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ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 838–846
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Nuclear magnetic resonance–determined lipoprotein abnormalities in nonhuman primates with the metabolic syndrome and type 2 diabetes mellitus

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Received 29 April 2006; accepted 19 January 2007

Abstract

The lipid profile in patients with the metabolic syndrome (MetS) and type 2 diabetes mellitus (T2DM) is commonly characterized by increased levels of triglycerides and decreased levels of high-density lipoprotein (HDL) cholesterol. However, within each lipoprotein class, the changes are more complex. The present study defined the characteristics of dyslipidemia among nonhuman primates, using nuclear magnetic resonance (NMR) spectroscopy as well as the classic β-quantification method, and examined the pattern of multiple lipoprotein fractions in relation to the main factors identified with the MetS. Seventy-three rhesus monkeys were classified into 3 groups: healthy monkeys, monkeys with MetS, and monkeys with T2DM. Characteristics of dyslipidemia in the MetS and T2DM groups included increased levels of triglyceride-rich very low-density lipoprotein, intermediate-density lipoprotein, and small, dense, low-density lipoprotein (LDL) particles. Reduced concentrations of large LDL and large HDL particles together with reduction of LDL and HDL particle sizes were also observed. Correlation analysis revealed that poor glycemic and lipid profiles, glucose intolerance, and insulin resistance were associated with an atherogenic NMR profile. Compared with the conventional lipid panel, the NMR lipoprotein profile presented in greater detail distinctive differences between the dyslipidemia of the MetS and that of diabetes and demonstrated significant and divergent shifts in both particle size and number within lipoprotein classes between those 2 groups. Detailed lipoprotein profiling may provide additional indicators for more timely intervention. Rhesus monkeys are likely to provide an excellent model for novel drug testing designed to address the specific differences in lipoprotein fraction profile across these 3 groups that reflect the progression of pathophysiology from normal to overt diabetes.

1. Introduction

The metabolic syndrome (MetS) is characterized by a cluster of key features that include abdominal obesity, insulin resistance, hypertension, and dyslipidemia, in addition to chronic inflammation, procoagulation, and impaired fibrinolysis [1,2]. It is estimated that the MetS is present in 20% to 30% of middle-aged adults in the United States [3,4] and is associated with an increased risk for the development of cardiovascular disease (CVD) [5,6] and type 2 diabetes mellitus (T2DM) [7,8]. The dyslipidemic components of the MetS, which include hypertriglyceridemia and reduced levels of high-density lipoprotein cholesterol (HDL-C), are probably the features most closely linked with insulin resistance and risk of diabetes and CVD [9-11]. The MetS-related dyslipidemia is seen in most patients with diabetes. It is likely that the earlier phases of dyslipidemia differ in significant ways from the dyslipidemia of severe diabetes.

Lipoproteins are composed of a heterogeneous spectrum of particles that differ in size, density, chemical composition, and atherogenicity and which contribute differently to the development of CVD or diabetes [12,13]. Although it is well known that both low-density lipoprotein (LDL) [14] and HDL [15,16] can be subdivided into subclasses that are thought to differ in atherogenic or antiatherogenic properties, little information concerning lipoprotein subclass distribution is gained from the conventional lipid profiles when using the traditional β-quantification method to examine lipids and

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0026-0495/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2007.01.015

lipoproteins. However, nuclear magnetic resonance (NMR) spectroscopy offers an additional means of quantifying lipoprotein particle concentration and particle size in plasma and has been used to reveal potentially adverse or favorable changes in lipoprotein subclass distributions, alterations of which may improve the prediction of CVD and diabetes in individuals beyond the risk assessment provided by a conventional lipid panel [13,17,18].

Our previous prospective longitudinal studies have identified the natural progression of metabolic changes leading to the spontaneous development of diabetes in rhesus monkeys (*Macaca mulatta*) and have characterized distinct phases in this process [19,20]. The close similarity between nonhuman primates and humans has also been well demonstrated [21-23]. Although Hannah et al [24] reported that lipoprotein abnormalities were associated with the early development of T2DM before the clinical diagnosis of the disease, dyslipidemia in the monkey has not been fully characterized, particularly regarding the lipoprotein subclasses. Therefore, detailed characterization of naturally occurring dyslipidemia in the MetS and in T2DM may provide more information for implementing prevention strategies, predicting the onset of diabetes, and understanding the mechanisms by which the atherogenic lipid profile contributes to CVD risk and diabetes.

In the present study, we have sought to identify what, if any, additional understanding of the dyslipidemia of the MetS and of T2DM may be elucidated by the addition of NMR spectroscopy to describe the differences in lipoprotein distributions in 3 groups of monkeys, ranging from metabolically healthy to fully established MetS to overt spontaneous diabetes. The relationships between lipoprotein profiling and a range of metabolic variables were further investigated.

