Longer Life Foundation Project Progress Report 2019
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1. Overview

Increasing age and excess adiposity cause alterations in cardiometabolic function, including impaired glucose homeostasis, atherogenic dyslipidemia, and nonalcoholic fatty liver disease (NAFLD), which are important risk factors for type 2 diabetes (T2D), cardiovascular disease (CVD), frailty, and premature mortality[1, 2, 3]. Insulin resistance is central to many of the metabolic and medical complications associated with aging and excess adiposity because insulin is a key regulator of liver glucose production, liver triglyceride (TG) synthesis, plasma TG concentration, and skeletal muscle glucose uptake[3-9]. Accordingly, insulin resistance is implicated in the pathogenesis of T2D, dyslipidemia, hypertension, and coronary heart disease[3-6, 8-10]. Insulin resistant people have a 4- to 11-fold increased relative risk of T2D than healthy normal-weight subjects[11, 12]; experience a greater decline in muscle mass, muscle strength, and functional capacity with aging[13, 14]; have a 2- to 4-fold increased risk for sarcopenia [15], cognitive impairments/Alzheimer's disease[16], [17] and certain cancers (e.g., colon and breast) [6, 7]

The purpose of this study is to increase our understanding of why some people with obesity are metabolically normal and others are metabolically abnormal.

The following three distinct groups of subjects, separated based on metabolic health and adiposity, were studied:

Metabolically-normal lean (MNL) group: i) body mass index (BMI) ≥18.5 and ≤24.9 kg/m^2; ii) liver fat content ≤3%; iii) fasting plasma glucose concentration <100 mg/dl; iv) 2-hr oral glucose tolerance (OGTT) plasma glucose concentration <130 mg/dl; and v) hemoglobin A1C ≤5.5%

Metabolically-normal obese (MNO) group: i) BMI ≥30.0 and ≤48.0 kg/m^2; ii) liver fat content ≤3%; iii) fasting plasma glucose concentration <100 mg/dl; iv) 2-hr oral glucose tolerance (OGTT) plasma glucose concentration <130 mg/dl; and v) hemoglobin A1C ≤5.5%

Metabolically-abnormal obese (MAO) group: i) BMI ≥30.0 and ≤48.0 kg/m^2; ii) liver fat content ≥5.6%; iii) hemoglobin A1C ≥5.7%; and iv) fasting plasma glucose concentration ≥100 mg/dl, or 2-hr OGTT plasma glucose concentration ≥140 mg/dl.

2. Study subjects

The baseline characteristics of the enrolled study subjects enrolled are shown in Table 1. These data demonstrate excellent separation of characteristics among groups needed to test our study hypotheses. The metabolically abnormal obese (MAO) group demonstrate clear characteristics of metabolic dysfunction compared with the metabolically normal lean (MNL) and metabolically normal obese (MNO) groups (i.e. increased liver fat content, higher fasting blood glucose and blood glucose 2 hours after ingesting an oral glucose load during the oral glucose tolerance test (OGTT), abnormal blood lipids and higher hemoglobin A1c (HbA1c).

Table 1. Subject characteristics

	MNL (n=24)	MNO (n=24)	MAO (n=24)
Age (years)	30	36	44
Sex (M/F)	12/12	3/36	8/24
Body mass index (kg/m^2)	22.6	37.4	39.8
Body fat (% of body weight)	28.1	47.5	48.0
Liver fat content (%)	1.8	2.3	14.5
Plasma concentrations			
Fasting glucose (mg/dL)	85	86	100
2-hour OGTT glucose (mg/dL)	97	109	169
Triglycerides (mg/dL)	65	67	127
HDL-cholesterol (mg/dL)	64	53	46
LDL-cholesterol (mg/dL)	98	97	109
HbA1c (%)	5.0	5.0	5.7

Results

a. Cardiopulmonary fitness (VO_2 peak). Cardiopulmonary fitness was determined by the subject's ability to consume oxygen during maximum treadmill exercise (continually increased treadmill speed and incline until volitional exhaustion). The metabolically normal obese group demonstrated better cardiopulmonary fitness than the metabolically abnormal obese group, and both obese groups were less "fit" than the lean group (Table 2).

Table 2. VO_2peak

	Metabolically Normal Lean	Metabolically Normal Obese	Metabolically Abnormal Obese
VO_2peak (ml O_2/kg fat-free mass/min)	51.4	46.1	39.2

b. Body composition.

i) Whole-body dual energy x-ray absorptiometry (DXA) assessment (Table 3) revealed marked differences between lean and obese individuals but the same lean body mass, fat mass and bone mass in MNO and MAO subjects.

