

## Non-alcoholic fatty liver disease, bulb carotid intima-media thickness and obesity phenotypes: results of a prospective observational study

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### Abstract

**Aims:** The objective of this prospective study was to assess the correlation between carotid intima-media thickness at the common carotid (CIMTc) and carotid bifurcation (CIMTb) level, hepatic fat accumulation, and obesity phenotypes. **Material and methods:** Two hundred obese adults, in which CIMTc and CIMTb thickness was determined, were included. According to body mass index (BMI) and presence of metabolic syndrome (MetS), patients were classified as metabolically healthy obese (MHO, obesity without MetS) and metabolically unhealthy obese (MUHO, obesity with MetS). MHO patients were further classified as MHO1 (obese with increased waist circumference) and MHO2 (obese with increased waist circumference plus one of the 4 criteria for MetS). Non-alcoholic fatty liver disease (NAFLD) presence was assessed by fatty liver index (FLI). **Results:** CIMTc and CIMTb increased with obesity phenotypes from 0.74 mm and 1.04 mm in MHO1 to 0.84 mm and 1.23 mm in MHO2 and 0.88 mm and 1.74 mm in MUHO. Obesity phenotypes were significantly correlated with CIMTb. NAFLD frequency increased from 66.0% in the MHO1 to 73.0% in the MHO2 and 84.2% in the MUHO ( $p < 0.05$ ). Independent of age, BMI, total cholesterol, HbA1c, and HOMA-IR, the CIMTc was significantly associated with FLI in all obesity phenotypes and CIMTb only in MHO2 and MUHO. **Conclusions:** Our results suggest that subclinical atherosclerosis varies according to obesity phenotypes and is correlated with the hepatic fat accumulation.

**Keywords:** carotid intima-media thickness; non-alcoholic fatty liver disease; obesity phenotypes

### Introduction

Obesity represents a public health problem due to its increasing prevalence despite public awareness programs [1]. Based on cardiovascular (CV) risk factors, clinical studies have identified 2 types of obesity – metabolically healthy obesity (MHO) and metabolically unhealthy obesity (MUHO). MHO are characterized by the presence of obesity as defined by a body mass index (BMI) equal

or over 30 kg/m<sup>2</sup> without metabolic CV risk factors. MUHO associates obesity with the presence of metabolic CV risk factors and an increased risk of diabetes and CV diseases [2-4]. Studies assessing the health risks associated with MHO have shown conflicting results, some showing similar or lower risk of CV disease and diabetes when compared to MUHO. Despite numerous clinical cross-sectional and prospective epidemiological studies evaluating the CV risk associated with this obesity phenotype and its clinical implications, controversies surrounding the health risks associated with MHO remain [5-7]. Therefore, it still under debate whether MHO represents a distinct phenotype compared with MUHO (lower health associated risks during lifetime or just a MUHO precursor) [8].

In parallel with the increasing prevalence of obesity an increased prevalence of nonalcoholic fatty liver disease (NAFLD) has been reported [9]. Obesity and ab-

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dominal obesity, metabolic syndrome, and type 2 diabetes have been identified among risk factors for NAFLD [10], which in turn is associated with an increased risk of fatal and non-fatal CV events and increased risk of total and CV diseases mortality [11]. The gold standard for NAFLD diagnosis is the liver biopsy but its invasive nature limits its use. Fatty liver index (FLI) score [12] is a noninvasive method widely used in epidemiological studies for NAFLD screening, showing good sensitivity compared with magnetic resonance spectroscopy for detecting fatty liver [13].

Carotid intima-media thickness (CIMT) is a simple and non-invasive method of the assessment of subclinical atherosclerosis and has been shown to be an independent predictor of CV disease risk [14-16]. Evaluation of CIMT includes evaluation of common carotid artery (CIMTc), bifurcation (bulb; CIMTb), and internal carotid artery and it has been shown that the association of the CIMT with CV risk factors varies according to the segment assessed [17]. The association between CIMT and NAFLD has been reported in the past years and this association was independent of other CV risk factors [18,19].

Currently, limited data are available on the relationship between hepatic fat content and atherosclerosis according to obesity phenotypes. A recent study showed that MHO participants had significantly lower levels of CIMT and intrahepatic triglycerides content compared with the MUHO participants and intrahepatic triglycerides content was independently associated with metabolic syndrome (MetS) components and increased CIMT [20].