2. Research design and methods

2.1. Animals

The study population included a cohort of 73 (68 males and 5 females) adult rhesus monkeys (*Macaca mulatta*), ranging widely in age (5.2-30.1 years) and body weight (5.4-27.2 kg). The monkeys were housed individually and maintained under a 12-hour light-dark cycle, with an ambient temperature of 22°C. Fresh water and diet were provided ad libitum. The diet consisted of standard laboratory primate chow (LabDiet 5038 from Purina Mills, St Louis, MO), which provided 18% of energy as protein, 13% as fat, and 69% as carbohydrate. Housing and experimental procedures were in accordance with the principles of laboratory animal care of the National Academy of Sciences/National Research Council and were approved by the Institutional Animal Care and Use Committee of the University of Maryland, Baltimore.

The monkeys were classified into the following 3 groups: healthy monkeys, monkeys with MetS, and monkeys with T2DM. Diabetes was diagnosed according to the American Diabetes Association criteria for humans [25]. Diabetic monkeys had at least 2 fasting plasma glucose (FPG) levels of 126 mg/dL (6.99 mmol/L) or greater. For humans, a number of clinical criteria for the diagnosis of the MetS have been recommended [26-29]. These criteria are similar in many respects, but with varying emphasis on either the increased risk of CVD or heightened risk of diabetes in patients. Considering these various definitions of the MetS and their caveats in humans [8,30,31] and in view of the emphasis of the present study on diabetes rather than on CVD, we have identified the MetS in our monkey colony on the basis of the characteristics defined by the World Health Organization [28] and subsequent International Diabetes Federation publications [27], with some modifications based on our previous studies identifying reference ranges in monkeys compared to humans [32]. Furthermore, although the criteria for the MetS from these publications both explicitly include diabetes, we consider diabetes to be a frequent sequel to and not an essential component of the syndrome [4]. The criteria selected for use in defining the MetS specifically include the following: impaired fasting glucose (defined as an FPG of 80-125 mg/dL [4.44-6.99 mmol/L]) and/or impaired glucose tolerance (IGT, defined as a glucose disappearance rate $K_{Gluc} \leq 2.0\%$/min by intravenous glucose tolerance test [IVGTT]) and/or insulin resistance (defined as an insulin-stimulated peripheral glucose uptake rate during a euglycemic-hyperinsulinemic clamp [*M* rate] of <7.5 mg/kg fat-free mass [FFM] per minute) [21] plus 2 or more of the following: obesity, which in rhesus monkeys has been defined as body fat greater than 25%; raised blood pressure (BP), identified by a systolic BP (SBP) greater than 130 mm Hg or a diastolic BP greater than 80 mm Hg; hypertriglyceridemia, defined as a fasting plasma triglyceride (TG) greater than 80 mg/dL (0.9 mmol/L); or reduced HDL-C, defined as a fasting HDL-C less than 60 mg/dL (1.6 mmol/L, obtained by the β-quantification method). The IVGTT was used instead of the oral glucose tolerance test to assess glucose tolerance because it has greater precision and reproducibility in monkeys and allows detailed assessment of insulin response to glucose [19].

2.2. Procedures

Blood samples were obtained under light anesthesia (ketamine hydrochloride, 10 mg/kg body weight) after a 16-hour fast. Fresh plasma samples were used for the β-quantification assays and the remaining plasma samples were frozen and kept at −80°C for later assays.

An IVGTT (0.25 mg/kg) was carried out with sampling at 1, 3, 5, 7, 10, 15, 20, and 30 minutes after a 16-hour fast. K_{Gluc} was calculated by using the following formula: K_{Gluc} = ln(glucose level at 5 min) − ln(glucose level at 20 min)/15min × 100% [19].

Euglycemic-hyperinsulinemic clamps (2400 pmol/m^2 body surface area per minute insulin infusion) were carried

out to assess the sensitivity of peripheral tissues to maximal insulin stimulation [22]. Plasma glucose was maintained at approximately 4.7 mmol/L to estimate the insulin-stimulated glucose uptake rate (*M* rate). The *M* rate was corrected for FFM, an estimate of metabolically active mass. The percentage of body fat was determined by the tritiated water dilution method.

2.3. Lipid and lipoprotein assays

Fresh plasma was immediately fractionated by ultracentrifugation for lipoprotein analysis via β-quantification (Penn Med Laboratories, Washington, DC), relying on previously identified methods [24], and total TG, total cholesterol, LDL-C, HDL-C, very low density lipoprotein cholesterol (VLDL-C), and VLDL-TG were determined by this assay.

The NMR lipoprotein subclass profiles were determined (LipoScience, Raleigh, NC) as described previously [33,34], using EDTA plasma from the same blood sample as the β-quantification lipid panel (stored at -80°C). In brief, the NMR method uses the characteristic signals broadcast by lipoprotein subclasses of different sizes as the basis of their quantification. Each subclass signal emanates from the total number of terminal methyl groups on the lipids contained within the particle core (cholesterol ester and TG, each contributing 3 methyl groups) and on the surface shell (phospholipid and unesterified cholesterol, each contributing 2 methyl groups). The methyl NMR signal emitted by each subclass therefore provides a direct measure of the concentration of that subclass.

