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ORIGINAL ARTICLE

Duodenal adipose tissue is associated with obesity in baboons (*Papio* sp): a novel site of ectopic fat deposition in non-human primates

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Abstract

Aims Ectopic fat is a recognized contributor to insulin resistance and metabolic dysfunction, while the role of fat deposition inside intestinal wall tissue remains understudied. We undertook this study to directly quantify and localize intramural fat deposition in duodenal tissue and determine its association with adiposity.

Methods Duodenal tissues were collected from aged (21.2 ± 1.3 years, 19.5 ± 3.1 kg, $n = 39$) female baboons (*Papio* sp.). Fasted blood was collected for metabolic profiling and abdominal circumference (AC) measurements were taken. Primary tissue samples were collected at the major duodenal papilla at necropsy: one full cross section was processed for hematoxylin and eosin staining and evaluated; a second full cross section was processed for direct chemical lipid analysis on which percentage duodenal fat content was calculated.

Results Duodenal fat content obtained by direct tissue quantification showed considerable variability (11.95 ± 6.93%) and was correlated with AC ($r = 0.60$, $p < 0.001$), weight ($r = 0.38$, $p = 0.02$), leptin ($r = 0.63$, $p < 0.001$), adiponectin ($r = -0.32$, $p < 0.05$), and triglyceride ($r = 0.41$, $p = 0.01$). The relationship between duodenal fat content and leptin remained after adjusting for body weight and abdominal circumference. Intramural adipocytes were found in duodenal sections from all animals and were localized to the submucosa. Consistent with the variation in tissue fat content, the submucosal adipocytes were non-uniformly distributed in clusters of varying size. Duodenal adipocytes were larger in obese vs. lean animals (106.9 vs. 66.7 μm^2, $p = 0.02$).

Conclusions Fat accumulation inside the duodenal wall is strongly associated with adiposity and adiposity related circulating biomarkers in baboons. Duodenal tissue fat represents a novel and potentially metabolically active site of ectopic fat deposition.

Keywords: Non Human Primates; Baboons; Insuline resistance; Ectopic fat deposition; Adipose tissue; Duodenum; Gastrointestinal tract

Introduction

The ectopic deposition of triglyceride is an important contributor to obesity associated insulin resistance metabolic dysfunction [1]. Adverse metabolic effects of excess ectopic fat in liver and skeletal muscle are well documented [2, 3]. Additional sites of ectopic fat deposition continue to be identified [4, 5] and evidence suggests that fat accumulation at these sites also contributes to metabolic dysfunction [4–6].

Obesity associated ectopic fat deposition in intestinal tissues is suggested by several studies [7–9]. Although the occurrence of fat inside the large intestine wall is a well-described computed tomography (CT) finding in inflammatory bowel disorders [10–12]—known as the fat halo sign—several reports have also described intramural fat deposition

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in the intestines of individuals with no inflammatory bowel disease [7, 8, 13, 14]. Several of these reports described a greater frequency of intestine wall fat among higher body weight individuals [7, 8]. Moreover, in a recent study, CT detected stomach wall fat was more common in individuals with BMI greater than 25 kg/m^2 and was linked to higher visceral fat, hepatic steatosis, and the presence of intramural fat in the ileum and colon [9]. Detailed investigations of intestinal fat deposition involving direct quantification of intramural fat and its associations with adiposity have not been undertaken.

We investigated duodenal tissue fat deposition and its relationship to adiposity and circulating metabolic biomarkers in a large non-human primate model. Our objectives were to: (1) directly quantify intramural fat content in the duodenum and determine its association with adiposity; and (2) identify and the location of and characterize the nature of the fat deposited in the duodenum. We found that duodenal wall fat exhibited considerable inter-animal variation, was strongly associated with adiposity and adiposity related biomarkers, and occurred inside variably sized clusters of adipocytes that were distributed throughout the submucosal region.

Subjects and methods

Non-human primates

Samples were obtained from 39 female baboons (*Papio Sp.*) housed at the Southwest National Primate Research Center (SNPRC), San Antonio, TX. Female baboons were studied to avoid the confounding effects of sex differences in adiposity and metabolic disease risk factors [15, 16].

All baboons used were housed in social groups either in large 2.4 ha outdoor corrals or in 95 m^2, 9 m-high covered outdoor cages according to established National Research Council guidelines. Animals were fed a commercial monkey chow diet (5LE0 solid feed, LabDiet, PMI, St. Louis, MO) containing 3.26 kcal/g with 13.8% fat, 67.2% carbohydrate (3% sugar), and 19% protein as a percentage of total energy. Water was provided ad libitum and the diet was supplemented with grains, fruits, and vegetables.

