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Review article

Understanding type 2 diabetes and aging: lessons from nonhuman primates

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Background: The increase in global prevalence of obesity and diabetes, and the growth of the elderly population worldwide emphasize the biomedical research need for an animal model which exhibits close similarity to human disease and aging processes. The rhesus monkey develops obesity and type 2 diabetes spontaneously and naturally when *ad libitum* fed, within a lifespan which is about a third that of the human.
Objective: To characterize the genetic, structural, biochemical and physiological changes occurring in monkeys who age successfully and in those who develop obesity and type 2 diabetes.
Results: The rhesus monkey demonstrates the same signs and symptoms of type 2 diabetes, including macro- and microvascular complications, as observed in humans. Age-related changes, potential biomarkers, and proposed biochemical pathways of aging can be readily investigated, with outcomes very similar to those in humans.
Conclusion: The rhesus monkey model imparts valuable insights to normal and pathological processes accompanying aging and type 2 diabetes. It also provides a valuable tool by which to test novel therapeutic interventions which otherwise can not be performed in humans due to ethical considerations, but where results are highly translatable.

Keywords: Aging, diabetes, metabolic syndrome, obesity, nonhuman primates, rhesus macaques.

Because the prevalence of both obesity and diabetes are increasing at rates which have been likened to a global epidemic [1], it becomes increasingly imperative to understand their natural history and progression in order to offer and implement meaningful approaches towards their treatment and prevention. The need becomes more evident as estimates have been made that the global prevalence of diabetes was 171 million in 2000 [2] and the number of people afflicted with diabetes may reach the 380 million mark by the year 2025 [3]. This has been attributed to population growth, urbanization, increased prevalence of obesity, physical inactivity, as well aging.

Toward obtaining a thorough understanding of the pathophysiology of the disease, an animal model demonstrating close similarity to the human type 2 diabetes, with regard to signs, symptoms and development, becomes extremely relevant. The nonhuman primate model is unique in that, in addition to the close phylogenetic relationship to humans, monkeys spontaneously develop obesity and diabetes which follow the exact trajectory as the human disease, including its long-term macro- and microvascular complications. The possible etiologic factors that are responsible for both the initiation of the disease process and its complications can be rigorously investigated within a period about 70 % shorter than the human lifespan. Even more important is the ability rendered by the nonhuman primate model to examine type 2 diabetes during its transitional phases, such as in prediabetes and early diabetes, thus providing valuable information as to which parameters are not only highly predictive of disease progression, but also possibly reversible. When longitudinally observed under the controlled environment of a research laboratory, nonhuman primates even offer a distinct advantage over human studies because of the absence of extraneous sources of variations such as diet, lifestyle, drug history and ethnicity. Needless to say, the close affinity between humans and primates

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makes genetic studies on monkeys also more readily translatable to humans.

Both the proportion of the older population and the absolute numbers of middle-aged and elderly continue to increase globally. Thus, the availability of this animal model for studies related to normal aging becomes extremely valuable. Nonhuman primates age very similarly to humans. No animal model is perhaps more appropriate for providing the ability to detect changes that are pathological (e.g., due to diabetes), *vs*. those that can be ascribed to biological aging, than the nonhuman primate model. Disease processes in nonhuman primates are likely to be more "homologous" with humans rather than "analogous" as when studied in lower forms of animals. Nonhuman primate aging demonstrates close parallelisms with the endocrine, metabolic, immunologic, cardiovascular and other organ system changes seen in humans, as discussed in our recent review [4]. Since nonhuman primate lifespan is about a third or a fourth that of humans, prospective and retrospective analyses of data, evaluation of pharmacologic and non-pharmacologic interventions, and examination of naturally occurring age-associated alterations and disease development can be observed within a compressed timeframe.

We have previously described the spontaneous development of both obesity and diabetes in the rhesus monkey [5-9] and recently updated our findings on this species [10]. However, diabetes has also been described in other species of nonhuman primates (**Table 1**). In this review, we characterize the genetic, structural, biochemical and physiological changes occurring in monkeys who age successfully and in those who develop obesity and type 2 diabetes.