Nuclear magnetic resonance spectra of each plasma specimen (0.4 mL) were acquired by use of an automated 400-MHz lipoprotein analyzer, and the lipid methyl signal envelope was decomposed computationally to give the amplitudes of the contributing signals of 15 subclasses (6 VLDL, 1 intermediate-density lipoprotein [IDL], 3 LDL, and 5 HDL). Conversion factors relating signal amplitudes to subclass concentrations expressed in particle concentration units were then applied. Particle concentrations (nanomoles per liter for VLDL, IDL, and LDL; micromoles per liter for HDL) were calculated for each subclass standard by measuring the total concentration of core lipid (cholesterol ester plus TG) and dividing the volume occupied by these lipids by the core volume per particle calculated from the particle's diameter.

The 15 measured subclasses were grouped for analysis into the following categories: 3 VLDL subclasses (large, 60-200 nm; intermediate, 35-60 nm; small, 27-35 nm), IDL (23-27 nm), 3 LDL subclasses (large, 21.3-23 nm; intermediate, 19.8-21.2 nm; small, 18.3-19.7 nm), and 3 HDL subclasses (large, 8.8-13 nm; intermediate, 8.2-8.8 nm; small, 7.3-8.2 nm). Weighted average VLDL, LDL, and HDL particle sizes (nanometers diameter) were computed as the sum of the diameters of each subclass multiplied by its relative mass percentage as estimated from the amplitude of its methyl NMR signal.

2.4. Other assays

Plasma glucose concentrations were determined by the glucose oxidase method by using a glucose autoanalyzer (Beckman Instruments, Fullerton, CA). Fasting plasma insulin (IRI) levels were determined by radioimmunoassay (Linco Research, St Charles, MO). Glycated hemoglobin (HbA_{1c}) levels were obtained by using a Bayer DCA2000 analyzer (Pittsburgh, PA).

2.5. Statistical analyses

Data are presented as mean $\pm$ SE or median and interquartile range. Differences in normally distributed data were assessed by a 1-way analysis of variance (ANOVA), followed by a Tukey-Kramer multiple comparison test. For the nonnormally distributed values, differences between groups were analyzed using the nonparametric Kruskal-Wallis test. In multivariate analyses, Spearman correlation coefficients (*r*) were used to describe the association between independent variables of interest; the partial correlation coefficient was used to control for the effect of age and sex. All statistical analyses were performed using NCSS 2000 software (NCSS, Kaysville, UT) and a *P* value less than .05 was considered statistically significant for all analyses.

3. Results

3.1. The metabolic characteristics of the study subjects

Table 1 shows the metabolic characteristics of the 73 monkeys: 35 healthy monkeys, 20 with the MetS ,and 18 with overt T2DM. The mean 25th and 75th percentiles for each of the variables in the full population are also presented in Table 1. Compared with healthy monkeys, monkeys with the MetS had significantly higher levels of IRI, HbA_{1c}, and percent body fat, lower K_{Gluc}, and lower *M* rate. Monkeys with T2DM had markedly higher FPG and HbA_{1c} levels and lower K_{Gluc} values than both healthy and MetS monkeys. The highest mean IRI was observed in the MetS group. There were no differences in percent body fat and *M* rate between the MetS and T2DM groups. Blood pressure did not exhibit statistically significant differences among the 3 groups, although the values in the MetS and T2DM groups tended to be increased relative to the healthy group.

3.2. Lipoprotein subclass particle concentrations and particle sizes determined by NMR spectroscopy

NMR-determined lipoprotein subclass concentrations are shown in Table 2. Compared with the healthy group, total VLDL particles were significantly elevated in the MetS and T2DM groups, with higher levels of large, intermediate, and small VLDL subclasses. Furthermore, monkeys with diabetes had higher total VLDL than the MetS group. The data indicated that the increase in total VLDL in the T2DM group is mainly due to significant increases in concen-

Table 1
Characteristics of healthy, MetS, and T2DM rhesus monkeys

Variables	Healthy (n = 35)	MetS (n = 20)	T2DM (n = 18)	25-75th Percentiles
Age (years)	15.4 ± 0.9[dm]	18.7 ± 1.2	22.7 ± 1.3[h]	13.0-23.1
Body fat (%)	22.8 ± 1.5[ms, dm]	31.0 ± 2.0[h]	30.9 ± 2.1[h]	20.1-33.8
IRI (pmol/L)	241.0 ± 73.8[ms]	906.0 ± 97.6[dm, h]	163.3 ± 102.8[ms]	150.3-444.2
FPG (mmol/L)	3.7 ± 0.3[dm]	4.2 ± 0.4[dm]	14.7 ± 0.5[ms, h]	3.7-6.9
HbA_{1c} (%)	4.3 ± 0.2[ms, dm]	6.1 ± 0.3[dm, h]	9.7 ± 0.3[ms, h]	4.4-8.1
K_{Gluc} 5-20 (%/min)	2.9 ± 0.1[ms, dm]	2.3 ± 0.2[dm, h]	1.1 ± 0.2[ms, h]	1.6-3.1
M rate (mg/kg FFM per minute)	12.9 ± 0.5[ms, dm]	5.3 ± 0.6[h]	5.8 ± 1.1[h]	5.6-13.1
SBP (mm Hg)	122.6 ± 4.4	135.9 ± 5.9	134.4 ± 6.7	110.0-148.5