Our study was designed to utilize an ongoing colony management protocol to obtain duodenal tissues in animals selected to undergo necropsy. All sample collections were conducted over a 3 month period between late May and late August. Veterinary health assessments on all animals used in the study, including blood chemistry and hematology profiles, were found to be normal. Electronic health and research procedure records were searched to confirm that the animals were not subject to any pharmacological or surgical interventions over their lifespans. Animals were consuming the same low-fat maintenance diet (as described above) for at least 2 years prior to necropsy. Gross examinations and histological assessments showed that animals included in the final analysis were found to be free of major pathologies, including gastric, small intestine, and large intestine pathologies, as determined by a Board Certified Veterinary Pathologist (E.J.D., M.O.). Study procedures were approved by the Institutional Animal Care and Use Committee of the Texas Biomedical Research Institute, San Antonio, TX. This research was undertaken in compliance with National Guidelines and with American Society of Primatologists Principles for the Ethical Treatment of Nonhuman Primates.

Body weight and abdominal circumference measurements

Animals were sedated with ketamine hydrochloride (VEDCO, St. Joseph, MO), at 10 mg/kg and body weight was measured three times and averaged to the nearest 0.1 kg using a calibrated electronic weighing scale (GSE 665, Texas Scales Inc., Cibolo, TX). After euthanasia, with the animal supine, abdominal circumference (AC) was measured three times and averaged to the nearest centimeter at the midpoint between the lowest rib and the iliac crest, using a calibrated fixed tension plastic measuring tape (Gulick 2 Plus, Creative Health Products, Ann Arbor, MI).

Clinical pathology and circulating biomarkers

After a 12 h overnight fast, baboons were anesthetized with ketamine hydrochloride (VEDCO, St. Joseph, MO), at 10 mg/kg and blood samples were collected from the femoral vein and processed for plasma and serum. Fasting serum chemistries (including glucose, triglyceride, total cholesterol, and HDL cholesterol) were analyzed using an ACE Clinical Chemistry Analyzer (Alfa Wassermann Diagnostic Technologies, LLC, West Caldwell, NJ). Serum insulin and C-peptide were measured by automated radioimmunoassay (Immulite™1000 Immunoassay System, Siemens Medical Solutions Diagnostics, Los Angeles, CA). Fasting plasma leptin and adiponectin (26,414 Da form) concentrations were analyzed in duplicate using commercially available ELISA kits (EMD Millipore, Billerica, MA). Intra-assay CVs for leptin and adiponectin were 2.2 and 5.2%, respectively.

Necropsy tissue collection, histology and immunocytochemistry

Animals were euthanized using an intravenous injection (0.2 mg/kg) of pentobarbital sodium (Fatal-Plus Solution™, Vortech Pharmaceuticals, Dearborn, MI) and necropsy was initiated with open laparotomy. A full cross section of duodenum was systematically collected from each animal

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at the level of the major duodenal papilla as identified by veterinary pathologists (E.J.D., M.O.). All extraneous tissues, including mesenteric adipose tissue, were carefully dissected and removed from the duodenal wall. The cleaned section was then divided into two additional cross sections. The first section was fixed in 10% neutral buffered formalin, processed conventionally, embedded in paraffin, cut at 5 µM, and stained with hematoxylin and eosin (H&E). Slide images were captured using a Nikon DXM1200C camera mounted on a Nikon Eclipse 80i microscope (Nikon Instruments Inc., Melville, NY). The remaining full-thickness tissue cross section (~2 cm) was placed in a cryovial, immediately frozen in liquid nitrogen, and stored at − 80 °C for direct lipid quantification. After completion of duodenal tissue collection, additional gastric, jejunal, and colonic tissues were collected and processed for histology assessments as described above. Immunocytochemistry for CCK e GLP-1 was performed as previously described [17].

Quantification of duodenal tissue fat content

Direct chemical analysis of fat content (total lipid) was undertaken by SDK Laboratories (Hutchison, KS) using the direct ether extraction technique. Analyses were performed on two 5-g cross sections taken from each duodenal sample. The technique was performed according to the Asso[illegible]C) Method [illegible]ay

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CV for two repeat measurements on samples from 12 different animals was found to be 10.5%. The average of the measured fat content from each of the two tissue cross sections was used to represent duodenal tissue fat content (DFAT) was expressed as a percentage of total duodenal tissue mass (%DFAT). (Table 1).