In this context, we aimed to investigate the correlation between subclinical carotid atherosclerosis assessed by CIMT at common carotid and carotid bifurcation level, hepatic fat, and obesity phenotypes.

## Material and methods

This was a prospective study performed in the Emergency County Clinical Hospital Cluj-Napoca, Romania. We included 200 obese patients as defined by a BMI  $\geq 30$  kg/m<sup>2</sup>, who presented, between February 2014 and November 2015 for nutritional and metabolic status evaluation in the Diabetes, Nutrition and Metabolic Diseases Clinic. We excluded from the study patients under 18 years of age, with prior diagnosis of autoimmune, viral (hepatitis virus B, C, D), toxic or uncertain etiology hepatitis, with high alcohol consumption (>140 g/week), diabetes mellitus, pregnancy, with hypolipemic and/or weight loss medication. The patients signed an informed consent prior to enrollment and Institutional Ethics Committee approval was obtained.

## Clinical assessments

We recorded for all patients demographic and clinical variables such age, weight, height, waist circumference, BMI [calculated as weight (kg)/height (m<sup>2</sup>)], associated disease (hypertension, dyslipidemia, etc), and their medication.

Waist circumference (WC) was measured with the patients standing, with a measuring tape halfway between the ribcage and the iliac crest, horizontally, at the end of a complete expiration.

Blood pressure (BP) was measured according to the guidelines [21] after a 5 minute resting, in sitting position. Two measurements were performed for each arm at 2 minutes interval. The arm with the highest BP was chosen and the average value of the measurements was computed.

## Metabolic syndrome definition

Classifying patients as MHO or MUHO was performed after a series of explorations to identify the MetS components according to the International Diabetes Federation criteria [22]: 1) abdominal obesity: WC >94 cm (men) and >80 cm (women); 2) hypertriglyceridemia:  $\geq 150$  mg/dl; 3) low levels of HDL-C: <40 mg/dl (men) and <50 mg/dl (women) or specific treatment; 4) hypertension:  $\geq 130/85$  mmHg or specific treatment; 5) high fasting glucose:  $\geq 100$  mg/dl.

## Assay and indices assessment

Blood samples were drawn from the cubital vein after a 12-h fasting. Fasting plasma glucose (FPG), triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, gamma-glutamyl transferase (GGT), transaminases, uric acid, fasting insulinemia (for insulin resistance), glycated hemoglobin (HbA1c), apolipoprotein A1 and B were determined using a Beckman Coulter UniCel DxI 600. Insulin resistance was estimated using homeostasis model assessment (HOMA-IR) as [fasting glucose (mg/dL)  $\times$  fasting insulin ( $\mu$ UI/mL)]/405 [23].

Based on FPG values, patients were classified as having dysglycemia (prediabetes) if they had an FPG level from 110 to less than 126 mg/dl [24]. NAFLD was diagnosed using the fatty liver index (FLI) score. FLI requires for calculation BMI, WC, triglycerides, and GGT [13]. A FLI score > 60 is considered to be suggestive for the presence of NAFLD [13].

## Carotid Ultrasound Measurements

Ultrasound evaluations were performed by a single examiner (with a 15-year expertise, certified for carotid ultrasonography) using a 3-10 MHz VF8-3 linear Transducer (ACUSON X300 Ultrasound System). The examination was performed with the patient lying in supine position, with a lateral probe position using a standardized protocol [25]. Wall thickness was measured in lon-

itudinal view, at the far wall level, with the transducer positioned strictly perpendicular to this wall (the lumen-intima and media-adventitia interfaces were clearly defined), including the carotid bifurcation in the image plane. CIMT was measured at 10, 15, and 20 mm below the end of the common carotid artery (CCA), at a plaque-free point on the far wall and the average was considered the CIMTc on that side. Any atherosclerotic thickening  $\geq 1.5$  mm was considered a plaque. CIMTc was calculated as the average of the CIMT for left and right CCA. CIMTb was calculated as CIMT average in the thickest point (including plaque) of the left and right internal carotid bulb. Variation coefficients of the measurements for the examiner were  $< 5\%$ .

