Consumption of two meals per day is associated with increased intrapancreatic fat deposition in patients with type 2 diabetes: a retrospective study

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ABSTRACT

Introduction This study aimed to identify the associations between lifestyle factors and intrapancreatic fat deposition in patients with type 2 diabetes.

Research design and methods The participants were 185 patients with type 2 diabetes who were hospitalized at Osaka University Hospital between 2008 and 2020 and underwent abdominal CT during hospitalization. Information regarding lifestyle factors, including the number of meals consumed per day, snacking habits, exercise habits, exercise at work, smoking habits, alcohol intake, insomnia, sleep apnea syndrome, and night-shift working, was acquired from self-administered questionnaires or medical records. We measured the mean CT values for the pancreas (P), liver (L), and spleen (S), and the visceral fat area (VFA), and quantified intrapancreatic and liver ectopic fat accumulation as P−S and L−S, respectively.

Results After adjustment for age, sex, hemoglobin A1c, and body mass index (BMI), participants who consumed two meals per day had significantly lower P−S (higher intrapancreatic fat deposition, p=0.02) than those who consumed three meals per day. There were no significant associations between the number of meals consumed and liver ectopic fat accumulation and VFA (p=0.73 and p=0.67, respectively).

Conclusions Patients with diabetes who consumed two meals per day showed greater intrapancreatic fat deposition than those who consumed three meals per day, even after adjustment for BMI. These findings support the current guideline for diabetes treatment that skipping meals should be avoided.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Fatty pancreas disease is known to be associated with higher risk of diabetes mellitus; however, the associations between lifestyle factors and intrapancreatic fat deposition remain unclear.

WHAT THIS STUDY ADDS

⇒ We found that patients with type 2 diabetes who consumed two meals per day showed greater intrapancreatic fat deposition than those who consumed three meals per day, even after adjustment for hemoglobin A1c and body mass index.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ These findings support current diabetes treatment guidelines that skipping meals should be avoided and may lead a novel approach to treatment of diabetes focusing on intrapancreatic fat deposition.

INTRODUCTION

With the recent dramatic increase in the number of patients with obesity and diabetes, organ dysfunction associated with ectopic fat accumulation is receiving a great deal of attention. Ectopic fat is defined as the accumulation of fat in or around organs that normally contain only small amounts of fat, rather than in adipose tissue. The accumulation of ectopic fat in the liver and muscle is associated with insulin resistance and development of type 2 diabetes.[1] In addition, ectopic fat accumulation has been demonstrated in the pancreas, and this has been termed 'intrapancreatic fat deposition' (continuous variable) and 'fatty pancreas disease' (categorical variable).[2] We recently showed that intrapancreatic fat deposition, quantified using abdominal CT, is significantly associated with glucose intolerance[3] and a longitudinal decrease in the endogenous capacity for insulin secretion.[4] In addition, Singh et al[5] have shown that the presence of fatty pancreas disease is associated with significantly higher risk of diabetes mellitus, arterial hypertension, and metabolic syndrome (risk ratios 2.08, 1.67, and 2.37, respectively). Furthermore, intrapancreatic fat deposition

is the risk factor for pancreatic ductal adenocarcinoma in animals[6] and humans.[7]

Several previous studies have shown that lifestyle factors such as diet[8 9] and smoking[10 11] are associated with risk of liver ectopic fat accumulation. It was also shown that diet and exercise interventions reduce the amount of ectopic fat in the liver and skeletal muscle.[1] Several previous studies have reported about lifestyle factors and intrapancreatic fat deposition.[2] Stuart *et al*[12] showed that tobacco smoking was associated with greater intrapancreatic fat deposition. Stuart *et al*[13] also investigated the association between cannabis use and intrapancreatic fat deposition and reported that regular cannabis users did not differ from never users in terms of intrapancreatic fat deposition.[13] However, it remains unclear whether other lifestyle factors, including diet or exercise, are associated with intrapancreatic fat deposition.

In the present study, we evaluated the relationships between lifestyle factors and intrapancreatic fat deposition in patients with type 2 diabetes. We hypothesized that there would be a lifestyle factor that specifically alters the risk of fatty pancreas disease and may represent a target for the development of novel approaches for the prevention and treatment of type 2 diabetes.

RESEARCH DESIGN AND METHODS
Study sample and design
The participants were patients with type 2 diabetes who were hospitalized at the Department of Metabolic Medicine, Osaka University Hospital, between January 2008 and April 2020 and who had undergone abdominal CT scanning during hospitalization. From the 595 patients who met these criteria, we excluded those who had been diagnosed with pancreatic diseases, such as pancreatic tumors and pancreatitis; severe hepatic diseases, including viral hepatitis, liver cirrhosis, and hepatocellular carcinoma; malignant tumor of other organs; endocrine diseases; chronic renal failure (estimated glomerular filtration rate of $<30 \, mL/min/1.73 \, m^2$); neuromuscular disease; heart disease, including symptomatic cardiovascular disease and heart failure (New York Heart Association grade ≥2); acute infection; diabetes of genetic origin; and those

taking glucocorticoids. Ultimately, a total of 185 patients with type 2 diabetes were enrolled in the study (figure 1).