Results are presented as mean ± SE. One-way ANOVA was performed, followed by Tukey-Kramer multiple comparison test for determining differences between groups. Superscripts [h, ms, dm] indicate the group(s) from which each group differs significantly ($P < .05$); h indicates healthy; ms, metabolic syndrome, dm, type 2 diabetes mellitus. The mean 25th and 75th percentiles for the entire population are given for each of the variables.

trations of large and intermediate VLDL, whereas the small VLDL level in the diabetes group did not notably differ from levels in the MetS group.

Compared with the other 2 groups, IDL particle concentration increased markedly in T2DM monkeys. Modest but significant increases in total LDL particle concentrations were observed in the MetS and T2DM groups (Table 2), but LDL subclass distributions among the 3 groups differed substantially in their trends. Median levels of large LDL decreased in a stepwise fashion from healthy to MetS to T2DM (388.2, 198.6, and 0 nmol/L, respectively). In contrast, small LDL progressively increased, from a median value of 0 nmol/L among healthy monkeys to 39.6 nmol/L among the MetS to 1803.0 nmol/L among the T2DM. More interestingly, the median values of intermediate LDL particle concentration followed an inverted U-shaped curve with 180.7 in healthy monkeys, an elevated level of 388.4 in MetS, and a reduction to a median value of 0 nmol/L in T2DM. The increment of total LDL particle concentrations in the monkeys with the MetS and T2DM was attributed mainly to increase of small LDL. In particular, for the T2DM monkeys, small LDL was the absolute contributor to the high level of total LDL particle, even as large LDL concentration was significantly lower than that in the healthy monkeys. When compared with MetS monkeys, median values for small LDL particle concentrations were higher, whereas large LDL and intermediate LDL levels were lower in T2DM monkeys, with the result that the total LDL particle concentrations did not differ significantly between these 2 groups.

A marked decrease in total HDL particle concentrations was observed only in the MetS group and there was no significant difference between the healthy and T2DM groups due to the increase in small HDL concentrations specifically in the T2DM group. Similar to the LDL subclass profiles, large, intermediate, and small HDL particle concentrations also changed in different directions and nonlinearly across the 3 groups. A stepwise decline of the concentration of large HDL was observed from healthy to MetS to T2DM status (19.0 ± 1.2, 13.3 ± 1.6, and 8.8 ± 1.7 μmol/L in healthy, MetS, and T2DM groups, respectively). On the other hand, the intermediate HDL concentrations presented progressive increases across the 3 groups, and a significant increase of small HDL was found in the T2DM group.

In addition to providing lipoprotein particle concentration, NMR profiles also revealed changes in lipoprotein particle size. As indicated in Fig. 1, VLDL particle sizes tended to be increased in the MetS and T2DM groups, but

Table 2
NMR-determined lipoprotein particle profile in healthy, MetS, and T2DM rhesus monkeys

NMR lipoprotein subclass	Healthy (n = 35)	MetS (n = 20)	T2DM (n = 18)
Large VLDL (nmol/L)	1.3 (0.4-2.6)[ms,dm]	6.6 (4.7-15.7)[dm, h]	21.2 (8.7-54.7)[ms, h]
Intermediate VLDL (nmol/L)	1.6 (0-7.0)[ms, dm]	20.2 (2.7-42.9)[dm, h]	76.7 (39.7-135.5)[ms, h]
Small VLDL (nmol/L)	0 (0-4.2)[ms,dm]	22.9 (0-87.7)[h]	13.1 (0-58.3)[h]
Total VLDL (nmol/L)	7.3 (2.3-15)[ms, dm]	48.7 (11.4-165.1)[dm, h]	126.8 (66.8-222.8)[ms, h]
IDL (nmol/L)	0 (0-13.9)[dm]	9.6 (0-40.5)[dm]	66.0 (11.3-111.7)[ms, h]
Large LDL (nmol/L)	388.2 (95.7-576.2)[dm]	198.6 (0-472.6)[dm]	0 (0-67.1)[ms, h]
Intermediate LDL (nmol/L)	180.7 (0-356.8)	388.4 (82.1-742)[d]	0 (0-368.8)[ms]
Small LDL (nmol/L)	0 (0-0.8)[ms, dm]	39.6 (0-638.5)[dm, h]	1803.0 (797.1-3080.5)[ms, h]
Total LDL (nmol/L)	619.6 (508.5-770.1)[ms, dm]	1060.4 (798-1729)[h]	1950.5 (1419.9-3522.1)[h]
Large HDL (μmol/L)	19.0 ± 1.2 (21.0)[ms, dm]	13.3 ± 1.6 (15.7)[h]	8.8 ± 1.7 (7.0)[h]
Intermediate HDL (μmol/L)	4.5 ± 0.9 (2.2)[dm]	6.5 ± 1.2 (7.5)	10.2 ± 1.3 (10.3)[h]
Small HDL (μmol/L)	13.5 ± 0.9 (14.0)[dm]	11.4 ± 1.3 (11.1)[dm]	17.9 ± 1.3 (16.6)[ms, h]
Total HDL (μmol/L)	36.9 ± 1.0 (36.9)[ms]	31.3 ± 1.4 (31.2)[dm, h]	36.9 ± 1.5 (35.6)[ms]