Characteristics of the colony

We describe a colony of rhesus monkeys in the Obesity, Diabetes and Aging Research Center currently situated at the University of South Florida, USA, which provides an ideal model for studying the development of obesity, diabetes and their interrelationships with normal aging. *Ad libitum* feeding of a heart- healthy, low fat diet leads, over the course of years, to the *spontaneous* and natural development of obesity and metabolic syndrome in some of the monkeys and, from this group, some progress to overt diabetes. No artificial intervention was ever used to induce the diabetes. The colony was established in 1969 and has expanded over the last 30 years under the directorship of one of the authors (BCH). As such, extensive medical records exist for all of the monkeys

Table 1. Primate species where diabetes has been identified [14].

Cetus apella	Capuchin
Cercopithecus aethiops	African green monkey, vervet, or grivet
Cercopithecus cephus	Moustached green guenon
Cercopithecus diana	Diana monkeys
Cercopithecus mitis	Blue, Sykes (silver, golden) of Samango monkey
Cercopithecus mona	Mona monkey
Colobus polykomos	King Clolbus monkey
Galago crassicaudatus	African bushbaby
Macaca fasicularis	Cynomolgus or crab-eating macaque
Macaca cyclopis	Taiwan or Fornmcsan rock macaque
Macaca mulatla	Rhesus monkey
Macaca nemestrina	Pig-tailed macaque
Macaca radiata	Bonnet macaque
Mandrillus leucophaeus	Mandrill baboon
Papio hamadryas	Sacred baboon
Sagninus fuscicollia	Tarnarin
Saquinna cedipus	Tarnarin
Saimiri aciureus	Squirrel monkeys
Pan troglodytes	Chimparrzee
Pan paniscus	Bonobo

in the colony, including longitudinal and serial observations brought about by the frequent assessments of the metabolic status of each individual in the colony, including daily food intake and random glucose values, performed under the highest standards of husbandry and care. The colony serves as a resource for studies related to aging and the natural development of disease and is supported in part by the NIH-National Institute on Aging, USA. The deterioration from normoglycemia to diabetes in this colony has been extensively characterized [5, 8] and can be divided into 9 phases, with phase 1 comprising normal, lean, young adult monkeys and phase 9 composed of those that have progressed to severe insulin-treated diabetes (see **Fig. 1**).

Diagnosis of diabetes in our colony is based on the criteria set for humans by the Expert Committee of the American Diabetic Association (ADA), first issued in 1997 and modified in 2003 [11, 12]. These were defined in humans as: 1) a fasting plasma glucose value of ≥ 126 mg/dL (7.0 mmol/L) after at least an 8-hour fast; 2) a 2-hour plasma glucose ≥ 200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test; 3) a casual plasma glucose value ≥ 200 mg/dL (11.1 mmol/L) plus the classic symptoms of polyuria, polydipsia, and unexplained weight loss. For humans, values between 100 and 125 mg/dL constitute impaired fasting glucose, while impaired glucose tolerance denotes 2-hr plasma glucose values between 140 and 199 mg/dL. In monkeys, based on longitudinal and cross-sectional data from our colony, fasting glucose values are actually in the range of 55-80 mg/dl in normal, lean, young subjects. Fasting glucose values in the range of 80-105 mg/dL are, therefore, actually in the impaired fasting glucose category, and a level ≥ 105 mg/dL could be considered to be the threshold of the diabetic range [10]. However, for convenience, we continue to use the ADA definitions. Clinical diagnosis of diabetes per the ADA endorsed definition 2003, occurs at phase 8 of the above progression scheme.