The study was announced to the public on the website of our department at Osaka University Hospital and all patients could choose to participate or refuse to participate in the study.

Assessment of lifestyle factors
Information regarding lifestyle factors was acquired from self-administered questionnaires and interviews conducted by physicians and nurses at the time of admission. The accuracy of questionnaires was confirmed by the attending physicians and nurses. Dietary habit was additionally acquired from nutritional guidance records by registered dietitians. Using these records, we recorded the number of meals consumed per day, snacking habits (more than four times per week or less), exercise habits (performing exercise outside of work or not), exercise performed at work (standing and/or manual labor or none), smoking habits (never a smoker, previous smoker, or current smoker), alcohol intake (none, intermediate (≤20 g/day), or large (>20 g/day)), presence of insomnia (regular use of sleep-inducing agents), presence of sleep apnea syndrome (SAS; a score of >40 on the Apnea-Hypopnea Index), and whether the participant worked night shifts. Information about use of sleep-inducing agents was acquired from medication records.

Measurement of ectopic fat accumulation in the pancreas and liver
To quantify the degree of intrapancreatic fat deposition and hepatic ectopic fat accumulation, we used unenhanced CT values, which have been shown to closely correlate with the organ fat content determined histologically.[14] As previously reported,[4] we defined the pancreatic CT value (P) as the mean CT value of three $1 \, cm^2$ regions of interest in the head, body, and tail of the pancreas, with careful exclusion of the pancreatic duct and margins from the measurement areas. Similarly, we defined the liver CT value (L) as the mean CT value of three $1 \, cm^2$ regions of interest in the anterior, posterior, and lateral regions of the liver. We also defined the splenic CT value (S) as the mean CT value of three $1 \, cm^2$ regions of interest in the upper, middle, and lower regions of the spleen. Then, as in previous studies, intrapancreatic fat deposition was quantified as the difference between the mean pancreatic and splenic CT values (P–S),[4 15 16] and the liver ectopic fat accumulation was quantified as the difference between the mean liver and splenic CT values (L–S).[4 17 18] In general, air, water, and fat have unenhanced CT attenuation values of approximately −1000, 0, and −100 Hounsfield units (HU), respectively, with lower values implying more ectopic fat accumulation. All unenhanced CT scanning were performed at the setting of 5 mm slice thickness. Tube voltage was 120 kVp in 181 patients, 135 kVp in 1 patient, and 140 kVp in 3 patients. The images were analyzed using Aquarius Net Viewer V.4.4 (TeraRecon, Tokyo, Japan). In addition,
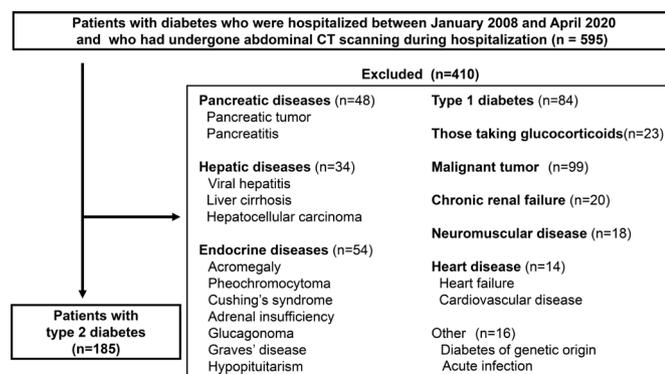
Figure 1 Flow chart of the recruitment of participants.

we measured the visceral fat area (VFA) and the subcutaneous fat area of the participants using the Synapse Vincent image analysis system (Fujifilm, Tokyo, Japan).

Assessment of covariates

We obtained the following data at the time of hospitalization from participants' medical records: age; sex; height; body mass; waist circumference; duration of diabetes; medication for diabetes at the time of admission (use of insulin and glucagon-like peptide-1 (GLP-1) receptor agonist); hemoglobin A1c (HbA1c); fasting plasma glucose (FPG), total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol concentrations; aspartate transaminase, alanine transaminase, γ-glutamyl transpeptidase, and amylase activities; and blood urea nitrogen, creatinine, uric acid, and C reactive protein concentrations. Blood samples were collected before breakfast on the day following admission. Body mass index (BMI) was calculated as body mass in kilograms divided by the square of the height in meters.