Measurement of submucosal adipocyte size

Adipocyte cell sizing was undertaken on duodenal slide sections from a subsample of lean ($n=3$) and obese ($n=3$) baboons selected based on AC measurements. Lean and obese animals were selected based on AC less than the 25th percentile and greater than the 75th percentile of the sample, respectively. Images for cell sizing from each H&E-stained slide were captured using an Olympus Trinocular Microscope (Olympus Corporation of the Americas, Center Valley, PA) and BioQuant Osteo II (version 8.10.20, BioQuant Image Analysis Corporation, Nashville, TN). Two-hundred adipocytes per animal were randomly selected across the slides to remove any site-specific bias. Cell size analysis was undertaken using ImageJ software [19] with 64-bit Java 1.8.0_77. Pixels were converted to µm by applying the pixel/µm ratio of the microscope using the "SCALE" function on ImageJ. The final cell size data used for analysis represent the mean of 200 individual adipocytes from each of the baboons ($n=6$). The analyst was blinded to the obesity status of the animals.

Table 1 Distribution statistics and percentiles of the directly quantified duodenal fat content variable, $n=38$

	Mean	SD	SEM	Min–max
DFAT (g)	0.60	0.34	0.06	0.07–1.35
%DFAT	11.95	6.93	1.13	1.38–27.05
DFAT percentiles (g)		Value		95% CI
10th		0.13		0.07–0.29
25th		0.31		0.13–0.43
50th		0.54		0.39–0.73
75th		0.82		0.69–1.03
90th		1.06		0.92–1.35
%DFAT percentiles		Value		95% CI
10th		2.6		1.4–5.7
25th		6.3		2.6–8.7
50th		10.9		7.7–14.5
75th		16.3		13.8–20.7
90th		21.2		18.4–27.1

DFAT duodenal tissue fat mass in grams, *%DFAT* fat mass as a percentage of the total duodenal tissue mass, *CI*

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Statistical analysis

Distributional characteristics of the newly generated duodenal tissue fat content variable (DFAT/%DFAT) were analyzed using Shapiro–Wilk and Anderson–Darling tests for deviation from the Gaussian normal distribution and a two-tailed classical Grubbs test at an alpha level of 0.05 for outlier identification. %DFAT percentiles were calculated using the weighted average at X_{np} method with normal distribution-based confidence intervals. Relationships among the variables were determined using Pearson correlation and coefficients of determination (R^2) from ordinary least squares regression. Mean values comparisons were undertaken using two-tailed independent samples *t* tests. Principal component regression analysis was used to model the relationship between leptin and the inter-correlated adiposity variables: accounting for multicollinearity among the predictor variables [20]. Statistical analysis was undertaken using XLSTAT version 19.01.42700 (Addinsoft, Paris, France) and the R package version 3.4.0 (The R Foundation for Statistical Computing, http://www.r-project.org).

Results

All animals (21.2 ± 1.3 years, 19.5 ± 3.1 kg) had normal renal function and liver enzyme profiles according to age (Suppl. Table 1a).

All duodenal tissues were normal based on histological assessments by Board Certified Pathologists (EJD, MO). Descriptive data for the metabolic variables studied are given in Supplemental Table 1b. Significant quantities of fat were present in the duodenal tissues of all animals ($n = 39$) and showed considerable variation. One outlier was identified in the %DFAT data ($G = 3.97$, $p < 0.001$, Suppl. Figures 1A & 1B). After removing the outlier, %DFAT had a normal distribution (Suppl. Figures 1c–e), with a mean and standard deviation of 11.95 ± 6.93%. Detailed descriptive and percentile data for %DFAT (with outlier removed) are given in Table 2. %DFAT was significantly correlated with both body weight and with AC (Fig. 1). A stronger correlation between %DFAT and AC than between %DFAT and body weight was observed. These relationships were present regardless of whether the previously detected outlier was included (Fig. 1a–c) or excluded (Fig. 1d–f). This outlier was excluded from the subsequent analyses.