Table 3. Contribution from lean mass, fat mass and bone to total body weight

	% of total body mass		
	MNL	MNO	MAO
Lean body mass	68.0%	49.7%	49.4%
Fat mass	28.1%	47.5%	48.0%
Bone mass	3.9%	2.8%	2.6%

ii) Body fat distribution. Figure 3 provides examples of: 1) liver fat content measured by using magnetic resonance imaging (MRI) and the Lipoquant method developed by our collaborators at UCSD (top left panel); 2) subcutaneous and intra-abdominal fat volume measured by using MRI (top right panel); and 3) muscle, subcutaneous and inter-muscular (between muscle fibers) thigh and calf fat by using MRI (bottom left panel). These results show: i) similar liver fat content in MNL and MNO subjects, but much greater liver fat in MAO subjects; ii) greater abdominal subcutaneous adipose tissue (fat below the skin) in MNO and MAO subjects than MNL subjects, but no difference between MNO and MAO groups; iii) greater intra-abdominal adipose tissue (fat inside the abdomen) in MNO and MAO subjects than MNL subjects, but much greater intra-abdominal adipose tissue in the MAO than the MNO group; iv) greater subcutaneous adipose tissue in the thigh and calf in MNO and MAO subjects than in MNL subjects; and v) similar intermuscular adipose tissue (IMAT) mass in MNL, MNO and MAO groups. The similarity of IMAT (fat that accumulates between muscle fibers) in all groups is a novel finding, because data from previous studies concluded that IMAT is a risk factor for poor blood glucose control, insulin resistance and type 2 diabetes. However, a significant limitation in previous studies is the imprecision in measuring IMAT. As part of this study, we developed methods that greatly improved the accuracy in assessing IMAT. Our data demonstrate that IMAT does not contribute to poor glucose control and insulin resistance.