Statistical analysis

The P–S and L–S values were log-transformed to normalize their distributions and then the outliers (less than the first quartile−1.5×(third quartile−first quartile); or greater than the third quartile+1.5×(third quartile−first quartile)) were excluded to minimize measurement error. Multiple regression analyses were used to evaluate the relationships between lifestyle factors and log-transformed P–S or L–S. The least squares geometric mean concentrations were estimated for each lifestyle factor and these were adjusted for covariates including age (continuous), sex (male or female), HbA1c (continuous), and BMI (continuous).

Differences in P–S, L–S, and VFA for each lifestyle factor were tested using Welch's t-test for two groups and one-way analysis of variance for three groups. Differences in lifestyle factors and clinical indicators between patients who consumed two or three meals per day were tested using Mann-Whitney U test for continuous data and Fisher's exact test for categorical data. Statistical analyses were performed using R V.4.0.3 (https://www.r-project.org/) and $p<0.05$ was considered to represent statistical significance.

RESULTS
Participant characteristics

The clinical characteristics of the participants at hospitalization are shown in table 1. Their median age was 63 years, their median BMI was 26.5, and their median HbA1c was 74.0 mmol/mol (8.9%). With respect to their dietary habits, 22 participants (13.3%) had two meals per day and the others had three meals per day; 147 participants (87.0%) consumed snacks. Of the 22 participants who had two meals per day, 14 skipped breakfast, 5 skipped lunch, 2 skipped dinner and 1 ate at 18:00 and 0:00. With respect to exercise, 68 participants (43.9%)

undertook daily exercise and 20 (14.8%) performed manual labor. Of the participants, 34 (18.4%) were current smokers and 36 (19.5%) consumed >20 g/day alcohol. With respect to their sleeping habits, 22 participants (12.4%) regularly experienced insomnia, 33 (20.6%) had SAS, and 6 (4.4%) undertook night shifts. The median VFA of the participants was 118.1 cm^2.

Table 1 Clinical characteristics of the participants

Age (years)	63.0 (50.0, 71.0)
Sex (male/female)	101/84
Number of meals per day (2/3) (n=165)	22/143
Snack (no/yes) (n=169)	22/147
Exercise (no/yes) (n=155)	87/68
Exercise at work (no/yes) (n=135)	115/20
Smoke (never/previous/current)	93/58/34
Alcohol intake (none/intermediate/large)	100/49/36
Insomnia (no/yes) (n=178)	156/22
Sleep apnea syndrome (no/yes) (n=160)	127/33
Night-shift work (no/yes) (n=136)	130/6
P (HU)	37.8 (31.2, 43.5)
L (HU)	54.1 (45.9, 60.0)
S (HU)	48.5 (44.9, 52.0)
Visceral fat area (cm^2) (n=164)	118.1 (87.8, 160.7)
Subcutaneous fat area (cm^2) (n=164)	174.6 (110.7, 248.7)
Body mass index (kg/m^2)	26.5 (23.9, 31.2)
Waist circumference (cm) (n=137)	97.0 (88.0, 106.0)
Duration of diabetes (years) (n=164)	10.0 (4.0, 18.0)
HbA1c (mmol/mol)	74.0 (61.0, 95.0)
HbA1c (%)	8.9 (7.7, 10.8)
Fasting plasma glucose (mmol/L) (n=182)	8.2 (6.8, 10.1)
AST (U/L)	22.0 (17.0, 36.0)
ALT (U/L)	25.0 (16.0, 48.0)
γ-GTP (U/L) (n=183)	36.0 (22.0, 61.0)
AMY (U/L) (n=164)	55.0 (44.0, 73.0)
BUN (mmol/L) (n=184)	5.4 (4.3, 6.1)
Cre (μmol/L) (n=184)	61.9 (53.0, 79.6)
UA (μmol/L) (n=178)	339.0 (279.6, 398.5)
CRP (mg/L) (n=180)	0.9 (0.4, 3.0)
T-Cho (mmol/L) (n=175)	4.9 (4.3, 5.7)
HDL-C (mmol/L) (n=178)	1.1 (1.0, 1.4)
LDL-C (mmol/L) (n=176)	2.9 (2.3, 3.6)
TG (mmol/L) (n=178)	1.6 (1.1, 2.2)

Values are median (first quartile, third quartile) for continuous variables and number (percentage) of participants for categorical variables. ALT, alanine transaminase; AMY, amylase activities; AST, aspartate transaminase; BUN, blood urea nitrogen; Cre, creatinine; CRP, C reactive protein concentrations; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HU, Hounsfield units; L, mean CT values for the liver; LDL-C, low-density lipoprotein cholesterol; P, mean CT values for the pancreas; S, mean CT values for the spleen; T-Cho, total cholesterol; TG, triglyceride; UA, uric acid; γ-GTP, γ-glutamyl transpeptidase.