Results are presented as mean ± SE (median) or median (interquartile range). One-way ANOVA was performed for normally distributed values and Kruskal-Wallis test was performed for nonnormally distributed data. The superscripts [h, ms, dm] indicate the groups that differ significantly from each other ($P < .05$); h indicates healthy; ms, metabolic syndrome, dm, T2DM.

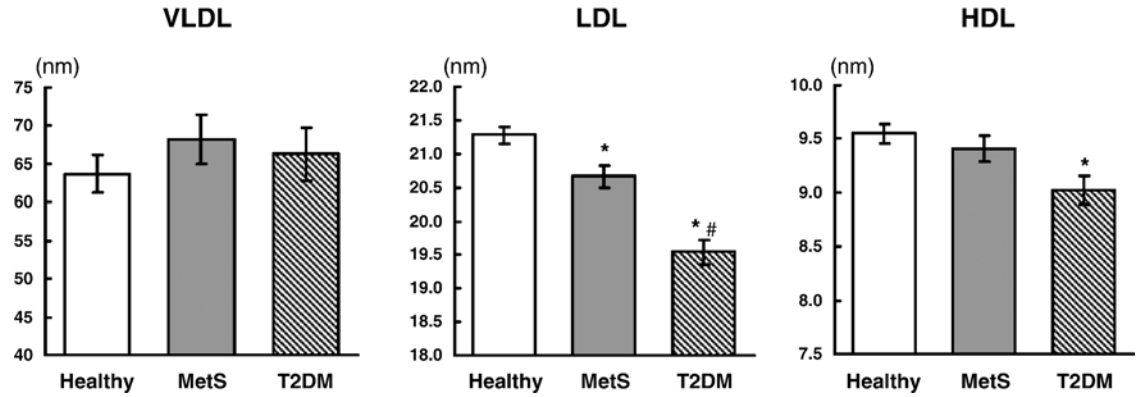

Fig. 1. Comparison of lipoprotein particle size determined by NMR spectroscopy. Three groups were studied: healthy monkeys, monkeys with MetS, and monkeys with T2DM. Results are presented as mean ± SE. P values from 1-way ANOVA. *$P < .05$, significantly different vs healthy monkeys; $^{\#}P < .05$, significantly different vs the MetS group.

did not achieve statistical significance across the groups. LDL particle sizes were markedly smaller in the MetS and T2DM monkeys compared with healthy monkeys. T2DM monkeys had significantly further lower LDL particle size than the MetS monkeys (19.5 ± 0.2 vs 20.7 ± 0.2 nm, $P <$ .05). HDL particle size did not differ between the healthy and MetS groups, whereas a significant decline was found in the T2DM group.

3.3. Conventional lipid panel determined by the β-quantification method

The conventional lipid panel measured using β-quantification is shown in Table 3. Between the healthy and the MetS groups, total cholesterol did not differ, but significant differences were observed in total TG, LDL-C, HDL-C, VLDL-TG, and VLDL-C. When comparing the MetS with the T2DM group, total cholesterol, total TG, VLDL-TG, and VLDL-C were all found to be markedly higher in the T2DM group, whereas there were no significant differences in levels of LDL-C (2.14 ± 0.21 mmol/L in MetS vs 2.49 ± 0.22 mmol/L in T2DM) and HDL-C (1.09 ± 0.10 mmol/L in MetS vs 1.35 ± 0.10 mmol/L in T2DM).

