Enteroendocrine and adipokine associations with type 2 diabetes: phenotypic risk scoring approaches

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Abstract

Background and Aim: The contribution of gut-derived factors to the mechanisms linking obesity and metabolic disease remain under-investigated. The aim of the current study was examine the associations between glucagon and enteroendocrine signaling and type 2 diabetes (T2D) using a derived risk score approach. To compare the relative importance of the enteroendocrine system, associations between adipokine measures and T2D were also investigated

Methods: A total of 130 individuals with T2D and 161 individuals without T2D were included in the study. Circulating concentrations of enteroendocrine (glucagon, ghrelin, glucagon-like peptide-1 and gastric inhibitory peptide) and adipokine mediators (adiponectin, leptin, resistin, visfatin, adipsin) were measured. Standard scores (Z-scores) were determined for each measure and enteroendocrine (ERS) and adipokine (ARS) risk scores calculated based on summation of the component measures. Associations between both the ERS and ARS and T2D status were assessed using logistic regression models.

Results: The ERS was significantly associated with T2D status in an adjusted model (OR: 1.36; 95%CI: 1.08-1.72; p=0.009). Associations between the ARS and T2D status were not independent of age, sex and BMI (OR: 1.21; 95%CI: 0.99-1.47; p=0.06). Quantification of risk across ERS tertiles revealed that individuals with an ERS in the upper tertile were 10 times more likely (CI: 3.23-32.73; p<0.001) to have T2D.

Conclusions: These data support an association between enteroendocrine signaling and T2D. Use of the ERS as a potential tool for classifying individuals with Metabolic Syndrome as high or low risk for T2D development is being considered.

Key Words: gut hormones, obesity, diabetes

INTRODUCTION

It is widely recognized that the complex interplay between host and environmental factors underpins risk for obesity-associated disease. Among host factors, inflammatory signaling continues to receive attention as a link between obesity and metabolic disease predicated on the recognized cross talk between inflammatory and insulin signaling pathways.[1] We have previously proposed a key role of the gut in driving the chronic low grade inflammation associated with obesity.[2] However, given the complex biological networks linking the gut, inflammatory and metabolic signaling pathways, further examination of the associations between gut-associated markers and metabolic disease is needed.

A central role of the gut in linking obesity and metabolic disease is supported by multiple lines of evidence. Alterations in the composition of the intestinal microbiome, including the abundance of key bacteria, have been noted in obesity and metabolic disease.[3 4] Microbial metabolites promote the integrity of the intestinal mucosal and regulate intestinal permeability[5] with maintenance of barrier exclusion essential in isolating the gut microbiota to the intestinal lumen. Alterations in intestinal permeability in obesity can result in the appearance of microbial components, such as lipopolysaccharide (LPS), in the circulation and subsequent activation of innate immune/inflammatory pathways underpinning metabolic disease.[2] In the large FINRISK97 cohort, circulating LPS concentrations were predictive of type 2 diabetes (T2D) development over the 10-year follow-up period.[6] In addition, our own previous work has also demonstrated a significant association between increases in intestinal permeability and T2D[7] further supporting a mechanistic link between gut-associated factors and risk for metabolic disease.

Beyond the gut microbiota, potential contributions of the enteroendocrine system to risk for metabolic disease are also of interest. The incretin mediators, glucagon-like peptide(GLP)-1 and gastric inhibitory polypeptide (GIP) are secreted in response to luminal nutrient loads and among their

multiple endocrine effects, contribute to the regulation of insulin and glucagon secretion. Indeed the "-gliptin" class of diabetes medications inhibit dipeptidyl peptidase-4 (DPP4), the key enzyme involved in GLP-1 inactivation, in order to promote insulin secretion and glucose homeostasis.[8] Data from bariatric surgery studies lend further support to the potential role of gut-sensing mechanisms in underpinning risk for metabolic disease. Multiple studies have documented improvements in various metabolic indices in as short as one week following bariatric surgery and independent of weight loss[9-11] implicating the local environment within the gut, including energy sensing mechanisms, as a key regulator of metabolic control. The potential for the gut microbiota to signal to enteroendocrine cells within the gut mucosa may provide an additional mechanism linking obesity and metabolic disease[12], but remains largely uninvestigated.