The median CT radiodensity values for the pancreas, liver, and spleen were 37.8 HU, 54.1 HU, and 48.5 HU, respectively, which were consistent with those obtained in previous studies.[4 19] There were 40 patients treated with insulin and 7 patients treated with GLP-1 receptor agonist at the time of admission to the hospital. There were missing data about the number of meals consumed per day (n=20), snack (n=16), exercise (n=30), exercise at work (n=50), insomnia (n=7), SAS (n=25), and night-shift work (n=49). Missing data were not included in the statistical analyses. The correlations among intrapancreatic fat deposition (P–S), liver ectopic fat accumulation (L–S), and VFA are shown in online supplemental table S1. Ectopic fat accumulation in both the pancreas and liver correlated with VFA, but the correlation between liver ectopic fat accumulation and VFA (r=0.35, p<0.001) was slightly stronger than that between intrapancreatic fat deposition and VFA (r=0.27, p<0.001). However, there was no correlation between intrapancreatic fat deposition and liver ectopic fat accumulation (r=0.02, p=0.82).

Lifestyle factors and intrapancreatic fat deposition

The relationships between lifestyle factors and intrapancreatic fat deposition (P–S) are shown in table 2. Compared with the participants who consumed three meals per day, those who consumed two meals per day had significantly lower P–S (higher intrapancreatic fat deposition) after adjustment for age, sex, HbA1c, and BMI (p=0.02; table 2). No significant relationships were identified between other lifestyle factors and intrapancreatic fat deposition.

Lifestyle factors and liver ectopic fat accumulation

The relationships between lifestyle factors and liver ectopic fat accumulation (L–S) are shown in table 3. Compared with the participants who did not have SAS, those with SAS had significantly lower L–S (higher liver ectopic fat accumulation) after adjustment for covariates other than BMI (p=0.008; table 3). This relationship weakened by further adjustment for BMI (p=0.34). No significant relationships were identified between other lifestyle factors, including the number of meals consumed per day, and liver ectopic fat accumulation.

Lifestyle factors and VFA

We next evaluated the relationships between lifestyle factors and VFA (online supplemental table S2). Participants who undertook regular exercise had lower VFA than those who did not after adjustment for age, sex, and HbA1c (p=0.04), but this relationship disappeared after further adjustment for BMI (p=0.83). Participants with regular insomnia had higher VFA than those without insomnia after adjustment for age and sex (p=0.02), but this relationship weakened by adjustment for HbA1c (p=0.06) and further adjustment for BMI (p=0.11). Patients with SAS had higher VFA than those without after adjustment for age, sex, and HbA1c (p<0.001), but this relationship was abolished after adjustment for

BMI (p=0.93). No significant associations were identified between other lifestyle factors, including the number of meals consumed per day, and VFA.

Sensitivity analyses

As shown in table 4, we next compared the clinical characteristics of the participants who consumed two meals per day and those who consumed three meals per day. The former were younger than the latter (49.5 years vs 64.0 years, respectively, p=0.001), tended to be less likely to exercise regularly (23.8% vs 48.0%, p=0.06), were more likely to do night shifts (23.5% vs 1.8%, p=0.003), had higher FPG concentrations (10.3 mmol/L vs 8.0 mmol/L, p=0.04), had lower amylase activities (43.0 U/L vs 56.0 U/L, p=0.03), and had lower HDL-C concentrations (1.0 mmol/L vs 1.2 mmol/L, p=0.03). The inverse association between the number of meals consumed per day and intrapancreatic fat deposition persisted when we additionally adjusted for exercise habits, FPG, amylase activity, HDL-C concentration, insulin use, GLP-1 receptor agonist use, and tube voltage (all p≤0.03; online supplemental table S3). When we further adjusted for night-shift working, the relationship weakened (p=0.14; online supplemental table S3), although the number of participants working night shift was very few (only six cases). The associations of other lifestyle factors with intrapancreatic fat deposition and hepatic ectopic fat accumulation remained unchanged after additional adjustment for night-shift working (online supplemental table S4).

CONCLUSIONS

In the present study, we have shown that patients with type 2 diabetes who consumed two meals per day had significantly greater intrapancreatic fat deposition than those who consumed three meals per day. There were no significant associations between the number of meals consumed and liver ectopic fat accumulation; however, obesity-related factors, including SAS, were significantly associated with higher liver ectopic fat accumulation before adjustment for BMI. Similarly, the number of meals consumed per day was not associated with VFA, whereas exercise and SAS were significantly associated with VFA before adjustment for BMI.