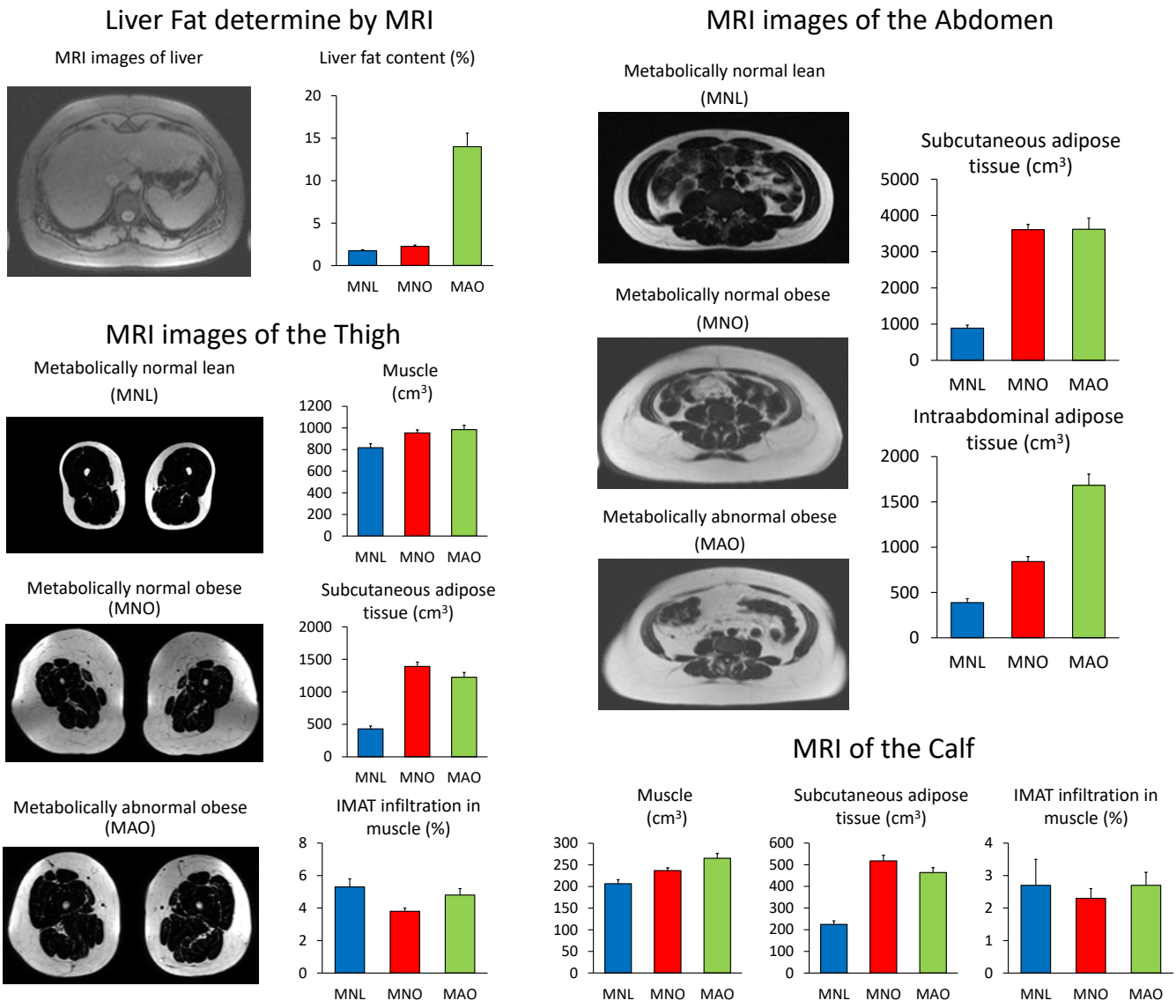

Figure 3. Body fat distribution assessed using Magnetic Resonance Imaging (MRI)

c. *Response to glucose ingestion.* Plasma glucose and insulin concentrations in response to a glucose drink (oral glucose tolerance test) are shown in Figure 4. The amount of glucose in this drink is 75 g, which is about twice the amount of sugar found in a can of soda. The change in plasma glucose after consuming the glucose drink was the same in the MNL and MNO groups, however, the insulin response was different; greater insulin was secreted by the MNO than the MNL group, which was needed to achieve the same level of glucose control. In MAO subjects, both plasma glucose and insulin concentrations were much higher than in MNO subjects, demonstrating significant insulin resistance so that even a large insulin response was not able to normalize plasma glucose concentrations.

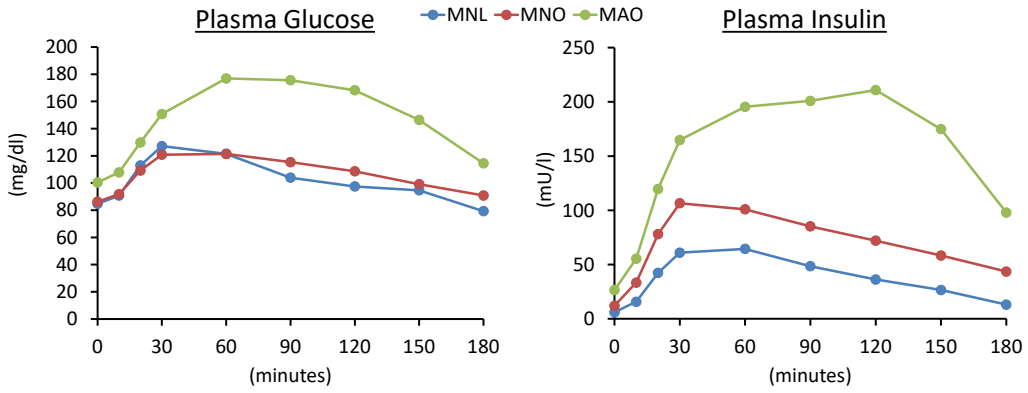

Figure 4. Plasma glucose and insulin before and after consuming a sugary drink

The concentrations of plasma glucose, insulin and other metabolites and hormones over 24 hours are being evaluated. During this study, subjects consumed breakfast, lunch and dinner with a third of daily calorie needs provided at each meal and standard macronutrient (carbohydrate, protein, fat) content. The data for plasma glucose are available and demonstrate marked differences in daily 24-hour plasma glucose excursion in MNO and MAO subjects (Figure 5).