This study aimed to use a phenotypic risk scoring approach to further investigate associations between enteroendocrine measures and T2D. To compare the relative importance of the enteroendocrine system, associations between adipokine measures and T2D were also investigated, based on reports of alterations in adipokine concentrations in states of obesity and identified roles of adipokine signaling in both the regulation of appetite and glucose control [13] and immune-regulatory effects.[14]

METHODS

Participants

This study included a subset of T2D-affected individuals (n=130) from the Diabetes Heart Study-MIND with ascertainment as described previously.[15] For the control group, a total of 161 individuals were recruited from the community. These individuals were aged 18-65 years, free from Metabolic Syndrome (MetS) as per the ATPIII criteria[16]; had no history of liver, kidney, thyroid, cardiovascular or gastrointestinal disease; and were not using anti-hypertensive, cholesterol lowering or immunomodulating medications or supplements (including probiotics and fish oil).

The institutional Human Research Ethics Committee provided ethical approval for this study (approval: MSC/04/15/HREC), all study procedures were carried out in accordance with the Declaration of Helsinki, and all subjects provided written informed consent prior to participation.

Laboratory Analyses

Fasting blood samples were collected from all participants and serum separated by centrifugation and stored frozen until analysis. Standard laboratory analyses included HbA1c, triglycerides, total cholesterol, HDL cholesterol, glucose, and CRP. LDL cholesterol concentrations were calculated using the Friedewald equation.[17]

A commercially available, off-the-shelf, multiplex suspension array assay kit (Diabetes 10-plex; Bio-rad Laboratories, Berkeley, California, USA) and Bioplex suspension array system (Bio-rad Laboratories, Berkeley, California, USA) were used to determine the concentrations of insulin, glucagon, leptin, resistin, visfatin, ghrelin, GLP-1, GIP. The assay was completed according to the manufacturers' instructions. All samples were analyzed in duplicate and samples from the two study groups were distributed across assay plates in a randomized and counterbalanced manner. The coefficient of variation for sample replicates was accepted at less than 10% and intra-assay variability was on average: insulin 3.6%; glucagon 3.8%; ghrelin 2.6%; GIP 3.4%; GLP-1 3.9%; leptin 2.9%; visfatin 3.1%; resistin 0.8%. Inter-assay variability was: insulin 9.0%; glucagon 11%; ghrelin 5.2%; GIP 9.9%; GLP 9.4%; leptin 8.0%; visfatin 7.2%; resistin 9.6%.

Adiponectin and adipsin concentrations were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits (AdipoGen LifeSciences, San Diego, California, USA and RayBiotech, Norcross, Georgia, USA respectively). Assays were completed according to the manufacturers' instructions. All samples were analyzed in duplicate and samples from the two

study groups were distributed across assay plates in a randomized and counterbalanced manner. The coefficient of variation for sample replicates was accepted at less than 10% and intra-assay variability was on average: adiponectin 2.3%; adipsin 3.8%. Inter-assay variability was: adiponectin 9.1%; adipsin 8.1%.

Risk Score Calculation

To allow for a more integrated assessment of the associations between related measures and disease status, derived risk scores (dRS) were calculated. The enteroendocrine risk score (ERS) included glucagon, ghrelin, GLP-1 and GIP based on recognized secretion from specialized cell types within the gastrointestinal tract and contributions to regulation of satiety and glucose homeostasis.[8] The adipokine risk score (ARS) included adiponectin, leptin, resistin, visfatin, and adipsin based on recognized secretion from adipose tissue and roles in satiety and the regulation of insulin sensitivity.[13 18]

To account for differences in the absolute values of individual variables, standard scores (Z scores) were determined for each measure and the dRS calculated as the sum of the component variables. For the ERS, the reciprocal of both GLP-1 and Ghrelin concentrations were calculated for inclusion in the dRS based on reports that lower concentrations are associated with risk for metabolic aberration.[19 20] This was to ensure alignment of the direction of the effect across the four component variables and mitigate the possibility of a net zero value on summation of the components. Similar to the ERS and given the acknowledged lower adiponectin concentrations with increased adiposity[13], the reciprocal of adiponectin concentrations were determined for inclusion in the ARS calculation to align the direction of effect with other variables i.e. higher concentrations in states of increased adiposity. For each of the ERS and ARS, individuals were

assigned an ordinal value based on tertile stratification (scored 1-3 based on increasing values) to allow for determination of relative risk as described below.

Statistical Analysis

Summary statistics were determined for key demographic and outcome measures; for dichotomous/ordinal measures these are presented as counts and percentages and for continuous measures, as both mean ± standard deviation and median and interquartile range. Continuous variables (clinical chemistry, enteroendocrine and adipokine measures) were log-transformed as appropriate to approximate conditional normality. Differences in demographic data, standard laboratory measures, enteroendocrine and adipokine measures between the two groups were assessed initially using a t-test for unpaired samples. Correlation between individual enteroendocrine or adipokine measures was assessed using a Pearson's correlation. The associations of each of the dRS with T2D were assessed using logistic regression models, which were subsequently adjusted for (i) age and sex, and (ii) age, sex and BMI. Risk for T2D was further quantified across the increasing risk score tertiles, again using unadjusted and adjusted logistic regression models. Associations are presented as odds ratios and 95% confidence intervals. Logistic regression models were evaluated with receiver-operator characteristic (ROC) curves. The difference in the areas under the curve (AUC) was compared using Delong's method.[21] All analyses were completed using SPSS Statistics v21 (IBM Corporation, Armonk, USA) with the exception of the AUC comparisons which were performed using R.[22] Statistical significance was accepted at p<0.05.