Several mechanisms may explain the relationship between the number of meals consumed per day and intrapancreatic fat deposition. First, patients who consumed two meals per day might have had less healthy diets, containing larger amounts of sugars and fats per meal, than those consumed by patients who consumed three meals per day. High sugar intake causes substantial insulin release from the pancreas. Hyperinsulinemia has been reported to be associated with intrapancreatic fat deposition.[20] It might be possible that local insulin hypersecretion after high sugar intake might affect intrapancreatic fat deposition. In addition, the participants who consumed two meals per day had lower amylase activities

Table 2 Relationships between lifestyle factors and intrapancreatic fat deposition (P–S)

	P–S value (95% CI)			P value
Number of meals (/day)	2	3		
MV1	−14.4 (−20.0 to −9.9)	−9.9 (−11.5 to −8.4)		0.07
MV2	−15.8 (−21.8 to −10.9)	−9.7 (−11.3 to −8.2)		0.02
MV3	−15.8 (−21.8 to −10.9)	−9.7 (−11.3 to −8.2)		0.02
MV4	−15.8 (−21.8 to −10.9)	−9.7 (−11.1 to −8.2)		0.02
Snack	no	yes		
MV1	−8.1 (−12.2 to −4.7)	−10.5 (−12.2 to −8.9)		0.27
MV2	−7.8 (−12.0 to −4.6)	−10.5 (−12.2 to −8.9)		0.21
MV3	−7.8 (−11.8 to −4.6)	−10.5 (−12.2 to −8.9)		0.21
MV4	−8.4 (−12.4 to −4.9)	−10.5 (−12.2 to −8.9)		0.33
Exercise	no	yes		
MV1	−10.1 (−12.2 to −8.2)	−8.8 (−11.1 to −6.8)		0.38
MV2	−10.5 (−12.6 to −8.5)	−8.2 (−10.5 to −6.3)		0.13
MV3	−10.5 (−12.6 to −8.5)	−8.2 (−10.5 to −6.3)		0.14
MV4	−10.3 (−12.4 to −8.4)	−8.4 (−10.7 to −6.3)		0.22
Exercise at work	no	yes		
MV1	−10.0 (−11.8 to −8.4)	−11.2 (−16.3 to −7.1)		0.58
MV2	−9.5 (−11.3 to −7.8)	−12.9 (−18.5 to −8.4)		0.21
MV3	−9.5 (−11.3 to −7.8)	−12.6 (−18.2 to −8.2)		0.24
MV4	−9.5 (−11.3 to −7.8)	−13.3 (−19.1 to −8.7)		0.15
Smoke	Never	Previous	Current	
MV1	−10.9 (−13.1 to −8.9)	−10.1 (−12.9 to −7.6)	−8.7 (−12.0 to −5.8)	0.38
MV2	−11.1 (−13.3 to −9.1)	−9.5 (−12.2 to −6.9)	−8.7 (−12.2 to −5.8)	0.23
MV3	−11.1 (−13.3 to −9.1)	−9.5 (−12.2 to −6.9)	−8.7 (−12.2 to −5.8)	0.24
MV4	−11.1 (−13.6 to −9.1)	−8.9 (−11.8 to −6.6)	−9.1 (−12.6 to −6.1)	0.25
Alcohol intake	None	Intermediate	Large	
MV1	−10.9 (−13.1 to −8.9)	−10.1 (−13.1 to −7.5)	−8.6 (−11.8 to −5.8)	0.44
MV2	−11.1 (−13.1 to −9.1)	−10.3 (−13.3 to −7.6)	−7.6 (−10.9 to −4.7)	0.11
MV3	−11.1 (−13.1 to −9.1)	−10.3 (−13.3 to −7.6)	−7.6 (−10.9 to −4.7)	0.11
MV4	−10.7 (−12.9 to −8.7)	−10.5 (−13.6 to −7.8)	−8.0 (−11.3 to −5.0)	0.23
Insomnia	no	yes		
MV1	−10.4 (−12.2 to −8.9)	−9.4 (−14.0 to −5.6)		0.66
MV2	−10.5 (−12.0 to −8.9)	−9.5 (−14.0 to −5.6)		0.67
MV3	−10.5 (−12.0 to −8.9)	−9.5 (−14.0 to −5.6)		0.68
MV4	−10.5 (−12.0 to −8.9)	−9.1 (−13.8 to −5.5)		0.6
Sleep apnea syndrome	no	yes		
MV1	−10.4 (−12.2 to −8.7)	−10.4 (−14.3 to −7.3)		0.97
MV2	−10.1 (−12.0 to −8.5)	−11.1 (−15.3 to −7.8)		0.6
MV3	−10.1 (−12.0 to −8.5)	−11.1 (−15.3 to −7.8)		0.61
MV4	−10.7 (−12.6 to −8.9)	−9.1 (−13.3 to −5.6)		0.52
Night-shift work	no	yes		
MV1	−10.3 (−12.0 to −8.5)	−9.6 (−20.3 to −2.7)		0.9
MV2	−10.1 (−11.8 to −8.4)	−12.0 (−24.5 to −4.2)		0.68
MV3	−10.1 (−11.8 to −8.4)	−11.5 (−23.8 to −3.7)		0.76
MV4	−9.9 (−11.8 to −8.4)	−13.1 (−26.2 to −4.7)		0.53

Least squares mean (95% CI) values for P–S are shown.
MV1 model: not adjusted; MV2 model: adjusted for age and sex; MV3 model: further adjusted for hemoglobin A1c; MV4 model: further adjusted for body mass index.
MV, multivariate-adjusted model.