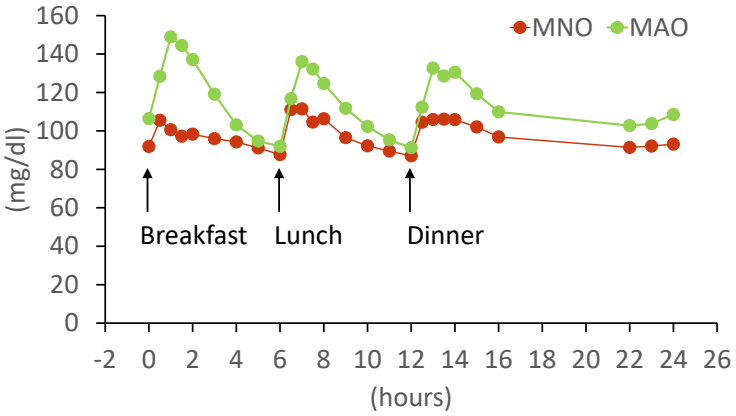

Figure 5. 24-hour glucose concentrations

d. *Insulin sensitivity.* Insulin sensitivity was assessed by determining the rate of glucose infusion needed to maintain normal blood glucose during an insulin infusion (known as the hyperinsulinemic-euglycemic clamp procedure). Insulin sensitivity (glucose infusion rate) was highest in MNL, lower in MNO and lowest in MAO subjects (Figure 6). This data demonstrates that even though MNO subjects appear metabolically healthy based on standard bloodwork, liver fat content and plasma glucose response to a glucose drink (see Table 1 and Figure 4) MNO subjects are insulin resistant compared with MNL subjects. Individuals with MAO were considerably more insulin resistant than the other groups.

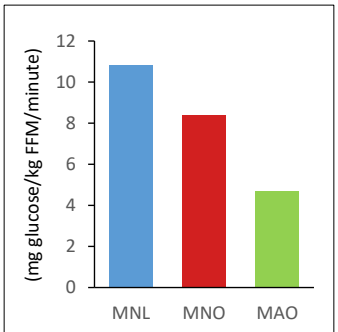

Figure 6. Glucose infusion rate during insulin infusion

e. *Adipose tissue oxygenation.* Recent studies have suggested that adipose tissue hypoxia (reduced oxygen in fat tissue), induced by a rapid expansion of adipose tissue that outpaces the growth of new blood vessels, initiates adipose tissue inflammation and, ultimately, leads to whole body insulin resistance. The adipose tissue hypoxia pathogenesis of insulin resistance has been observed in rodents, yet direct observation of a suppressed oxygen partial pressure (pO_2) in humans is lacking. We quantified pO_2 in the adipose tissue by using sterile OxyLite (Oxford Optronix, Ltd.) oxygen-sensitive fiber-optic probes. These probes are inserted into the subcutaneous adipose tissue through a small incision and the level of adipose oxygenation measured for ~2 minutes. As shown in Figure 7, adipose tissue pO_2 was lowest in MAO subjects and highest in MNL subjects, and pO_2 was positively associated with insulin sensitivity assessed by the glucose infusion rate required to maintain plasma glucose during insulin infusion. To the best of our knowledge, this is the first direct (pO_2) evidence of the hypoxia-driven insulin resistance hypothesis in humans.

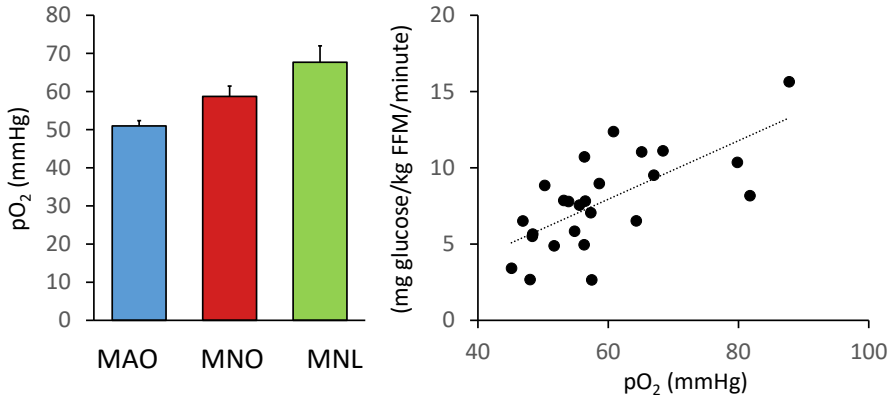

Figure 7. Adipose oxygen levels.

f. *Adipose tissue immune function and inflammation.* Abdominal subcutaneous adipose tissue was obtained by liposuction biopsy from MNL, MNO, MAO subjects and an additional group of subjects with obesity and type 2 diabetes (T2D). The non-lipid (fluid) portion of fat tissue, which surrounds the individual fat cells (i.e. stromal vascular fraction, SVF), was isolated by an enzymatic digestion procedure that we developed for this study. The SVF of fat tissue contains immune/inflammatory cells, which have been proposed as being a cause of insulin resistance, T2D and even cardiovascular disease associated with obesity. We examined whether differences in macrophages (immune cells that play a key role in the immune response) were present in the SVF. Macrophages come in 2 forms; one, which is proinflammatory (called M1 macrophages) and others that are thought to reduce inflammation (called M2 macrophages). A progressive increase in the number of the M1 inflammatory macrophages and the proportion of M1 to the total number of macrophages was observed with progressive increases in metabolic dysfunction (from MNL through T2D) (Figure 8). These findings are novel and support the notion that adipose tissue macrophages could contribute to the metabolic dysfunction observed in subjects with MAO and T2D.