Metabolic syndrome is often observed in the transition from normal to overt type 2 diabetes. In close analogy to the criteria used in humans, metabolic syndrome in monkeys is defined as the presence of impaired fasting glucose, impaired glucose tolerance (defined as a glucose disappearance rate of < 2.0 %/min during an intravenous glucose tolerance test), or insulin resistance (an insulin-sensitivity (M-rate) of < 7.5 mg/kg FFM/min;FFM = fat free mass) accompanied by 2 or more of the following features: hyperinsulinemia (fasting insulin values >70 microunits/ml), hypertension (systolic blood pressure (BP) ≥ 130 mm Hg; diastolic BP ≥ 80 mmHg), adiposity (body fat >22 %), and dyslipidemia (fasting triglycerides ≥ 80 mg/dl or HDL-C < 60 mg/dL). Metabolic syndrome

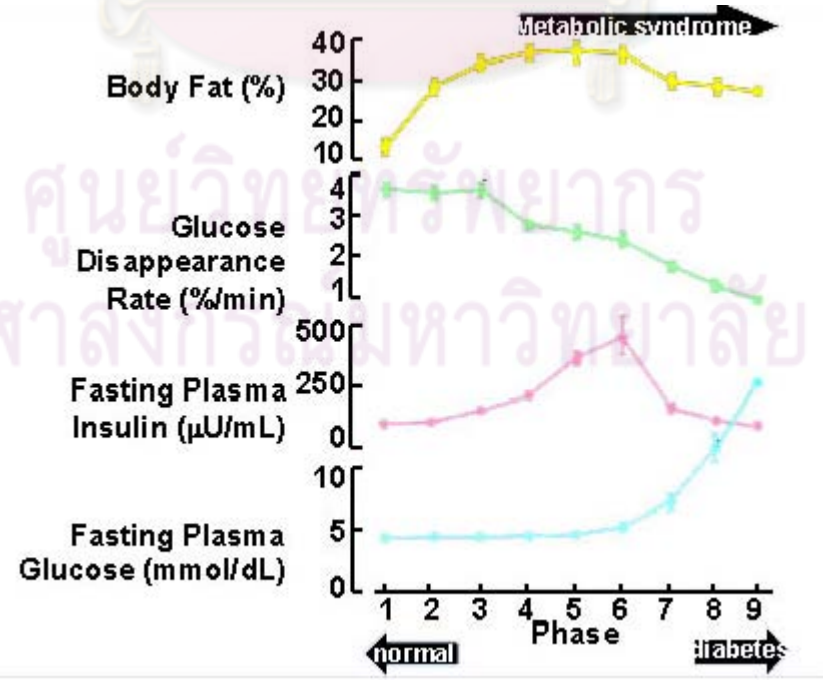


Fig. 1 Natural progression of type 2 diabetes mellitus in rhesus monkeys [5]. µU=microunits.

is clearly a prediabetic stage which occurs between phases 3 and 7 of the disease trajectory. Thus, we can identify subjects who, although still in the normoglycemic range, are actually prediabetic. Our previous publication on age-related changes identifies and separates disease-related perturbations in common clinical variables in contrast to those that occur in the course of natural aging [13, 14]. Therapeutic trials involving novel interventions can be initiated prior to the conversion to type 2 diabetes to examine whether diabetes or any of its accompanying metabolic dysregulation can be prevented or retarded. For instance, a group of monkeys was selected to remain weight-stabilized at 10 kg (the optimum weight for an adult monkey) by careful titration of their food intake, in contrast to the rest of the colony which was *ad libitum* fed. This "calorie-restricted (CR)" group was observed over many years until their death. In a 25-year study involving a total of 117 monkeys some of which were calorie restricted, none of the CR monkeys developed diabetes, and their average lifespan was longer than that of the *ad libitum* fed cohort (32 years *vs* 25 years) [15, 16]. Of the total population of *ad libitum* fed monkeys in the colony at any given time, 45 % are either diabetic or are on their way to becoming overtly diabetic.