3.4. Multivariate correlation analyses

Table 4 shows correlations between the NMR-determined lipoprotein profile and other metabolic parameters including FPG, K_{Gluc}, HbA_{1c}, M rate, percent body fat, plasma TG, HDL-C, and SBP. Glycemic indices, FPG and HbA_{1c}, were positively correlated with large VLDL, intermediate VLDL, IDL, small LDL, and small HDL particle concentrations, whereas both were inversely related to large LDL and large HDL particle concentrations as well as to LDL particle size and HDL particle size. K_{Gluc} and M rate, which reflect glucose tolerance and insulin sensitivity, respectively, were positively associated with the concentrations of large LDL and large HDL subfractions as well as with LDL particle size. HDL particle size was strongly and positively correlated with K_{Gluc} ($r = 0.36$, $P < .01$), but not with M rate. Conversely, K_{Gluc} was negatively correlated with concentrations of large and intermediate VLDL, IDL, small LDL, and intermediate HDL particles (all $P < .01$), whereas M rate was inversely associated with concentration of all 3 VLDL subclasses, IDL, intermediate LDL, and small LDL. Because in this study we used conventional total TG and HDL-C in defining the MetS, we also investigated the correlations between NMR lipoprotein profiling and TG and HDL-C. As shown in Table 4, total TG level was positively correlated with concentrations of VLDL, IDL, and small LDL and negatively correlated with concentrations of large LDL and large HDL particles, as well as with LDL and HDL particle sizes (all $P < .01$). HDL-C was inversely correlated with concentrations of all 3 VLDL subclasses and, interestingly, with intermediate HDL

Table 3
β-Quantification–determined conventional lipid panel in healthy, MetS, and T2DM rhesus monkeys

Conventional lipid panel (mmol/L)	Healthy (n = 35)	MetS (n = 20)	T2DM (n = 18)
Total TG	0.80 ± 0.47$^{ms,\ dm}$	3.30 ± 0.62$^{dm,\ h}$	6.46 ± 0.66$^{ms,\ h}$
Total cholesterol	3.37 ± 0.24^{dm}	4.18 ± 0.32^{dm}	5.58 ± 0.34$^{ms,\ h}$
LDL-C	1.49 ± 0.16$^{ms,\ dm}$	2.14 ± 0.21^{h}	2.49 ± 0.22^{h}
HDL-C	1.65 ± 0.07$^{ms,\ dm}$	1.09 ± 0.10^{h}	1.35 ± 0.10^{h}
VLDL-TG	0.22 ± 0.24$^{ms,\ dm}$	1.67 ± 0.32$^{dm,\ h}$	3.14 ± 0.34$^{ms,\ h}$
VLDL-C	0.23 ± 0.13$^{ms,\ dm}$	0.95 ± 0.17$^{dm,\ h}$	1.75 ± 0.18$^{ms,\ h}$

Results are presented as mean ± SE. One-way ANOVA was performed, followed by Tukey-Kramer multiple comparison test for determining differences between groups. The superscripts $^{h,\ ms,\ dm}$ indicate the groups that differ significantly from each other ($P < .05$); h indicates healthy; ms, metabolic syndrome, dm, T2DM.

Table 4
Correlations between NMR-determined lipoprotein profile and the parameters associated with the MetS—FPG, intravenous glucose tolerance (K_{Gluc}), HbA_{1c}, insulin sensitivity by euglycemic-hyperinsulinemic clamp (M rate), percent body fat, TG, total HDL-C, and SBP

Lipoprotein subclass	FPG		K_{Gluc}		HbA_{1c}		M rate		Body fat (%)		TG		HDL-C		SBP	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P
Large VLDL	0.37	<.01	−0.43	<.01	0.66	<.01	−0.66	<.01	0.14	.25	0.90	<.01	−0.46	<.01	0.30	<.05
Intermediate VLDL	0.53	<.01	−0.48	<.01	0.47	<.01	−0.46	<.01	0.15	.21	0.75	<.01	−0.31	<.01	0.23	.06
Small VLDL	0.13	.26	−0.16	.17	0.24	<.05	−0.45	<.01	0.04	.70	0.50	<.01	−0.29	<.05	0.20	.09
IDL	0.32	<.01	−0.37	<.01	0.39	<.01	−0.33	<.05	0.19	.11	0.42	<.01	0.05	.66	−0.14	.83
Large LDL	−0.35	<.01	0.39	<.01	−0.40	<.01	0.27	<.05	−0.02	.83	−0.43	<.01	0.53	<.01	−0.03	.82
Intermediate LDL	−0.09	.45	0.06	.62	−0.01	.98	−0.33	<.05	−0.01	.98	0.01	.92	−0.22	.06	0.09	.49
Small LDL	0.55	<.01	−0.54	<.01	0.43	<.01	−0.36	<.01	0.24	<.05	0.52	<.01	−0.24	<.05	0.07	.56
Large HDL	−0.26	<.05	0.45	<.01	−0.45	<.01	0.44	<.01	−0.18	.14	−0.53	<.01	0.77	<.01	0.03	.79
Intermediate HDL	0.29	<.05	−0.33	<.01	0.21	.08	−0.23	.08	0.10	.62	0.22	.06	−0.31	<.01	0.04	.77
Small HDL	0.27	<.05	−0.15	.20	0.31	<.01	0.16	.24	−0.06	.63	0.11	.38	0.04	.72	0.08	.54
VLDL size	0.04	.75	0.13	.26	0.11	.35	0.01	.97	0.03	.83	0.19	.11	−0.13	.28	−0.03	.78
LDL size	−0.50	<.01	0.53	<.01	−0.45	<.01	0.41	<.01	−0.14	.24	−0.57	<.01	0.47	<.01	−0.06	.64
HDL size	−0.27	<.05	0.36	<.01	−0.24	<.05	0.20	.13	−0.04	.75	−0.31	<.01	0.51	<.01	0.05	.72

Spearman rank correlation coefficients were obtained for examining the relationships between independent variables adjusted for age and sex. Values of $P < .05$ are considered significant.