#### ***Establishing the groups of patients according to obesity phenotype***

According to BMI and presence of MetS [22], participants were classified as MHO (obese without MetS) or MUHO (obese with MetS). MHO patients were further classified as MHO1 (obese with increased WC) and MHO2 (obese with increased WC plus one of the criteria for MetS).

#### ***Statistical analysis***

Statistical analysis was performed with SPSS version 20. Kolmogorov-Smirnov tests were used to evaluate the distribution of investigated variables. Data was presented as proportions for qualitative variables, mean and standard deviation (SD) or median for continuous variables. The chi-square-test was used to compare categorical variables and t-test for continuous variables. Mann-Whitney test or Kruskal-Wallis test was used for non-normal distribution variables. Correlations were assessed with Spearman or Pearson coefficients, according to the variables distribution.

Univariate linear regression was performed for the relation between CIMT and FLI score in the whole sample and by obesity phenotype, multiple regression analysis was applied for the relation between CIMT and FLI adjusted for age, BMI, total cholesterol, HbA1c, and HOMA-IR. A two-sided p value  $\leq 0.05$  was considered statistically significant.

## **Results**

Baseline characteristics for the 200 patients who met the inclusion criteria and were included in the study are shown in Table I. MHO patients were younger, had significantly lower BMI, WC, systolic and diastolic BP comparing with MUHO. Comparing with MHO, the MUHO patients had significantly lower HDL-cholesterol levels, higher triglycerides, uric acid, and CRP levels (p  $< 0.05$  for all). The frequency of dysglycemia was 11.5% in the

MHO2 group and 74.0% in the MUHO group. In the MHO2 group 21 patients (40.4%) had increased WC plus hypertension, 18 patients (34.6%) plus low HDL-cholesterol, 7 patients (13.5%) plus high triglycerides, and 6 patients (11.5%) plus dysglycemia. CIMTc, CIMTb and FLI score were significantly higher in MUHO patients. .

Comparing MHO1 and MHO2 patients, those with MHO2 had significantly higher WC, glycemia, HbA1c, insulinemia, C-peptide, HOMA-IR, total and LDL-cholesterol, triglycerides, CRP and uric acid. CIMTc and CIMTb were significantly higher in MHO2 patients than in MHO1 patients. NAFLD frequency was 66.0% in the MHO1 and 73.0% in the MHO2 (p  $< 0.05$ ). FLI score increased from 63.44 in the MHO1 to 68.01 in the MHO2.

In all groups both CIMTc and CIMTb were directly and significantly correlated with FLI score. Correlation coefficients between CIMTc and FLI score were 0.343, 0.425, and 0.343 (MHO1, MHO2, and MUHO, respectively); 0.763, 0.443, and 0.754 for CIMTb and FLI score in the three groups (Table II).

Obesity phenotypes were statistically significant correlated only with CIMTb (p  $< 0.05$  for all; Table III).

In the univariate regression model, CIMTc was associated with the FLI score in the whole sample and CIMTb was associated with the FLI score only in the MHO1 group. After adjusting for age, BMI, total cholesterol, HbA1c, and HOMA-IR, a statistically significant association was observed between the CIMTb and FLI score in the MHO2 and MUHO, and CIMTc with the FLI score in all obesity phenotype groups (Table IV).

## **Discussions**

MHO prevalence varies largely, up to 40%, according to the clinical study and the definition criteria, being more common in younger persons [8,26].

Our observations are similar to previous reports – MHO has a lower CV risk profile compared to MUHO [26,27]. Elevated CIMTc and CIMTb, found in several MHO1 patients were correlated with ApoB/ApoA1 ratio, but not with LDL levels. Therefore, our results support the recommendations of the guidelines, to assess apoB even when LDL levels are normal because apoB correlates with atherosclerosis and predicts CV events [28].

Marini et al showed that CIMT increased from 0.68 mm in non-obese to 0.79 in MHO and 0.89 in obese with insulin resistance [27]. From our findings, increasing values were observed from MHO1 to MHO2, reaching the highest value in MUHO. Supposedly both CIMTc and CIMTb could increase with the number of MetS components. Previous studies have shown that CIMT and carotid plaque prevalence are associated with the

Table I. Clinical, anthropometric, and metabolic characteristics of patients grouped according to the presence MetS.