The metabolic syndrome as observed in monkeys

Impaired glucose tolerance is assessed by performing a 60-minute intravenous glucose tolerance test and calculating for the $K_{glucose}$ or glucose disappearance rate during the first 20 minutes. Rates above 2 %/min are considered normal, and suggest normal beta cell function. Insulin sensitivity/resistance, on the other hand, is measured by performing a euglycemic, hyperinsulinemic clamp, wherein supraphysiologic doses of insulin are administered to suppress both endogenous insulin secretion and hepatic glucose production. The amount of glucose infused to maintain euglycemia (at a level between 80-90 mg/dL) is indicative of the rate at which insulin-mediated glucose disposal into peripheral tissues is achieved. Insulin resistant monkeys are those with M-rates, or insulin sensitivity rates, less than 7.5 mg/kg FFM/min. Fasting plasma glucose values are assessed using the glucose oxidase method. Clinical chemistry and hematology are performed by a commercial laboratory, and fasting insulin levels are measured using radioimmunoassay techniques. Apart from the total circulating triglycerides and cholesterol obtained from the clinical chemistry, lipid analysis is also performed routinely using vertical spin density-gradient ultracentrifugation or VAP (Atherotech, Birmingham, ALA, USA) for detailed measurements of the lipoprotein subclasses. In some studies, nuclear magnetic resonance (NMR) lipoprotein profiling (Liposciences, Inc. Raleigh, NC, USA) were also performed to obtain comparative information. Free fatty acid determinations are also performed at the lipid laboratory, University of Washington, USA. Circulating cytokine levels are obtained either using ELISA assays in-house, or performed by other university laboratories (e.g. Cornell University, University of Maryland, USA). Assays for adiponectin, primate leptin and ghrelin are performed by Linco diagnostics (St. Charles, MO, USA), for the most part by radioimmunoassay. Body fat was initially calculated by subtraction of the lean body mass from the total weight, as estimated using the tritiated water dilution method [17]. In recent years we have used the Dual Energy X-ray Absortiometry for estimation of the percentage of body fat.

Figure 2 demonstrates the results from a cross-sectional study involving 47 monkeys, 5 of whom were calorie- restricted, 17 normoglycemic, 11 prediabetic (PreDM)/ metabolic syndrome (MetSyn) and 14 overtly diabetic. The differences in metabolic values between these 4 categories are clearly evident. Calorie-restricted subjects have the lowest degree of adiposity, and body fat increases progressively across the different groups, with the highest being in the diabetic subjects. Weight, however, is highest among the prediabetic subjects, and the characteristic loss of weight is seen in the overt diabetic group despite having the greatest elevation of body fat. Fasting plasma insulin levels are highest also among the PreDM/MetSyn monkeys, and drop to normal or below normal values with progression to overt type 2 diabetes mellitus (DM). It is quite apparent that despite the only slightly elevated fasting plasma glucose values in the PreDM group, weight, body fat and especially insulin levels are above the normal range.

Measurements of other physiologic variables provide further evidence that the prediabetic/ metabolic syndrome group is a separate category and distinguishes itself from the normal control group. This can be seen in **Fig. 3**. Glucose tolerance ($K_{glucose}$) and insulin-sensitivity (M-rate) are significantly lower in the PreDM and DM group compared to the

normal subjects. The figures clearly show that during prediabetes, long before diagnosis of type 2 diabetes, glucose homeostasis is already severely impaired. On the other hand, glucose tolerance and insulin sensitivity in the CR monkeys were comparable to the normoglycemic monkeys, despite the fact that the CR monkeys were twice the age of the normal subjects (mean age of CR= 31.4 years *vs* N=14.3 years).