($r = -0.31$, $P < .01$). Positive correlations were observed between HDL-C with concentrations of large LDL and large HDL, as well as with LDL and HDL particle sizes. With respect to other important factors of the MetS, percent body fat and SBP did not show strong correlations with most of the NMR lipoproteins, except for a positive correlation between percent body fat and small LDL and a positive correlation between SBP and large VLDL.

4. Discussion

People with the MetS are at increased risk of T2DM compared with people without the syndrome, and almost half of the population-attributable risk for diabetes can be explained by the presence of the MetS [29]. In both MetS and diabetes, dyslipidemia has been believed to be responsible for considerable CVD-related morbidity and mortality. Widely accepted is that dyslipidemia is characterized by increased levels of TG and decreased HDL-C; however, with recent advances that recognize the complexity of lipoprotein metabolism and the mechanisms of atherogenesis, it has become important to identify the specific perturbations of dyslipidemia, potentially leading to more targeted interventions at the pathway or molecular levels. Conventional lipid assays, including the β-quantification method used in this study, determined the total amount of cholesterol and TG carried within the lipoproteins. However, these measures are unable to provide any quantification of lipoprotein subclass concentrations and sizes. NMR techniques have provided an opportunity to obtain further information about subclass size distribution. Some studies using NMR analysis technology have been reported in humans [17,35,36], but NMR-determined lipoprotein profiling has not been fully characterized in the rhesus monkey, an excellent model that spontaneously develops the MetS and in some cases subsequent T2DM, and that exhibits strong similarity to humans. Therefore, in the present study, we used NMR spectroscopy to examine the naturally occurring dyslipidemia in the MetS and T2DM in monkeys under low fat and low cholesterol diet, and to identify the shift in these subclass distributions between the MetS and the onset of diabetes to improve the explanatory power for advancing epidemiologic and diagnostic applications.

Previous studies in our colony have shown that both VLDL-TG and VLDL-C are early predictors of development of diabetes [32]. Currently presented data further reveal a highly significant increase in VLDL subclasses among the MetS and T2DM monkeys. Furthermore, when comparing the MetS with the T2DM group, the increase in total VLDL in the T2DM group was not due to a uniform increase in all VLDL subclasses, but rather to an increase in the large and intermediate VLDL particles, without a consistent change in the small VLDL particle. As widely known, TG-rich larger VLDLs are produced primarily by the liver, whereas smaller VLDL is derived predominantly from the action of lipoprotein lipase on larger VLDL particles. This has suggested that production or synthesis of larger VLDL particles may be a primary abnormality associated with the more severe dyslipidemia in T2DM. Garvey et al [17] reported that in humans, the level of the large VLDL fraction increases in insulin resistance and diabetes, whereas no significant changes were seen with the intermediate and small VLDL subsets. This is generally consistent with our findings, although we did find statistically significant increases in the intermediate and small VLDL particle concentrations in monkeys with the MetS and T2DM.

It has been widely shown that the predominance of small dense LDL particles is associated with cardiovascular risk [37,38]. Besides elevated levels of TG and reduced levels of HDL-C, a predominance of small dense LDL

particles has been considered as one of the important characteristics of dyslipidemia in the MetS and T2DM, and about 40% to 50% of type 2 diabetics have a preponderance of small dense LDL [39]. Our data strongly confirmed this finding. Furthermore, in our study, although there was no significant difference observed between conventional LDL-C levels in the MetS and T2DM groups, there were, nevertheless, differences in NMR-determined lipoproteins: specifically, large LDL particle levels decreased, and small LDL particle levels increased progressively from the MetS to the diabetic states. Intermediate LDL, however, reached a peak above normal during the MetS state and showed an inverted U-shaped curve, dropping off in the T2DM group. Correspondingly, there was a progressive reduction in LDL particle size in MetS and T2DM monkeys. Previous human studies also showed a stepwise decrease in LDL size in subjects with IGT progressing to clinical diabetes [36,40] and a similar change of LDL subclass distribution in subjects with insulin resistance progressing to diabetes [17]. In nonhuman primates, as well as in humans, a shift from large LDL to small dense LDL occurs during the progressive development of the MetS and T2DM and may contribute to the increased risk of cardiovascular events in both. Although in humans LDL-C remains one of the most robust and powerful predictors of atherosclerosis, additional information about the LDL subclass distribution may be helpful for predicting potential CVD risk because the same level of LDL-C can be the result of either a small number of large LDL particles or a large number of small LDL particles. Some findings in humans suggest that LDL-C remains unchanged or only slightly increased in the MetS and T2DM [10,41]. However, others reported that LDL-C elevation is frequently present, although it is not a diagnostic criterion of the MetS. The subjects with the triad of elevated LDL-C, low HDL-C, and high TG were more likely to have other characteristics of the MetS and T2DM [6,42], as is the case with our monkeys (5 of 20 monkeys with MetS recruited in this study exhibited LDL-C elevation). In addition, the "healthy" group in most human studies may actually include some undiagnosed prediabetic subjects, whereas our reference primate group specifically excluded monkeys with prediabetes or early evidence of the MetS. The "purity" of our healthy group magnifies the metabolic differences between the normoglycemic and the MetS monkeys, thus explaining some of the observed differences between human and nonhuman primate results. Despite the limitation due to our sample size, our ability to observe differences in LDL-C level between metabolic categories warrants further investigation on the determinants of circulating LDL-C.