Table 3 Relationships between lifestyle factors and liver ectopic fat accumulation (L–S)

	L–S value (95% CI)			P value
Number of meals (/day)	2	3		
MV1	3.4(–1.8 to 7.8)	5.0 (3.2 to 6.7)		0.47
MV2	5.5 (0.6 to 9.7)	4.7 (2.9 to 6.4)		0.75
MV3	5.5 (0.3 to 9.7)	4.7 (2.9 to 6.4)		0.76
MV4	5.5 (0.9 to 9.5)	4.7 (2.9 to 6.4)		0.73
Snack	no	yes		
MV1	6.4 (1.5 to 10.3)	4.5 (2.6 to 6.2)		0.42
MV2	6.2 (1.5 to 10.1)	4.7 (2.9 to 6.2)		0.51
MV3	6.2 (1.5 to 10.1)	4.7 (2.9 to 6.2)		0.49
MV4	5.0 (0.3 to 9.1)	4.7 (3.2 to 6.4)		0.94
Exercise	no	yes		
MV1	3.7 (1.2 to 6.0)	5.6 (3.2 to 8.0)		0.28
MV2	4.0 (1.8 to 6.2)	5.2 (2.6 to 7.6)		0.49
MV3	4.0 (1.5 to 6.2)	5.2 (2.6 to 7.8)		0.46
MV4	4.7 (2.6 to 6.9)	4.5 (1.8 to 6.9)		0.79
Exercise at work	no	yes		
MV1	4.4 (2.3 to 6.4)	3.0(–2.5 to 7.6)		0.58
MV2	4.2 (2.1 to 6.0)	5.5 (0.6 to 9.7)		0.59
MV3	4.2 (2.1 to 6.0)	5.7 (0.6 to 9.7)		0.58
MV4	4.2 (2.3 to 6.2)	5.0(–0.3 to 9.1)		0.83
Smoke	Never	Previous	Current	
MV1	5.3 (2.9 to 7.4)	5.2 (2.3 to 7.8)	3.3(–0.9 to 6.9)	0.53
MV2	5.0 (2.6 to 7.1)	5.2 (2.1 to 8.0)	4.7 (0.6 to 8.2)	1
MV3	4.7 (2.3 to 7.1)	5.2 (2.1 to 8.0)	4.7 (0.6 to 8.2)	0.99
MV4	4.5 (2.3 to 6.7)	6.2 (3.4 to 8.7)	4.0 (0.04 to 7.6)	0.98
Alcohol intake	None	Intermediate	Large	
MV1	5.9 (3.7 to 7.8)	2.1(–1.5 to 5.2)	5.8 (2.1 to 8.9)	0.12
MV2	5.5 (3.4 to 7.6)	2.9(–0.6 to 6.0)	6.0 (2.1 to 9.3)	0.84
MV3	5.5 (3.4 to 7.6)	2.9(–0.6 to 6.0)	6.2 (2.1 to 9.5)	0.86
MV4	6.2 (4.2 to 8.0)	2.3(–1.2 to 5.2)	5.0 (1.2 to 8.5)	0.31
Insomnia	no	yes		
MV1	4.9 (3.2 to 6.4)	5.0 (0.04 to 9.1)		0.97
MV2	5.0 (3.4 to 6.7)	4.5(–0.3 to 8.5)		0.82
MV3	5.0 (3.4 to 6.7)	4.7(–0.3 to 8.7)		0.88
MV4	5.0 (3.4 to 6.4)	5.2 (0.6 to 9.1)		0.91
Sleep apnea syndrome	no	yes		
MV1	6.1 (4.2 to 7.8)	–1.6(–6.6 to 2.6)		0.002
MV2	6.0 (4.2 to 7.6)	–0.6(–5.5 to 3.7)		0.005
MV3	6.0 (4.2 to 7.6)	–0.6(–5.5 to 4.0)		0.008
MV4	5.2 (3.4 to 7.1)	2.9(–2.5 to 7.1)		0.34
Nightwork shift	no	yes		
MV1	4.3 (2.3 to 6.2)	1.7(–10.0 to 9.9)		0.64
MV2	4.2 (2.3 to 6.0)	6.7(–3.1 to 13.6)		0.59
MV3	4.2 (2.3 to 6.2)	6.7(–3.1 to 13.6)		0.6
MV4	4.5 (2.6 to 6.2)	5.5(–4.5 to 12.5)		0.82

Least squares mean (95% CI) values for L–S are shown.
MV1 model: not adjusted; MV2 model: adjusted for age and sex; MV3 model: further adjusted for hemoglobin A1c; MV4 model: further adjusted for body mass index.
MV, multivariate-adjusted model.