RESULTS

Key demographic and standard laboratory measures for the two groups are included in Table 1. An established history of T2D was reported among the T2D-affected individuals with disease

duration of 12.9 ±7.8 years and 88% reporting pharmacological treatment for T2D (of these 18% reported use of insulin). In addition, 56% reported using anti-hypertensive medications and 47% reported using lipid lowering medications. All T2D-affected individuals self-reported Caucasian ethnicity. The control group were more likely to be female than the T2D group (64.4% v 45.4% female respectively), tended to be younger, and 89% reported Caucasian ethnicity (with the remainder reporting Asian (5%), Middle Eastern (2%), and other (2%) ethnicities). As anticipated, known cardiometabolic risk factors were significantly different between the groups (Table 1), with a higher average BMI and waist circumference, a predominance of dyslipidemia, evidence of impaired glucose control and low-grade inflammation evident among the T2D-affected individuals. Based on self-reported medication use, none of the T2D-affected individuals reported using gliptin class medications (DPP-4 inhibitors).

Concentrations of all enteroendocrine measures were significantly different between the Healthy and T2D groups. As anticipated, higher concentrations of glucagon (~40%) and GIP (~42%), and lower ghrelin concentrations (~10%) were noted among the T2D-affected individuals (Table 1). GLP-1 concentrations also tended to be higher (~7%) among the T2D-affected individuals (Table 1). Among the adipokine measures, significantly higher leptin (~37%) and resistin (~9%) concentrations and significantly lower adiponectin concentrations (~32%) were observed among the T2D-affected individuals (Table 1). A trend for higher adipsin (~10%) concentrations among the T2D-affected individuals was also noted (Table 1). Only partial correlations were noted between individual enteroendocrine (r=0.27-0.53, p<0.05) or adipokine (r=0.18-0.43, p<0.05) measures (Table 2).

Despite the significant differences in enteroendocrine measures between the groups, associations between each of the enteroendocrine measures in isolation and T2D were not particularly

compelling following adjustment for age, sex and body size (Table 3). However, the regression analysis for the ERS revealed a significant association with T2D status; each SD increased in the ERS was associated with a 1.66-fold (CI: 1.39-1.97, p=8.2x10^{-9}) increase in risk for T2D. This association remained evident in age and sex adjusted models (Table 4) and was also independent of age, sex and BMI (OR: 1.36; CI: 1.08-1.72; p=0.009). A similar pattern was noted for the ERS tertiles (Table 4); in age, sex and BMI adjusted models, each increase in the tertile group was associated with an incremental 3.22-fold (CI: 1.81-5.73, p=7.0x10^{-5}) increase in risk for T2D. Further quantification of risk indicated that, relative to individuals with an ERS in the lower tertile, individuals in the upper tertile were 10.28-fold (CI: 3.23-32.73; p=8.0x10^{-5}) more likely to have T2D independent of age, sex and BMI (Figure 1). Despite these strong associations, ROC curve analysis demonstrated that prediction of T2D was not significantly improved when including the ERS to a regression model containing age, sex and BMI (AUC: 0.954, 95% CI: 0.933-0.976 versus AUC: 0.958, 0.938-0.979; p=0.14).

With the exception of Adiponectin, the adipokine measures in isolation were not significantly associated with T2D following adjustment for age, sex and body size (Table 3). However, logistic regression models revealed a significant association between the ARS, when considered as a continuous measure, and T2D; each SD increase in the ARS was associated with a 1.36-fold (95% confidence interval (CI): 1.21-1.52; p=2.0x10^{-7}) increase in risk for T2D. This association was preserved when adjusting for age and sex (Table 4), but was partially negated in models which also included adjustment for BMI (OR: 1.63; CI: 1.34-1.98; p=0.06). A similar pattern was evident when considering the ARS tertiles which were associated with an incremental 1.93-fold (1.43-2.61, p=2.1x10^{-5}) increase in risk for type 2 diabetes in unadjusted models. However, this association was attenuated in age, sex and BMI adjusted models (Table 4). Further quantification of risk indicated that, relative to individuals with an ARS in the lower tertile, individuals in the

upper tertile were 3.67-fold (2.01-6.71, p<0.001) more likely to have T2D. This pattern of increasing risk for T2D with increasing adipokine signaling was maintained following adjustment for age and sex with a 3.81-fold (1.43-10.17, p=0.007) increase in risk observed, but was not independent of BMI (Figure 2). ROC curve analysis demonstrated that prediction of T2D was not significantly improved when including the ARS to a regression model containing age, sex and BMI (AUC: 0.956, 95% CI: 0.935-0.978 versus AUC: 0.960, 0.940-0.980; p=0.15).