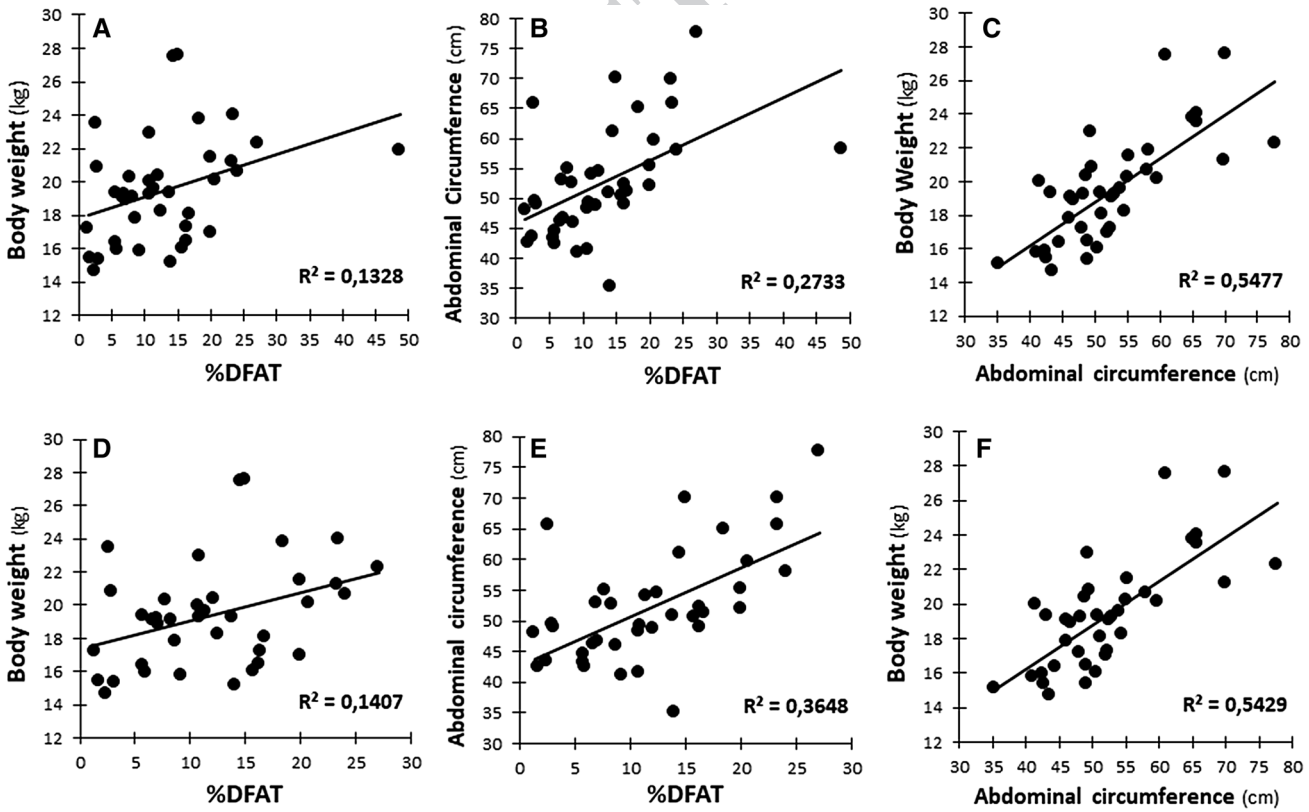

Fig. 1 Scatter plots of the relationships among percentage duodenal fat content (%DFAT), abdominal circumference, and body weight before (**a**–**c**, $n = 39$) and after outlier removal (**d**–**f**, $n = 38$)

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Table 2 Principal component regression analysis, dependent variable fasted leptin, $n = 38$

	Sum of squares	Mean squares	F	p
Model	49.24	16.41	15.42	< 0.0001
	Value	SE	t	p
Principal components model				
Intercept	2.72	0.17	16.22	< 0.0001
PC1	0.65	0.11	5.71	< 0.0001
PC2	0.27	0.21	1.30	0.202
PC3	− 1.27	0.37	− 3.54	0.001
Input variables model[a]				
Intercept	− 1.27	18.34	− 0.06	0.945
Weight	0.29	0.08	3.69	0.001
AC	− 0.07	0.03	− 2.02	0.052
%DFAT	0.14	0.03	4.52	< 0.0001

Dependent variable fasted plasma leptin regressed on %DFAT, body weight, and abdominal circumference, $n = 38$. Adjusted $R^2 = 0.54$, root mean square error = 1.03.

PC principal component, *%DFAT* percentage duodenal fat content, *AC* abdominal circumference

[a]Results of the principal component model transformed back into the original data space to show the model parameters as they correspond to the original input variables. The principal component analysis (that generated PC1-3) is detailed in Supplemental Table 4

H&E staining revealed a consistent presence of adipocytes in the submucosal layer of the duodenum in all animals. Clusters of adipocytes of varying size were observed to be diffusely deposited across the duodenal submucosa. Variation in the duodenal submucosal adipocyte deposition in four representative animals is shown in Fig. 2. Large clusters of adipocytes/adipose tissue deposits (Fig. 2d) were observed in several animals. Duodenal submucosal adipocytes from obese animals were 60% larger (106.9 ± 9.2 vs. 66.7 ± 3.4 μm^2, $p = 0.02$) than those from lean animals (Fig. 3), descriptive data for this subset of animals are provided in Suppl. Table 2. The variation in both adipocyte number and adipocyte size was consistent with the variation in directly quantified duodenal wall fat content. Further assessments of gastrointestinal tissues collected from these animals during necropsy revealed that adipocytes were distributed in similar diffuse patterns in the gastric, jejunal, and colonic submucosae (Suppl. Figure 3).