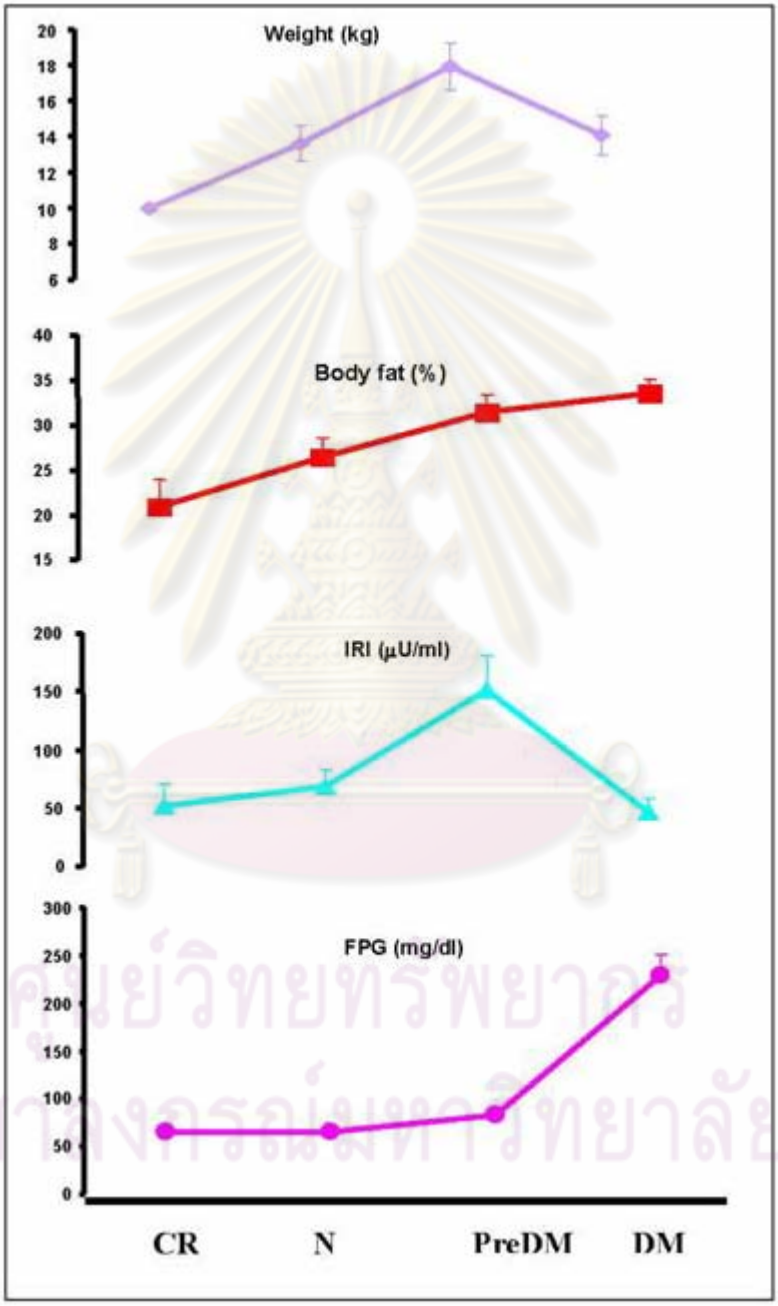


Fig. 2 The data showing body weight (kg), body fat (%), fasting insulin (μ units/ml) and fasting glucose (mg/dl) values in 4 groups of monkeys of differing metabolic states. CR = calorie-restricted, N = normoglycemic, PreDM = prediabetic/metabolic syndrome, DM = overt type 2 diabete. Values are presented as mean ± SE [17].