DISCUSSION

In efforts to better understand the complex biological networks linking the gut, inflammatory and metabolic signaling pathways, approaches that capture the cumulative impacts of multiple markers within a given system may be more informative than considering various measures in isolation. This study aimed to use a derived phenotypic risk scoring approach to further examine relationships between enteroendocrine and adipokine measures and T2D. Significant differences in enteroendocrine measures and some of the adipokine measures were noted between groups. However, only the derived ERS was significantly associated with T2D independent of age, sex and body size. These data support the need for further assessment of the mechanistic links between gut-associated factors and risk for metabolic disease.

In the current study it was interesting to note that the associations between each of the enteroendocrine measures in isolation and T2D (following adjustment for age, sex and body size) were not particularly compelling. However, capturing the cumulative activity via the derived ERS revealed significant and independent associations with T2D. Beyond recognized secretion in response to luminal nutrient loads, the potential for enteroendocrine measures to also provide a link between the gut microbiota and metabolic pathways has now been recognized.[12 23] The expression of receptors for short chain fatty acids (a key bacterial metabolite) on the surface on enteroendocrine cells, including GLP-1 secreting cells, isolated from human gut biopsy samples has been demonstrated.[24] This data suggests

the potential for activation of incretin secretion in response to gut microbial activity. This potential has been subsequently verified in a free fatty acid receptor knock-out mouse model where GLP-1 secretion was attenuated in knock-out animals following SCFA feeding.[25] Collectively, data such as these, in conjunction with our own findings, support the need for further consideration of gut-derived factors in underpinning risk for metabolic disease.

Similar to the enteroendocrine measures, but with the exception of Adiponectin, the adipokine measures, when considered in isolation, were not significantly associated with T2D following adjustment for age, gender and BMI. Interestingly, despite the volumes of evidence implicating adipokine signaling in underpinning risk for T2D[18 26], the ARS was not as strongly associated with T2D as the ERS. This observation further supports the possibility of an important contribution of the gut to risk for metabolic disease. Further evidence supporting a role for the gut as a key driver of risk for metabolic disease also comes from results of a small pilot trial by Kratz et al.[27] Improvements in glycemic control were noted two weeks following Roux-en-Y gastric bypass, despite persistent adipose tissue inflammation, suggesting local signaling from within the gut to be driving the observed improvements in metabolic control in the absence of significant alterations in circulating adiponectin and other inflammatory mediators.[27]

The use of risk scoring approaches based on phenotypic measures has been attempted by others previously, including in assessment of the relationships between adipose tissue depots and subclinical cardiovascular disease[28], investigation of the associations between the burden of vascular calcified plaque across multiple vascular beds and risk for mortality[29] and in evaluation of inflammatory status in T2D.[30] The magnitude of the correlation coefficients between enteroendocrine or adipokine measures reflect partial correlations and support the possibility that consideration of multiple measures in conjunction may capture more of the phenotypic variance

in the system/network and allow for improved risk prediction. In the current study, we again note the potential for this global assessment approach; stronger associations with T2D were noted for the derived RS than any single component variable in isolation. However, this pattern was less obvious for the ARS, where adiponectin concentrations were significantly associated with T2D status, but the association between the derived ARS and T2D was less compelling.

We acknowledge this study is not without its limitations. Assigning the direction of the effect in terms of the risk ascribed to each of the component dRS variables may impact whether the dRS accurately reflects the broader function of a complex biological network. For example, given the recognized role of leptin in contributing to satiety[13], low leptin concentrations could be theorized to increase risk of obesity and associated metabolic disease. However, this reasoning is complicated by recognized leptin resistance with excess body mass[31]; in the current study higher leptin concentrations were observed in the T2D-affected individuals and leptin concentrations were scored to reflect an increase in risk for disease during the risk scoring process. This scenario highlights a need for some knowledge of biological function when attempting phenotypic risk scores. In this study medication use was not standardized among the T2D-affected individuals and while this is representative of the population more broadly, in future studies, attempts could be made to reduce the confounding effects of this variable, although complete standardization would be difficult to achieve. Further, the limitation of trying to relate association and prediction has been recognized by others previously[32][33] and was also evident in this study; despite the strong association between the ERS and T2D, significantly improvements in prediction based on AUC were not observed. This however, may also be attributed to the relatively modest sample size. That said, the strong associations do support the need for additional consideration of gut-derived factors in the development of metabolic disease using designs that can further assess causality.

