Schooling CM. The impact of GDF-15, a biomarker for metformin, on the risk of coronary artery disease, breast and colorectal cancer, and type 2 diabetes and metabolic traits: a Mendelian randomisation study. *Diabetologia* 2019;62:1638–46.
11 Gerstein HC, Pare G, Hess S, *et al*. Growth differentiation factor 15 as a novel biomarker for metformin. *Diabetes Care* 2017;40:280–3.
12 Day EA, Ford RJ, Smith BK, *et al*. Metformin-Induced increases in GDF15 are important for suppressing appetite and promoting weight loss. *Nat Metab* 2019;1:1202–8.
13 Emmerson PJ, Wang F, Du Y, *et al*. The metabolic effects of GDF15 are mediated by the orphan receptor GFRAL. *Nat Med* 2017;23:1215–9.
14 Johnen H, Lin S, Kuffner T, *et al*. Tumor-Induced anorexia and weight loss are mediated by the TGF-β superfamily cytokine MIC-1. *Nat Med* 2007;13:1333–40.
15 Petry CJ, Ong KK, Burling KA, *et al*. Associations of vomiting and antiemetic use in pregnancy with levels of circulating GDF15 early in the second trimester: a nested case-control study. *Wellcome Open Res* 2018;3:123.
16 Karczewska-Kupczewska M, Kowalska I, Nikolajuk A, *et al*. Hyperinsulinemia acutely increases serum macrophage inhibitory cytokine-1 concentration in anorexia nervosa and obesity. *Clin Endocrinol* 2012;76:46–50.
17 Schernthaner-Reiter MH, Kasses D, Tugendsam C, *et al*. Growth differentiation factor 15 increases following oral glucose ingestion: effect of meal composition and obesity. *Eur J Endocrinol* 2016;175:623–31.
18 Kim JS, Kim S, Won CW, *et al*. Association between plasma levels of growth differentiation factor-15 and renal function in the elderly: Korean frailty and aging cohort study. *Kidney Blood Press Res* 2019;44:405–14.