Table 4 Comparison of the characteristics of participants who consumed two or three meals per day

Number of meals (per day)	2	3	P value
Age (years)	49.5 (39.3, 60.5)	64.0 (51.0, 71.0)	0.001
Sex (male/female)	16 (72.7)/6 (27.3)	74 (51.7)/69 (48.3)	0.07
Snack (no/yes)	2 (10.5)/17 (89.5)	18 (13.1)/119 (86.9)	1
Exercise (no/yes)	16 (76.2)/5 (23.8)	64 (52.0)/59 (48.0)	0.06
Exercise at work (no/yes)	13 (72.2)/5 (27.8)	94 (87.0)/14 (13.0)	0.15
Smoke (never/previous/current)	9 (40.9)/6 (27.3)/7 (31.8)	72 (50.3)/49 (34.3)/22 (15.4)	0.2
Alcohol intake (none/intermediate/large)	11 (50.0)/6 (27.3)/5 (22.7)	79 (55.2)/38 (26.6)/26 (18.2)	0.78
Insomnia (no/yes)	19 (86.4)/3 (13.6)	123 (89.1)/15 (10.9)	0.72
Sleep apnea syndrome (no/yes)	15 (78.9)/4 (21.1)	96 (77.4)/28 (22.6)	1
Night-shift work (no/yes)	13 (76.5)/4 (23.5)	107 (98.2)/2 (1.8)	0.003
P (HU)	35.8 (30.0, 41.7)	37.8 (31.3, 43.5)	0.25
L (HU)	55.8 (49.0, 59.9)	54.0 (43.8, 60.0)	0.76
S (HU)	49.8 (46.1, 53.2)	48.4 (44.7, 51.8)	0.26
Visceral fat area (cm^2)	102.4 (81.2, 132.9)	121.0 (86.0, 160.6)	0.27
Subcutaneous fat area (cm^2)	184.7 (143.8, 258.8)	181.6 (110.9, 245.0)	0.41
Body mass index (kg/m^2)	29.2 (25.6, 31.3)	26.5 (23.8, 31.4)	0.17
Waist circumference (cm)	100.4 (90.6, 105.9)	97.0 (89.0, 107.2)	0.65
Duration of diabetes (years)	10.0 (6.5, 18.5)	10.0 (3.0, 18.0)	0.57
HbA1c (mmol/mol)	87.0 (68.0, 103.0)	74.0 (62.0, 95.0)	0.11
HbA1c (%)	10.1 (8.4, 11.6)	8.9 (7.8, 10.8)	0.11
Fasting plasma glucose (mmol/l)	10.3 (7.7, 12.3)	8.0 (6.7, 9.8)	0.04
AST (U/L)	24.0 (17.3, 44.5)	23.0 (17.0, 35.5)	0.56
ALT (U/L)	37.5 (19.5, 66.8)	25.0 (16.0, 46.0)	0.12
γ-GTP (U/L)	29.0 (23.3, 46.8)	35.0 (20.0, 61.0)	0.78
AMY (U/L)	43.0 (38.8, 58.3)	56.0 (45.0, 73.5)	0.03
BUN (mmol/L)	5.0 (4.0, 5.7)	5.4 (4.3, 6.1)	0.61
Cre (μmol/L)	70.7 (53.0, 79.6)	61.9 (53.0, 79.6)	0.39
UA (μmol/L)	345.0 (321.2, 398.5)	333.1 (267.7, 398.5)	0.18
CRP (mg/L)	1.0 (0.5, 3.0)	0.8 (0.4, 3.0)	0.65
T-Cho (mmol/L)	5.0 (4.5, 6.1)	4.9 (4.3, 5.6)	0.31
HDL-C (mmol/L)	1.0 (0.9, 1.2)	1.2 (1.0, 1.4)	0.03
LDL-C (mmol/L)	3.1 (2.3, 4.0)	2.9 (2.3, 3.6)	0.35
TG (mmol/L)	1.5 (1.2, 3.4)	1.6 (1.1, 2.1)	0.39

Values are median (first quartile, third quartile) for continuous variables and number (percentage) of participants for categorical variables.
P value is calculated based on Mann-Whitney U test (for continuous variables) or Fisher's exact test (for categorical variables).
ALT, alanine transaminase; AMY, amylase activities; AST, aspartate transaminase; BUN, blood urea nitrogen; Cre, creatinine; CRP, C reactive protein concentrations; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; L, mean CT values for the liver; LDL-C, low-density lipoprotein cholesterol; P, mean CT values for the pancreas; S, mean CT values for the spleen; T-Cho, total cholesterol; TG, triglyceride; UA, uric acid; γ-GTP, γ-glutamyl transpeptidase.