To have some indication whether fat deposition in the duodenum can influence food intake and body weight, CCK and GLP-1 expression were evaluated by immunocytochemistry in duodenal sections of lean and obese animals (Suppl. Figure 4). While it was notable in these samples the expansion of adipose tissue of the submucosa in obese, there are no apparent differences in the number of GLP-1 immunoreactive cells between lean and obese animals. However, it seems that CCK cells are more numerous in the obese animals. A future systematic study in a larger number of samples will look to substantiate these possible changes and potential pathophysiological implications.

Relationships among %DFAT, AC, body weight, and circulating metabolic biomarkers concentrations are shown in Fig. 4. %DFAT was significantly correlated with fasting leptin ($r = 0.63$, $p < 0.001$, Fig. 4a), adiponectin ($r = -0.32$, $p < 0.05$, Fig. 4d), and triglyceride ($r = 0.41$, $p = 0.01$, Fig. 4g). Combined, the correlations suggested that leptin, adiponectin, and triglyceride were more strongly related to %DFAT than to AC (Fig. 4b, e, h) or body weight (Fig. 4c, f, i). %DFAT was not correlated with glucose, insulin, C–peptide, total cholesterol, or HDL–cholesterol. A matrix showing all correlations is provided in Suppl. Table 3. The relationship between %DFAT and leptin was strong and was similar in magnitude to the relationship between body weight and leptin (Suppl. Fig. 2). A regression model accounting for the collinearity among body weight, AC, and %DFAT revealed that %DFAT remained strongly associated with fasted leptin concentrations after accounting for variation in body weight and AC (Table 2, Suppl. Table 4).

Discussion

Findings from several studies suggest an association between obesity and the presence of intramural fat in the gastrointestinal tract. To date, direct investigation of intestinal wall fat in relation to adiposity has not been undertaken. Here, we present the first evidence to show that intramural fat in the duodenum is associated with adiposity, circulating adipokines, and triglyceride concentrations. Our histology findings indicate that duodenal fat is deposited predominantly inside adipocytes located in the submucosal tissue layer. Combined, our data are consistent with the duodenum as a novel and potentially metabolically active site of ectopic fat deposition.

The accumulation of triglyceride in tissues such as liver, skeletal muscle, vasculature, and pancreas is widely documented in human and animal models of obesity and, in some cases, is strongly linked to metabolic pathophysiology [1–6]. To date, a small number of intestinal imaging studies has suggested that there is also obesity associated fat deposition within the tissues of the gastrointestinal tract. Intramural fat accumulation in the colon has been widely reported as a distinct ring of low-density tissue on computed tomography scans; it is known as the fat halo sign and is a common finding in Crohn's disease and associated inflammatory bowel conditions [10–12]. Increased numbers of submucosal adipocytes are likely to underlie this phenomenon [12, 14]. Notably, fat halos have also been reported in the intestines of patients with no inflammatory bowel diseases and were found to be associated with body weight [7, 8, 13]. Although