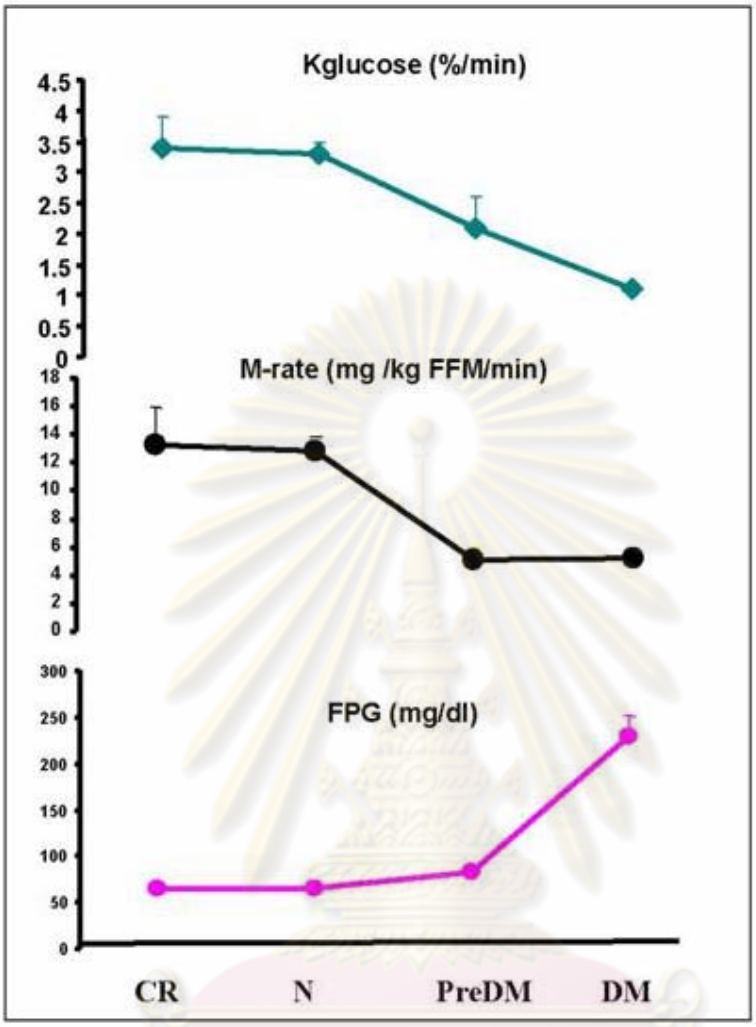


Fig. 3 Data demonstrating that in prediabetes, both insulin sensitivity (M-rate) and glucose tolerance ($K_{glucose}$) are already declining, even though fasting plasma glucose (FPG) values are well below the diabetic threshold. Calorie-restricted (CR) subjects, on the other hand, have values comparable to the normal group (N) despite being twice as old. Values are presented as mean SE [17].

Measurements of other variables associated with the metabolic syndrome

Lipids and lipoproteins

Dyslipidemia is a recognized predictor of both cardiovascular disease and diabetes in humans; it also occurs in rhesus monkeys, and demonstrates a profile very similar to that found in humans [18]. The following figures illustrate the evolution of dyslipidemia with progression of diabetes in the same group of monkeys as above (**Fig. 4A-D**). Total LDL-C (**Fig.4A**), triglycerides (**Fig. 4B**) and IDL-C (**Fig. 4C**) levels steadily escalate from the healthy to the overtly diabetic group, whereas HDL-C levels (**Fig. 4D**) steadily decline.

Data from our colony based on NMR analysis of lipoprotein subfractions demonstrate that together with the increased levels of VLDL, IDL and small dense LDL particles in metabolic syndrome and type 2 diabetes, concentrations of large LDL and large HDL particles are also reduced, as their particle sizes become smaller [18]. This can be seen in **Fig. 5.** Because this type of lipoprotein profile also predicts untoward cardiovascular events, dyslipidemia and adiposity appear to be common foundations for development of both type 2 diabetes and cardiovascular disease.

We have also performed measurements of circulating free fatty acids (FFA) in 92 monkeys, ages 7-37 years old, 5 of which had metabolic syndrome,

and 6 were overtly diabetic. FFA levels were determined using an enzymatic assay. While levels were significantly elevated among the diabetic group, values from the insulin-resistant prediabetic group did not differ significantly from the healthy subjects (data presented at the American Diabetes Association meeting, 2007). This result is intriguing because it contradicts the commonly held notion that beta cell failure and insulin resistance are consequent to elevated FFA.

Inflammatory markers

In addition, measurements of acute phase reactant proteins, such as high sensitivity CRP and interleukin 6 reveal significantly elevated levels in the prediabetic group as compared to the normal group (**Fig. 6**). Furthermore, this phase which apparently is a state of chronic inflammation, is also characterized by a hyperdynamic phase, where skin microvascular perfusion rate in response to thermogenic stimulation is also elevated (**Fig. 7**). We have, in fact, demonstrated that the enhanced capillary recruitment in the distal extremities of prediabetic subjects is significantly correlated with the circulating levels of hsCRP [20]. Our findings also suggest that these phenomena may be related to changes in the population of peripheral nerve fiber endings in the skin undergoing successive changes from the normoglycemic, through the dysmetabolic and finally to the overt diabetic phases [21] (**Fig. 8**).