In complex biological systems, a single maker alone may inadequately reflect the activities of the entire system and considering the cumulative impact of multiple makers through calculation of derived risk scores may provide an alternative approach to overcome this limitation. The current study applied this approach to investigate associations between enteroendocrine and adipokine measures and T2D. Results provide further evidence supporting the possibility of an important contribution of gut-derived factors to risk for metabolic disease. The application of this approach as a potential tool for classifying individuals with MetS as high or low risk for T2D development is being considered.

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Table 1

Demographic characteristics, standard laboratory measures, enteroendocrine measures and adipokine measures in individuals with (n=130) and without (n=161) type 2 diabetes. Data are presented as mean ± SD (median; interquartile range).

	Healthy (n=161)	T2DM (n=130)	p-value
Age (years)	37.4 ± 12.5 (37.2; 24.3-47.2)	57.5 ± 6.2 (59.3; 41.4-64.6)	<0.001
Sex (Female/Male)	104/57	59/71	0.001
BMI (kg/m^2)	25.1 ± 3.9 (24.1; 22.6-27.5)	30.4 ± 3.2	<0.001
Waist (cm)	84.4 ± 11.5 (83.5; 76.0—91.0)	105.1 ± 10.1 (105.8; 72.0-131.5)	<0.001
Dyslipidemia	49%	97%	<0.001
Systolic BP (mmHg)	120.1 ± 15.3 (118; 110-128)	126.3 ± 16.2 (125; 86-194)	0.001
Diastolic BP (mmHg)	77.7 ± 10.7 (77; 70-84)	74.3 ± 9.5 (75; 48-97)	0.005
Clinical Chemistry			
Triglycerides (mmol/L)	0.97 ± 0.42 (0.90; 0.67-1.19)	2.38 ± 2.23 (1.62; 1.24-2.80)	<0.001*
Cholesterol (mmol/L)	5.01 ± 0.91 (5.00; 4.39-5.52)	4.86 ± 1.27 (4.80; 4.00-5.55)	0.08*
HDL (mmol/L)	1.57 ± 0.37 (1.53; 1.28-1.79)	1.07 ± 0.36 (1.01; 0.82-1.25)	<0.001*
LDL (mmol/L)	2.99 ± 0.81 (2.89; 2.41-3.48)	2.73 ± 1.00 (2.63; 2.02-3.35)	0.005*
HbA1C (%)	5.4 ± 0.3 (5.4; 5.2-5.6)	7.8 ± 1.6 (7.4; 6.6-8.7)	<0.001*
Glucose (mmol/L)	5.09 ± 0.51 (5.08; 4.85-5.39)	8.74 ± 3.60 (7.80; 6.40-9.80)	<0.001*
Insulin (mIU/L)	6.8 ± 2.3 (6.3; 5.3-7.8)	24.4 ± 29.0 (15.2; 8.5-26.9)	<0.001*
CRP (mg/L)	1.76 ± 2.74 (0.82; 0.39-1.84)	3.64 ± 4.26 (2.42; 0.99-4.64)	<0.001*
Enteroendocrine Measures			
Glucagon (pg/mL)	107 ± 33 (104; 87-124)	149 ± 71 (129; 105-176)	<0.001*
Ghrelin (ng/mL)	1.86 ± 0.92 (1.64; 1.26-2.25)	1.67 ± 0.85 (1.44; 1.17-1.94)	0.04*
GLP-1 (pg/mL)	298 ± 43 (297; 271-320)	319 ± 79 (308; 275-340)	0.01*
GIP (pg/mL)	149 ± 115 (124; 87-152)	211 ± 130 (172; 122-249)	<0.001*
ERS	5.30 ± 1.56 (5.08; 4.33-5.96)	6.62 ± 1.75 (6.48; 5.33-7.50)	<0.001*
Adipokines			
Adiponectin (ug/mL)	18.8 ± 8.3 (17.2; 12.8-22.7)	12.4 ± 9.4 (10.1; 7.0-14.2)	<0.001*
Leptin (ng/mL)	5.19 ± 6.00 (3.22; 1.20-6.50)	7.1 ± 8.7 (4.87; 2.55-7.83)	0.001*
Resistin (ng/mL)	2.55 ± 0.42 (2.48; 2.29-2.73)	2.77 ± 0.82 (2.50; 2.25-3.11)	0.05*
Visfatin (ng/mL)	1.08 ± 0.32 (1.06; 0.87-1.20)	1.12 ± 0.39 (1.03; 0.85-1.32)	0.51*
Adipsin (ug/mL)	4.41 ± 1.41 (4.49; 3.44-5.44)	4.87 ± 2.10 (4.55; 3.38-5.74)	0.09*
ARS	7.47 ± 1.81 (7.16; 6.28-8.41)	9.25 ±3.11 (8.44; 7.13-10.97)	<0.001*