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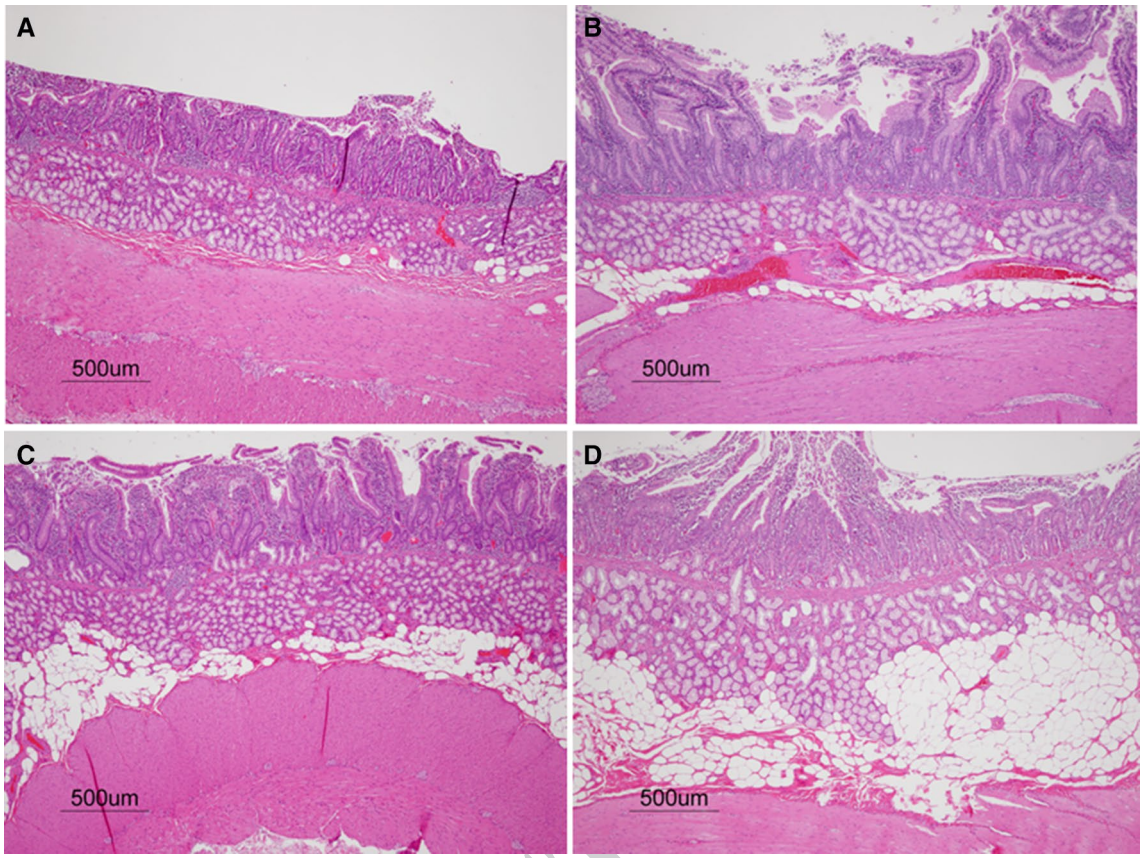

Fig. 2 Representative hematoxylin and eosin-stained duodenal wall sections from four baboons. Panels show the presence of adipocytes in the submucosa. Panels represent the variation seen in duodenal submucosal adipocyte accumulation in the sample studied: from small numbers of adipocytes (Panel A) to large clusters resulting in expansion of the submucosa (Panel D)

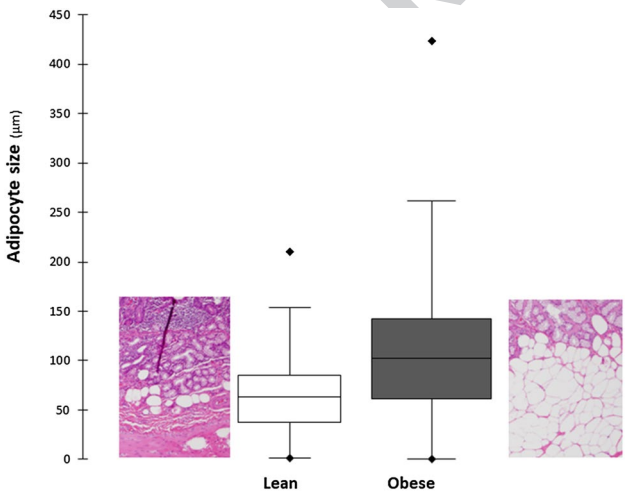

Fig. 3 Box-plots of adipocyte cell size variation in lean ($n=3$) and obese ($n=3$) baboons. Bars represent median and 25–75th percentiles. The representative images to show the adipocyte size are also provided

a recent histological evaluation of autopsy samples did not find a correlation between submucosal thickness—used as a surrogate for submucosal adipose deposition—and BMI in male patients [14], it is possible that the indirect submucosal thickness measurements did not sufficiently capture variation in intestine wall fat, particularly given the non-uniform distribution of submucosal adipocytes. It is also possible that intramural fat in the distal intestine is not as strongly associated with obesity as that of the proximal intestine. In support of this argument, results from a large study by Gervaise et al. [9] showed computed tomography evidence for stomach wall fat accumulation in a subset of individuals with higher BMIs, greater visceral fat, and no known gastrointestinal disease [8]. Fat deposition in relation to adiposity in the proximal small intestine wall has not been reported.