Assessed parameters	MHO			p*	MUHO	p#
	All N=100	MHO1 N=48	MHO2 M=52		N=100	
Age (years)	41.02±11.33	39.23±10.13	42.33±12.12	0.001	46.31±14.82	0.001
Weight (kg)	88.06±11.81	88.06±11.81	103.87±26.89	0.001	103.87±26.89	0.001
Waist (cm)	106.90±16.77	100.00±10.27	111.50±18.79	0.01	118.47±12.51	0.010
BMI (kg/m <sup>2</sup> )	31.72±5.42	29.98±4.45	32.8±15.77	0.23	36.40±5.75	0.006
SBP (mmHg)	127.13±22.64	113.24± 8.75	134.21±10.13	0.021	148.21±19.34	0.002
DBP (mmHg)	78.56±16.12	62.44 ±6.21	89.24±12.13	0.024	95.21±19.43	0.008
Total cholesterol (mg/dL)	186.39±43.21	179.60±43.37	196.80±42.28	0.023	206.63±50.23	0.120
LDL-cholesterol (mg/dL)	123.61±39.09	105.14±24.81	125.26±51.68	0.015	130.26±51.68	0.600
HDL-cholesterol (mg/dL)	49.18±10.70	52.13±8.02	45.26±11.91	0.01	41.20±11.84	0.019
Triglycerides (mg/dL)	106 (83.23; 120.4)	88 (75.5; 111.5)	112 (91; 122.5)	0.12	135 (77;191.5)	0.002
ApoB/ApoA1	1.43±0.550	0.85±0.33	0.71±0.14	0.23	1.44±.230	0.929
Glycemia (mg/dL)	76.83±37.30	72.75±26.94	79.56±38.08	0.01	97.28±36.13	0.052
HbA1c (%)	5.55 (5.3;5.9)	5.3 (5.20; 5.45)	5.6 (5.45; 5.95)	0.01	6.1 (5.85; 6.25)	0.001
Insulinemia (µUI/mL)	9 (7.5;11.3)	8.7 (7.60; 9.30)	10 (8.15; 16.65)	0.001	15.45 (13.3;23.85)	0.027
C-peptide (ng/mL)	2.25 (2.1;4.0)	2.1 (1.8; 2.2)	2.7 (2.2; 4.4)	0.009	3.25 (2.45; 5.65)	0.038
HOMA-IR	1.91 (1.02; 5.45)	2.43 (1.02; 3.25)	4.16 (2.25; 6.22)	0.001	6.12 (5.44; 7.51)	0.001
ASAT (IU/mL)	23.02±11.45	20.13±10.34	26.47±14.03	0.13	43.37±13.02	0.035
ALAT (IU/mL)	25.12±13.42	21.03±11.02	27.01±13.49	0.19	45.23±15.27	0.031
GGT (IU/mL)	34.25±10.89	24.38±11.02	35.67±14.89	0.21	48.38±16.72	0.037
Uric acid (mg/dL)	5.63±1.53	4.96±1.20	6.11±1.59	0.001	6.70±1.05	0.013
CRP (mg/dL)	1.90 (1; 3.3)	2.56 (0.95; 3.10)	1.40 (1.05; 3.75)	0.000	4.8 (2.3; 7.5)	0.035
CIMTc (mm)	0.81±0.19	0.74±0.17	0.84 ± 0.19	0.001	0.88±0.17	0.036
CIMTb (mm)	1.2 (1; 1.3)	1.2 (0.95; 1.3)	1.2 (1.1; 1.3)	0.01	1.5 (1.3; 2)	0.008
FLI score	66.65±26.52	63.44±25.32	68.01±26.82	0.009	79.89±26.97	0.002
NAFLD	70.0	66.7	73	0.005	84.2	0.013

The results are expressed as number (%), mean±SD or median (Q1;Q3). \*p values are provided for the comparison between MHO1 and MHO2.