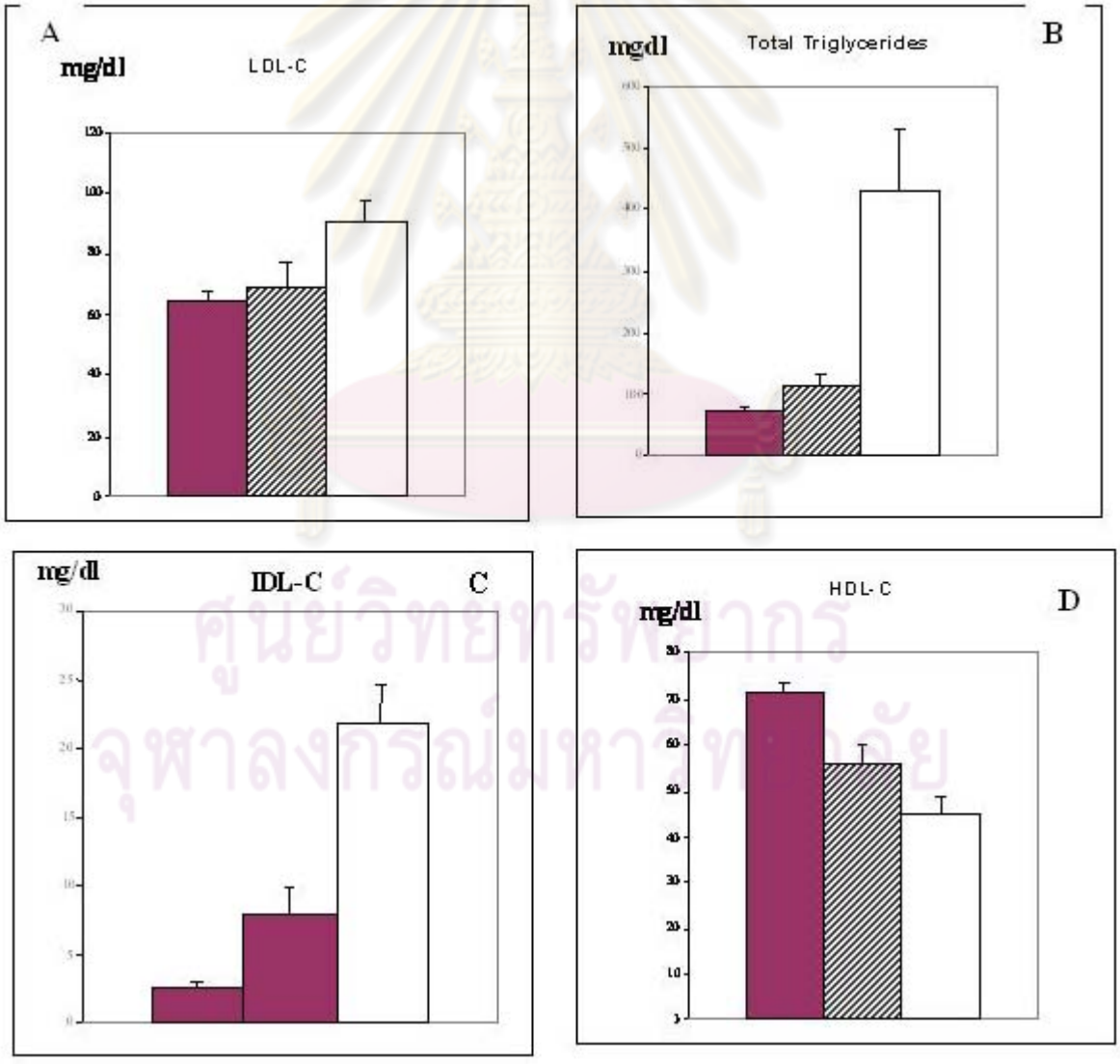


Fig. 4 Evolution of dyslipidemia as seen from the lipid profile of normal ( ), prediabetic ( ) and type 2 diabetic monkeys ( ). Results are presented as mean SE [18].