Our data show that duodenal tissue fat content, quantified by direct chemical analysis, is strongly associated with body weight and central adiposity in the highly clinically relevant baboon model [16, 21–24]. Baboons show considerable variation in body fat [21] and a form of diabetes, consistent with human type 2 diabetes occurs in baboons [16]. Furthermore, fat gain and dysmetabolism can be

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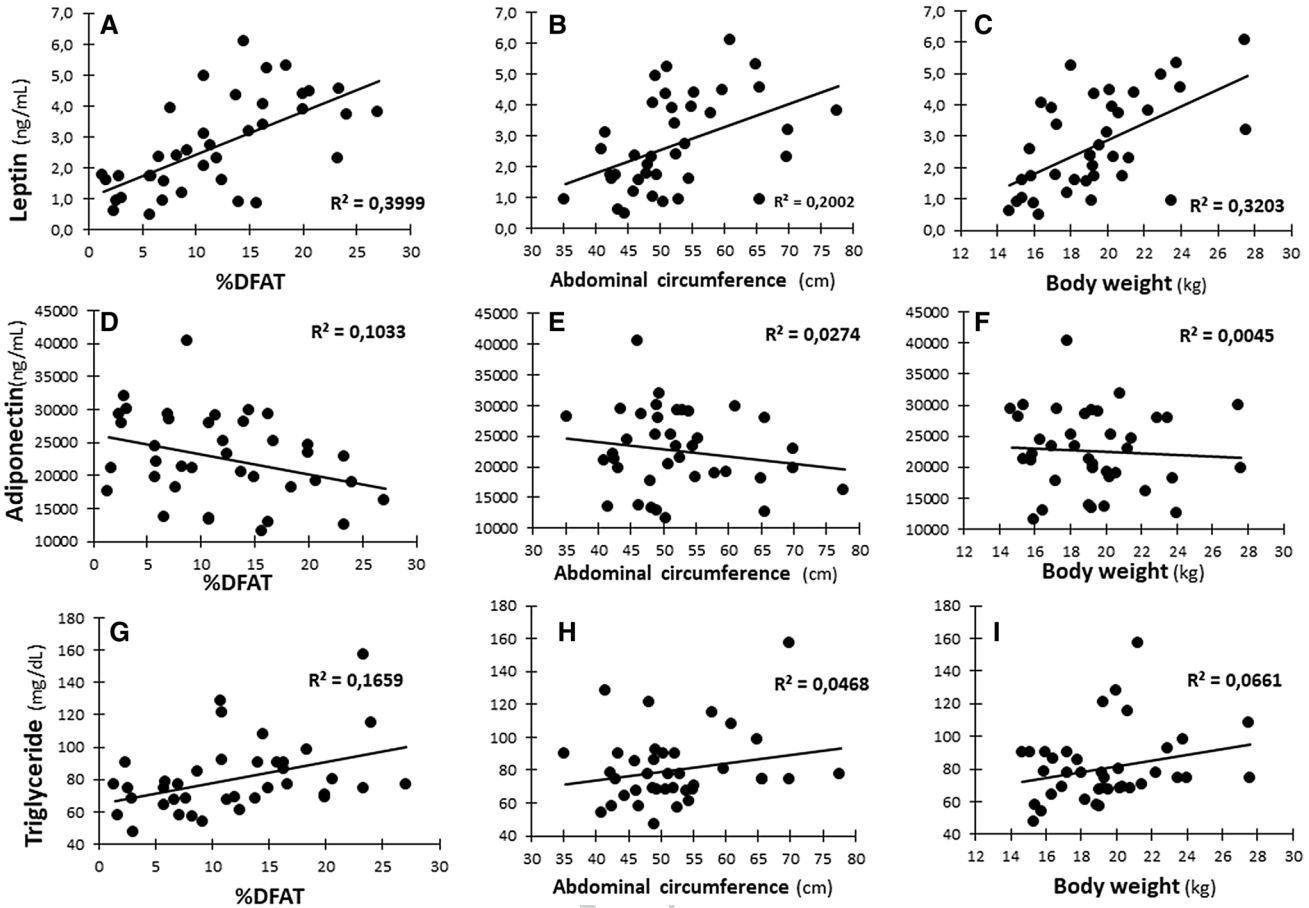

Fig. 4 Scatter plots of the interrelationships among leptin, adiponectin, and triglyceride and percentage duodenal fat content (%DFAT), abdominal circumference, and body weight

induced by diet in baboons [23]. Importantly, aged baboons (21 years) like those used in this study, reflects human older age (55–65 years), are insulin-resistant and have many of the molecular and cellular features of human skeletal muscle and adipose tissue insulin resistance [16, 25]. In addition, pancreatic islet pathology in diabetic baboons is similar to that of humans [22, 26, 27]. The pragmatic advantages offered by working with a large, long-lived, and tractable non-human primate, make the baboon a highly important and valid model for the study of obesity and related diseases. The animals used in this study are all female because they were present in large excess of males in the colony and were the focus of the management efforts. Although, the phenomenon of submucosal lipid deposits has also been found by our pathologists in male animals, we were unable to include enough males to have an appropriately balanced sample and therefore we chose to focus primarily on females.