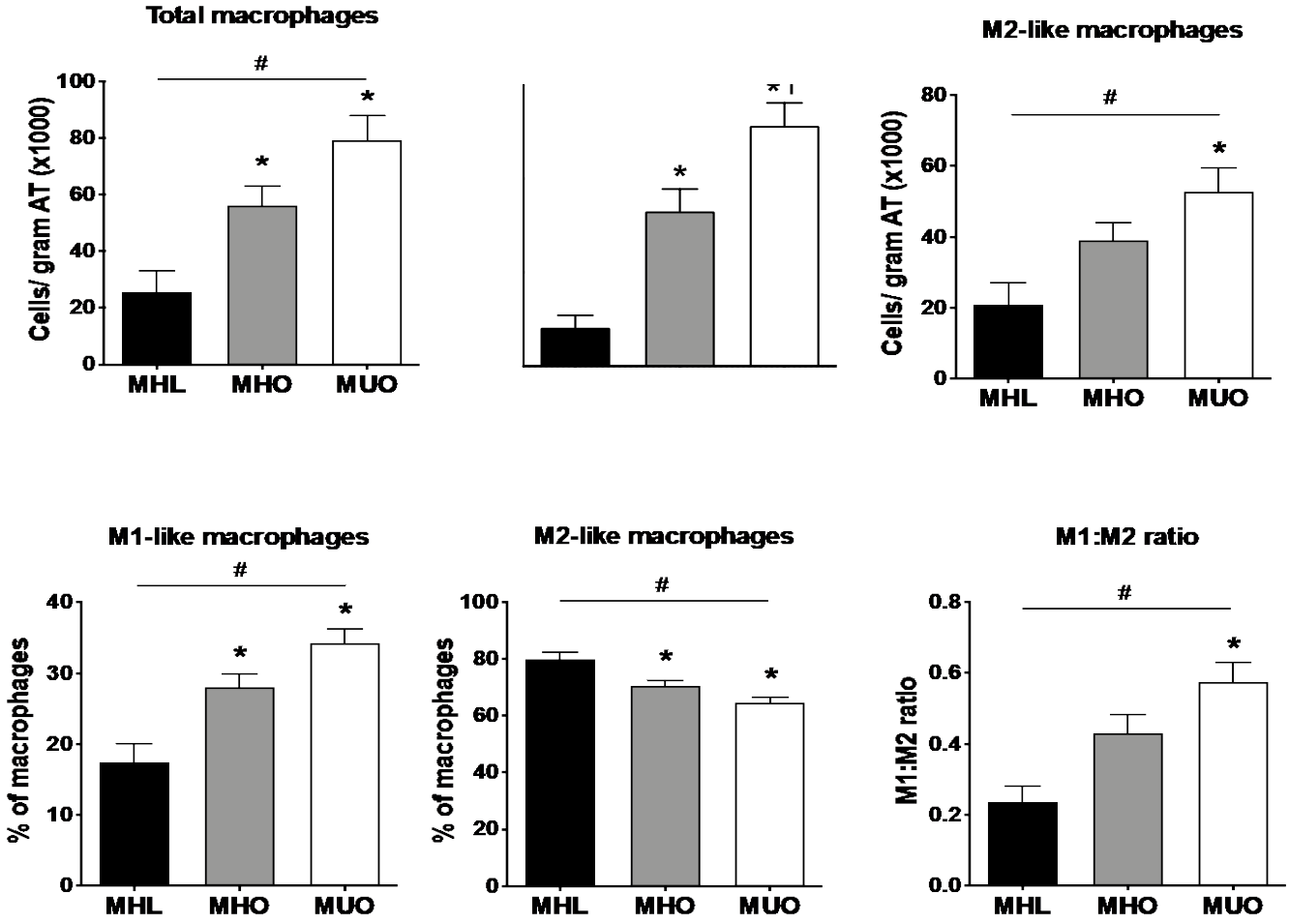

Figure 8. Adipose tissue macrophages in in MHL, MHO and MUO

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