than those who consumed three meals per day, which implies lower pancreatic exocrine function, perhaps due to acinar cell atrophy secondary to high fat intake.[21] Because we did not have data regarding the composition of the diets of the participants or the frequencies with which they consumed each component, further studies are required to evaluate the relationships between diet composition and intrapancreatic fat deposition. Another

potential mechanism is circadian misalignment. The circadian rhythm is affected by light and dietary stimuli and regulates metabolism.[22] Circadian misalignment has been reported to cause both hyperglycemia and hyperinsulinemia,[23] which may increase intrapancreatic fat deposition. In the present study, after additional adjustment for night-shift working, the association between the number of meals consumed per day and intrapancreatic

fat deposition weakened, and therefore the association between skipping meals and intrapancreatic fat deposition may at least in part be explained by circadian misalignment.

An interesting finding of the present study is that intrapancreatic fat deposition seems to be independent of obesity, which is known to be closely related to the development of non-alcoholic fatty liver disease.[24] We found that the association between the number of meals consumed per day and intrapancreatic fat deposition persisted after adjustment for BMI, whereas there were no significant relationships between obesity-related factors, such as SAS, and intrapancreatic fat deposition. In contrast, SAS was positively associated with liver ectopic fat accumulation and this association weakened after additional adjustment for BMI. In addition, the correlation between liver ectopic fat accumulation and VFA was slightly stronger than the correlation between intrapancreatic fat deposition and VFA (r=0.35 and r=0.27, respectively). These findings are consistent with those of previous studies. In a study of data from 56 participants with type 2 diabetes who were hospitalized twice between April 2008 and September 2018, we previously showed that intrapancreatic fat deposition was less strongly associated with obesity-related markers, such as BMI, previous highest BMI, and waist circumference, than liver ectopic fat accumulation.[4] Another study of 1478 participants showed that fatty pancreas disease occurred not only in patients with obesity, but also in lean individuals, and that intrapancreatic fat deposition is longitudinally associated with a higher risk of type 2 diabetes in lean individuals.[25] The lack of statistical significance between intrapancreatic fat deposition and liver fat was consistent with our previous study of patients with type 2 diabetes.[4] On the other hand, in other studies excluding or rarely including patients with diabetes, intrapancreatic fat deposition was related to liver fat.[26 27] As it has been reported that the intrapancreatic fat deposition in patients with diabetes was higher than in patients without diabetes,[19] the inconsistency of the associations might be due to patient characteristics (with type 2 diabetes in our study and without diabetes in other studies). In the current dietary guidelines for patients with diabetes, skipping meals is not recommended[28] because this worsens glycemic control[29] and increases the risk of arteriosclerosis.[30] The present findings are supportive of the current recommendations and future studies of dietary composition and frequency of consumption of specific components might aid deeper understanding of the mechanisms of intrapancreatic fat deposition.

The strengths of the present study include the simultaneous assessments of various lifestyle factors and the quantification of intrapancreatic fat deposition, liver ectopic fat accumulation, and VFA using abdominal CT. However, there are several limitations that should be noted. First, the present study was retrospective and the assessment of lifestyle was based on self-reporting via questionnaires and interviews. We do not have detailed dietary data, including energy consumption and the content, and detailed information on smoking, such as the number of cigarettes per day. In addition, the questionnaires used in this study have not been validated yet. However, the accuracy of the questionnaires was confirmed by the attending physicians and nurses, and all the interviews were conducted by trained doctors, nurses, or registered dietitians, which should have allowed us to collect information worth evaluating. Second, histological confirmation of the fat accumulation was not performed. However, the assessment method used in the study has been validated in several previous studies, which showed strong correlations between CT radiodensity values and the amount of fat, determined histologically, in both the pancreas[14 15] and liver.[17 18] Third, in this study unenhanced CT was performed using several scanners and we did not account for the difference between the scanners. Fourth, although we thoroughly excluded patients with multiple diseases that could affect lifestyle habits and intrapancreatic fat deposition, generalizability is limited because participants in the present study were patients who were hospitalized and underwent CT. The necessary exclusions of patients with multiple diseases resulted in the small number of participants in the study, and the small sample size of 22 patients who consumed two meals is also a limitation of this study. Future study is needed to evaluate the relationships between lifestyle factors and ectopic fat accumulation in outpatients in larger populations.

In conclusion, we found that patients who consumed two meals per day had higher intrapancreatic fat deposition than those who consumed three meals per day. This finding is supportive of the current diabetes guideline that skipping meals should be avoided. Future studies based on our results may lead a novel approach for treatment of diabetes focusing on intrapancreatic fat deposition.

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