Our findings advance those of previous imaging studies and demonstrate a robust association between adiposity and %DFAT. We show that duodenal fat content is a normally distributed variable with considerable inter-animal variability. This variation was consistent with histological observations of high variability in submucosal adipocyte accumulation in duodenal sections from the same animals. Our other histology findings suggest that submucosal adipocyte clusters of varying size are present throughout the length of the gastrointestinal tract. Intramural adipose in the submucosa of the gastrointestinal tract may represent a relatively large and underappreciated ectopic fat depot.

We found robust relationships between %DFAT and obesity associated circulating biomarkers. Although we did not see any relationships between %DFAT, glucose, and insulin, we found that fasting plasma leptin and adiponectin, and serum triglyceride concentrations were significantly correlated with duodenal tissue fat content. Taken together, the correlations suggest that circulating leptin, adiponectin, and triglyceride concentrations were more strongly related to %DFAT than to AC. Although we were limited to the use of AC as a measure of whole-body adiposity, we have previously shown that AC is a robust predictor of dual-energy X-ray absorptiometry measured body fat in baboons [16, 28] as both male and female

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baboons distribute excess body fat in the abdominal region [15]. However, we have previously shown that whole-body fat deposition negatively affects insulin sensitivity determined by the gold standard methodology, the euglycemic clamp as well as indirect measures of insulin sensitivity such as Homa-IR and insulin levels [16, 28]. Thus it is likely that also in this study, DFAT in obese animals might also contribute to whole-body insulin resistance. Moreover, the negative association between adiponectin and DFAT suggests that it may influence insulin sensitivity, at least indirectly.

The relationship between %DFAT and leptin was particularly strong. After accounting for the interrelationships among body weight, AC, and %DFAT in a regression model, %DFAT remained a strong predictor of fasted leptin concentrations. Stomach epithelial cells are known to produce leptin [29] and leptin itself exerts multiple actions on intestinal cells [30]. The strength of the relationship between duodenal fat content and circulating leptin may indicate involvement of submucosal adipose in regulation of circulating leptin concentrations or conversely, that circulating leptin/leptin resistance influences submucosal fat accumulation. Nonetheless, in the absence of a more direct measure of body composition, the association between leptin and %DFAT may be best understood as reflecting a strong relationship between %DFAT and whole-body fat stores. It will be necessary to further explore and unravel these relationships in future studies.

Our findings are consistent with duodenal fat accumulation occurring primarily inside submucosal adipocytes that expand in both number and size with increased duodenal fat content. It is known that ectopic fat deposition in the pancreas also occurs inside adipocytes [31, 32]. Small intestine submucosal adipocytes are frequently described in histology texts [33] but have not received much attention and their origin is unknown. The presence of large clusters of adipocytes resulted in duodenal submucosal expansion, as previously shown in human ileal and colonic submucosae [14]. We also found that submucosal adipocytes from obese baboons were significantly larger than those of their lean counterparts, indicating that, as with other adipose depots [34], submucosal adipose tissue expansion is likely to result from both an increase in cell size and cell number. The role of submucosal adipocytes in normal physiology is unknown. In addition to blood and lymphatic vessels, the submucosa contains Meissner's plexus, a branch of the enteric nervous system, which innervates the cells of the mucosa and the vasculature cells of the submucosa. Hanani et al. have reported that neurons of Meissner's plexus directly innervate submucosal adipocytes in the human colon [35]. These data suggest the intriguing possibility of a functional relationship between submucosal nerves and nearby adipocytes. Whether submucosal adipocytes engage in cross-talk with other intestinal cell types and what effect, if any, their expansion has on intestinal cell function will require investigation.

The data presented herein provide the first evidence for the presence of adiposity associated ectopic fat deposition in the duodenal tissue and provide a foundation for further investigation in baboons, which are extensively characterized non-human primate model for obesity, metabolic syndrome and type 2 diabetes mellitus. Further studies are needed to: (1) replicate the associations between adiposity and duodenal fat quantified using non-invasive intestinal imaging in humans; (2) quantify the deposition of intramural fat throughout the gastrointestinal tract and determine its association with adiposity; and (3) determine the role submucosal adipocytes and the effects of their increased accumulation on intestinal function. In conclusion, duodenal tissue fat represents a novel and a potentially important site of ectopic fat deposition. Duodenal fat, in addition to intramural adipose throughout the submucosa of the gastrointestinal tract, warrants further investigation.

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Compliance with ethical standards

Conflict of close.

Animal rights
This research was undertaken in compliance with the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985), as well as with the National Guidelines and the American Society of Primatologists principles for ethical treatment of Non-Human Primates.

Informed consent
For this type of study formal consent is not required

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