BMI: body mass index; BP: blood pressure; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; HbA1c: glycated haemoglobin; CRP: C-reactive protein; GLP-1: glucagon-like peptide-1; GIP: gastric inhibitory peptide; ERS: enteroendocrine risk score; ARS: adipokine risk score

* p-value is based on an unpaired t-test using log-transformed data

Table 2

Pearson's correlation coefficients for correlations (p-value) between standardized enteroendocrine measures and standardized adipokine measures.

Enteroendocrine Measures

	Glucagon	Ghrelin	GIP	GLP-1
Glucagon		-0.07 (0.25)	0.27 (<0.001)	0.53 (<0.001)
Ghrelin			-0.08 (0.15)	0.12 (0.04)
GIP				0.32 (<0.001)

Adipokine Measures

	Adiponectin	Leptin	Resistin	Visfatin	Adipsin
Adiponectin		0.19 (0.001)	-0.11 (0.06)	-0.11 (0.06)	0.18 (0.002)
Leptin			0.24 (<0.001)	0.08 (0.18)	0.30 (<0.001)
Resistin				0.43 (<0.001)	0.10 (0.09)
Visfatin					-0.04 (0.49)

GLP-1: glucagon-like peptide-1; GIP: gastric inhibitory peptide

Table 3

Associations between enteroendocrine and adipokine measures and type 2 diabetes based on logistic regression models. Odds ratios (OR) and 95% confidence intervals (CI) are reported.

	Unadjusted		Adjusted (age, sex)		Adjusted (age, sex, BMI)	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Enteroendocrine Measures*						
Glucagon	2.73 (1.90-3.92)	5.95×10^{-8}	2.21 (1.35-3.61)	0.002	1.91 (1.15-3.19)	0.013
GLP	1.45 (1.11-1.91)	0.007	1.06 (0.75-1.50)	0.73	1.00 (0.69-1.46)	0.98
GIP	1.79 (1.33-2.39)	9.56×10^{-5}	1.23 (0.88-1.71)	0.22	1.21 (0.85-1.71)	0.29
Ghrelin	0.80 (0.63-1.02)	0.07	0.60 (0.41-0.89)	0.01	0.78 (0.51-1.20)	0.26
Adipokine Measures*						
Adiponectin	0.39 (0.28-0.55)	5.45×10^{-8}	0.37 (0.23-0.58)	0.09	0.37 (0.24-0.58)	1.16×10^{-5}
Leptin	1.32 (1.02-1.70)	0.03	1.52 (1.43-1.60)	0.07	0.86 (0.56-1.31)	0.48
Resistin	1.43 (1.11-1.84)	0.005	1.40 (0.93-2.11)	0.11	1.33 (0.85-2.08)	0.21
Visfatin	1.11 (0.88-1.40)	0.40	0.99 (0.70-1.41)	0.97	0.97 (0.65-1.43)	0.87
Adipsin	1.30 (1.03-1.66)	0.03	1.16 (0.81-1.66)	0.41	0.89 (0.60-1.32)	0.56

*for each measure the standard score (Z-score) was used for analysis to allow for comparison of relative effects

GLP: glucagon-like peptide-1; GIP: gastric inhibitory peptide

Table 4

Enteroendocrine (ERS) and adipokine risk score (ARS) and risk score tertile associations with type 2 diabetes based on logistic regression models. Odds ratios (OR) and 95% confidence intervals (CI) are reported.