High-density lipoprotein is considered to be a cardioprotective lipoprotein, related to its reverse cholesterol transport, antioxidative, anti-inflammatory, and antiapoptotic activities, and decreased plasma HDL-C levels are recognized as a dominant feature of diabetic dyslipidemia and as an independent risk factor for CVD. Although some controversy remains, there is accumulating evidence suggesting that cholesterol ester–rich larger HDL particles have a greater contribution to the cardioprotective effects of increased HDL-C level than do small dense HDL particles that are functionally defective [43-45]. Thus, declines in the fraction concentration of the large antiatherogenic HDL subclass coupled with elevation of the less protective small dense HDL may result in a misleading favorable interpretation of the standard lipid profile. This hypothesis was supported by our HDL particle concentration data and HDL-C data. Large HDL particles carry much more cholesterol than small HDL particles. It was clearly seen that in the T2DM group, smaller HDL made a more profound contribution to the total HDL particle concentration than did large HDL. In contrast, large HDL made a much greater contribution to the total HDL mass concentration than did small HDL (data not shown; NMR-determined subclass mass concentration [milligrams cholesterol per deciliter] is consistent with the β-quantification–determined HDL-C level). Reduced levels of large HDL, especially in the T2DM group, accompanied by a significant increase in small and intermediate HDL, led to a similar total HDL particle concentration. Pascot et al [45] previously reported that the average HDL size was reduced in the subjects with metabolic abnormalities and suggested that small HDL was a new feature of atherogenic dyslipidemia; our data in monkeys support this finding.

Correlations between lipoprotein characteristics and some important disease-associated parameters further strengthened and supported our demonstrations above, especially regarding the LDL and HDL subclass distribution. Increased TG-rich VLDLs and IDL particle concentrations were highly correlated with hyperglycemia, IGT, insulin resistance, hypertriglyceridemia, and low HDL-C. However, interestingly, both large buoyant LDL and large HDL were positively associated with better glucose tolerance and insulin sensitivity, lower FPG and HbA_{1c}, and lower TG but higher HDL-C; conversely, smaller and denser LDL and smaller and denser HDL demonstrated associations in the opposite direction. These findings in monkeys are consistent with previous reports in humans that levels of small dense LDL are associated with an increase of insulin resistance in nondiabetic subjects [17,46]. Furthermore, our data show that the small HDL subclass is more closely associated with hyperglycemia and increased HbA_{1c} than with insulin resistance or glucose intolerance. Percent body fat and SBP did not show strong relationships to the lipoprotein distributions, except for a positive correlation with small LDL and large VLDL, respectively.

In summary, the present study shows that within lipoprotein classes there are significant and divergent shifts in both particle concentrations and sizes and that these shifts are evident in the progression to the MetS and diabetes. Although the clinical use of NMR technology is not yet clear, the results reported above provide some evidence of predictive use and suggest that measurement of lipoprotein

subclass distribution probably strengthens the power of diagnosis, and potentially identifies targets for intervention in disease. Lipid and lipoprotein abnormalities in diabetes are similar to those observed in the MetS, but of greater severity, thus conveying a greater risk of CVD. Although much has been learned about the association between the MetS, diabetes mellitus, and atherogenic dyslipoproteinemias, little is known about causation; thus, the causal relationship requires further investigation in longitudinal studies. Our data also demonstrate that rhesus monkeys exhibit a detailed lipoprotein fraction profile resembling those of humans, further emphasizing the strong similarity between the species and establishing that the spontaneously diabetic nonhuman primate provides an ideal model for investigating the progressive changes of dyslipidemia in the MetS and diabetes and the effects of interventions to mitigate these disorders.

Acknowledgments

This study was supported by National Institute on Aging grants NO1-AG-3-1012 (to BCH).

The authors thank Theresa Alexander, Holly Jermyn, Michelle Izuka, Wallace Evans, Karen Brocklehurst, and David Lamkin for their excellent technical expertise and animal care, the late Georgielle Gerzanich for her superior database management, and Rosemary Peternel for assistance with manuscript preparation. The consultation of Dr. J. D. Otvos with regard to the lipoprotein studies was greatly appreciated.

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