#p values are provided for the comparison between MHO and MUHO. N = number of participants; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; HbA1c – A1c glycated hemoglobin; HOMA-IR = homeostasis model assessment; ASAT = aspartate transaminase; ALAT = alanine transaminase; GGT = gamma-glutamyl transferase; CRP = C-reactive protein; CIMTc = carotid intima-media thickness measured at common carotid artery level; CIMTb = carotid intima-media thickness measured at carotid bulb level; FLI = fat liver index; NAFLD = non-alcoholic fatty liver disease; MHO = metabolically healthy obese; MHO1 = obese with increased WC; MHO2 = obese with increased WC plus one of the criterions for MetS; MUHO = metabolically unhealthy obese.

presence of MetS [23, 29] and the number of its components [29-33]. Each additional component of the MetS is associated with a 0.02 mm increase in the CIMTc, independent of age, gender, family history of CVD, and smoking [34].

We found that only CIMTb was correlated with the obesity phenotype and systolic and diastolic BP in all groups, suggesting that BP-induced shear stress could explain the higher yearly growth rate of CIMTb compared to CIMTc [35]. Polak et al [17] showed that FPG and diastolic BP had a stronger association with CIMTc while hypertension, diabetes, and smoking with CIMTb.

We showed that FLI score increased in parallel with obesity phenotype groups. The prevalence of NAFLD increased from MHO1 to MUHO. Similarly, Zhang et al showed that the intrahepatic triglyceride content was significantly lower in MHO compared to MUHO and this

content is a better predictor for MUHO than BMI, WC or percentage of body fat [20].

Intrahepatic fat accumulation and NAFLD are associated with a more adverse CV risk profile [36-40], while NAFLD is associated with insulin resistance, MetS and an atherogenic lipid profile [37, 41]. Furthermore, NAFLD patients have a higher prevalence of coronary artery lesions [36], higher CIMT, and atherosclerotic plaques [38], as well as a higher incidence of CVD, and increased CV mortality [39, 40]. We found significant correlations between the FLI score, presence of NAFLD, CIMTc, and CIMTb in all obesity phenotypes. Independent of age, BMI, total cholesterol, HbA1c, and HOMA-IR, the CIMTc was significantly associated with FLI in all obesity phenotypes, unlike CIMTb (only in MHO2 and MUHO), suggesting that hepatic fat accumulation plays a role in the determination of the obesity phenotype asso-

Table II. Correlations of carotid-intima media thickness with measured parameters according to the obesity phenotype.

Assessed parameters	MHO1		MHO2		MUHO	
	CIMTc	CIMTb	CIMTc	CIMTb	CIMTc	CIMTb
Age (years)	<b>0.569*</b>	<b>0.523*</b>	0.289	0.083	<b>0.678*</b>	0.550
Waist (cm)	0.523	0.110	<b>0.503*</b>	<b>0.336*</b>	-0.165	0.218
BMI (kg/m <sup>2</sup> )	0.368	0.667	0.137	-0.065	-0.337	-0.600
SBP (mmHg)	0.505	<b>0.514*</b>	0.523	<b>0.544*</b>	0.505	<b>0.603*</b>
DBP (mmHg)	0.489	<b>0.563*</b>	0.512	<b>0.570*</b>	0.489	<b>0.563*</b>
LDL cholesterol (mg/dL)	-0.413	-0.306	0.001	0.355	0.297	0.218
HDL cholesterol (mg/dL)	0.042	0.518	0.212	-0.315	0.338	0.327
Triglycerides (mg/dL)	0.200	-0.409	-0.056	0.222	0.463	0.491
ApoB/ApoA1	<b>-0.629*</b>	-0.691	-0.015	0.462	-0.036	-0.522
Glycemia (mg/dL)	<b>0.750*</b>	<b>0.710*</b>	0.083	-0.442	-0.493	0.327
HbA1c (%)	-0.209	-0.662	-0.110	-0.050	-0.021	0.806
HOMA-IR	-0.267	-0.564	0.332	-0.500	0.327	0.866
Uric acid (mg/dL)	0.407	0.218	0.231	0.077	0.528	0.794
CRP (mg/dL)	-0.360	0.051	0.389	<b>0.780*</b>	-0.118	0.500
FLI score#	<b>0.343*</b>	<b>0.763*</b>	<b>0.425*</b>	<b>0.443*</b>	<b>0.343*</b>	<b>0.754*</b>
NAFLD	<b>0.368*</b>	<b>0.792*</b>	<b>0.502*</b>	<b>0.434*</b>	<b>0.368*</b>	<b>0.783*</b>