	Unadjusted		Adjusted (age, sex)		Adjusted (age, sex, BMI)	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Enteroendocrine Measures						
ERS	1.66 (1.39-1.97)	8.21×10^{-9}	1.52 (1.20-1.93)	4.30×10^{-4}	1.36 (1.08-1.72)	0.009
ERS Tertiles	3.72 (2.63-5.26)	2.22×10^{-13}	3.90 (2.29-6.65)	5.67×10^{-7}	3.22 (1.81-5.73)	6.99×10^{-5}
Risk Score Tertiles						
T1	1	-	1	-	1	-
T2	3.70 (1.89-7.27)	1.43×10^{-4}	3.27 (1.23-8.71)	0.02	2.47 (0.86-7.14)	0.09
T3	13.84 (6.88-27.87)	2.78×10^{-13}	15.27 (5.24-44.53)	6.06×10^{-7}	10.28 (3.23-32.73)	8.00×10^{-5}
Adipokine Measures						
ARS	1.36 (1.21-1.52)	2.00×10^{-7}	1.37 (1.14-1.65)	0.001	1.21 (0.99-1.47)	0.06
ARS Tertiles	1.93 (1.43-2.61)	2.05×10^{-5}	1.95 (1.19-3.19)	0.007	1.34 (0.77-2.32)	0.29
ARS Tertiles						
T1	1	-	1	-	1	-
T2	1.48 (0.81-2.72)	0.20	2.04 (0.77-5.40)	0.15	1.54 (0.54-4.37)	0.42
T3	3.67 (2.01-6.71)	2.28×10^{-5}	3.81 (1.43-10.17)	0.007	1.80 (0.60-5.37)	0.29

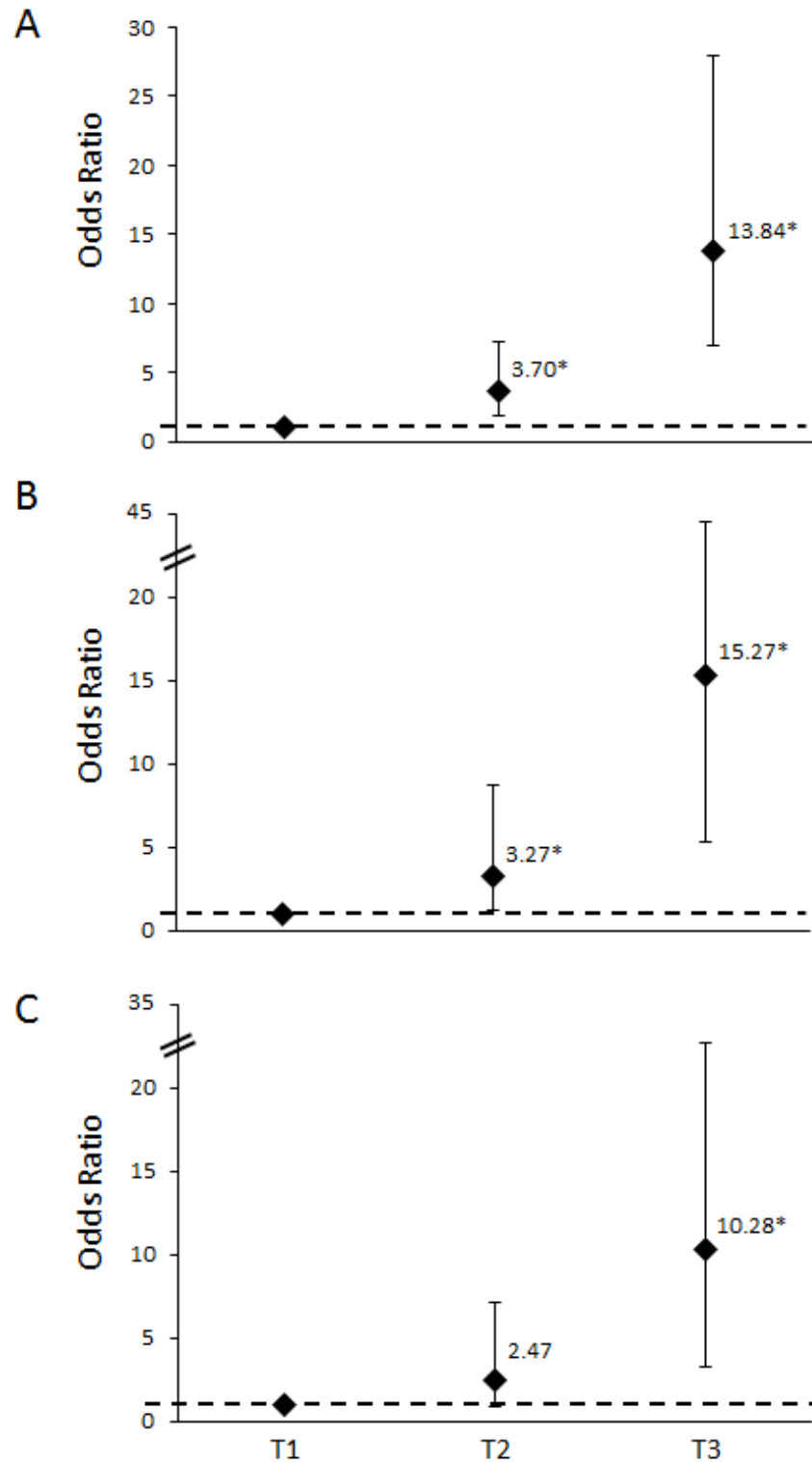T1: tertile 1; T2: tertile 2; T3: tertile 3

Figure 1

Odds ratios from logistic regression models for associations between the derived enteroendocrine risk score (ERS) tertiles (T1, T2, T3) and T2D (A), with adjustment for age and gender (B), and with adjusted for age, gender and BMI (C). *p<0.05
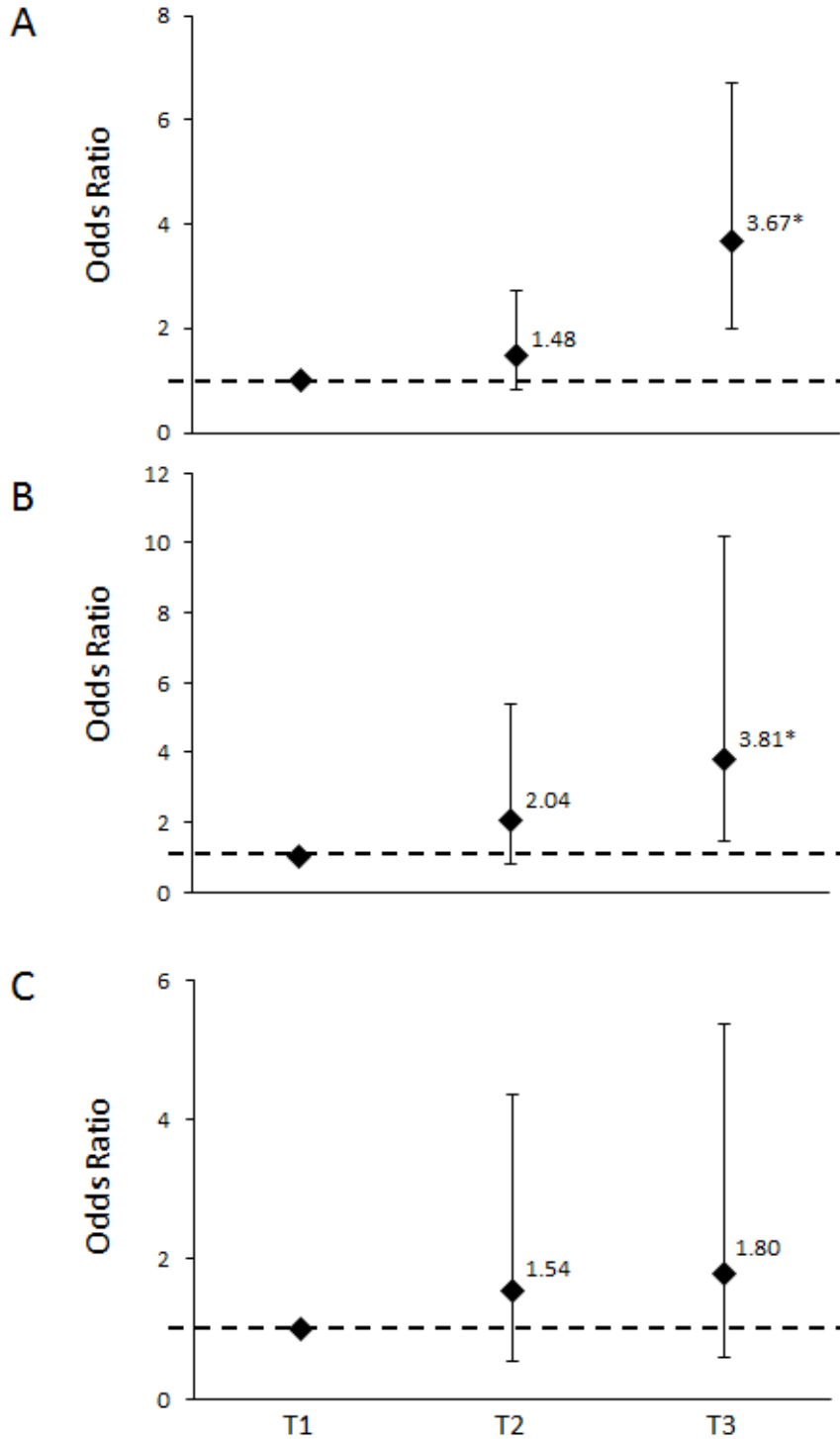
Figure 2

Odds ratios from logistic regression models for associations between the derived adipokine risk score (ARS) tertiles (T1, T2, T3) and T2D (A), with adjustment for age and gender (B), and with adjusted for age, gender and BMI (C). *p<0.05