\*p values <0.05 showing a statistically significant association; #FLI score included in the correlation analysis as a continuous variable; N = number of participants; BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; HbA1c – A1c glycated hemoglobin; HOMA-IR = homeostasis model assessment; CRP = C-reactive protein; CIMTc = carotid intima-media thickness measured at common carotid artery level; CIMTb = carotid intima-media thickness measured at carotid bulb level; FLI = fat liver index; NAFLD = non-alcoholic fatty liver disease; MHO = metabolically healthy obese; MHO1 = obese with increased WC; MHO2 = obese with increased WC plus one of the criterions for MetS; MUHO = metabolically unhealthy obese.

Table III. Correlations of the carotid-intima media thickness with the obesity phenotypes.

Assessed parameters	MHO/MUHO	MHO1/MHO2/MUHO
CIMTc	0.286 (p=0.060)	0.204 (p=0.184)
CIMTb	<b>0.483 (p=0.015)</b>	<b>0.518 (p=0.008)</b>

CIMTc = carotid intima-media thickness measured at common carotid artery level; CIMTb = carotid intima-media thickness measured at carotid bulb level; MHO = metabolically healthy obese; MUHO = metabolically unhealthy obese.

Table IV. Association of carotid-intima media thickness and with fatty liver index score in the whole sample and by obesity phenotypes

Assessed parameters	CIMTc		CIMTb	
	$\beta$	p	$\beta$	p
All sample	<b>0.283</b>	<b>0.005</b>	0.072	0.416
MHO1	0.160	0.351	<b>0.763</b>	<b>&lt;0.001</b>
MHO2	0.030	0.891	0.126	0.449
MUHO	-0.151	0.378	0.052	0.671
All sample#	0.116	0.226	0.058	0.763
MHO1#	<b>-0.577</b>	<b>0.001</b>	-*	-*
MHO2#	<b>-1.199</b>	<b>&lt;0.001</b>	<b>1.586</b>	<b>&lt;0.001</b>
MUHO#	<b>-7.713</b>	<b>&lt;0.001</b>	<b>-0.980</b>	<b>0.004</b>

#Models adjusted for age, BMI, total cholesterol, CRP, HbA1c, HOMA-IR. \*Could not be calculated due to high number of covariates compared to sample size;  $\beta$  – coefficient of correlation; CIMTc = carotid intima-media thickness measured at common carotid artery level; CIMTb = carotid intima-media thickness measured at carotid bulb level; MHO = metabolically healthy obese; MHO1 = obese with increased WC; MHO2 = obese with increased WC plus one of the criterions for MetS; MUHO = metabolically unhealthy obese.

ciated with subclinical atherosclerosis, probably through increased cytokines production [42]. Currently, there is scarce data on the association of CIMT and hepatic fat accumulation in obesity phenotypes. The only available

study we could identify showed that irrespective of obesity phenotype and independent of percentage of body fat, an increase in the intrahepatic triglyceride content was associated with a higher risk of increased CIMT [20].

Our study has some limitations that we must acknowledge. The sample size was relatively small and therefore, future prospective evaluation in a larger scale study is required. We could not collect accurate information on the smoking status for all patients (years of smoking, passive smoking, etc) and therefore we could not assess its impact on CIMT. NAFLD was assessed by the FLI score and not by liver biopsy nor by an ultrasound exam. However, FLI score was shown to have a good performance in NAFLD identification [43,44]. The study subjects were selected from ambulatory patients and not from the general population. Also we did not take into account the different effects of associated therapy on MetS components (anti-inflammatory drugs, contraceptives, alternative therapies etc). Because of the small number of patients, dividing them into groups according to the association of MetS component would not have allowed an accurate statistical analysis. Therefore we do not have results on the impact of each MetS component – WC pair on NAFLD and CIMT.

### Conclusions

Our results support previous findings suggesting the degree of subclinical atherosclerosis varies according to obesity phenotypes and is associated with hepatic fat accumulation. Hepatic fat accumulation increased according to the obesity phenotype and may represent a predictor of metabolic changes in obesity. Further studies investigating the association between NAFLD and CIMT progression in all obesity phenotypes are required.

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