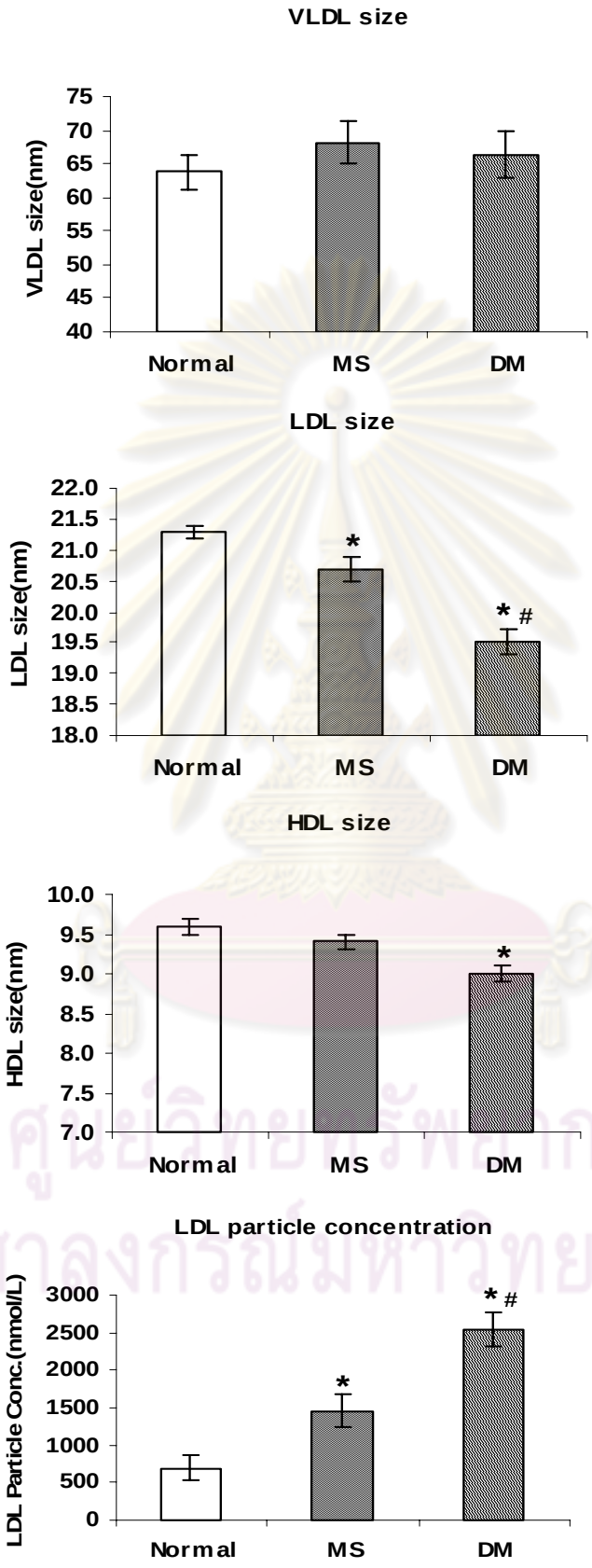


Fig. 5 Lipoprotein particle size as determined by NMR spectroscopy. Both LDL and HDL decrease in particle size. Values are presented as mean SE. *P<0.05, significantly different from healthy, normal group; # P<0.05, significantly different from the PreDM/MetSyn group [18].

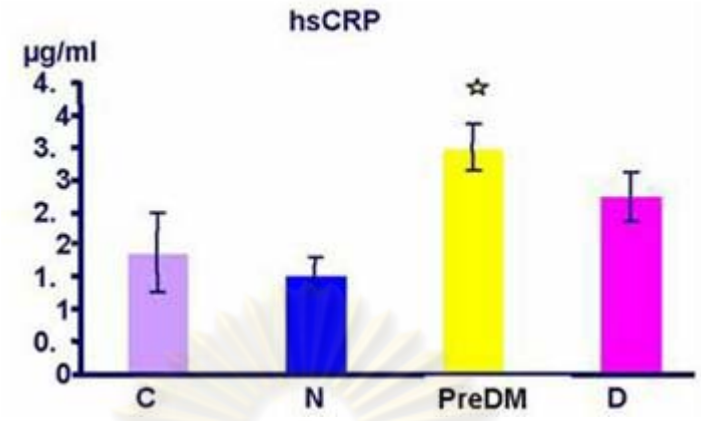


Fig. 6 Circulating levels of hsCRP are elevated in the prediabetic group compared to the normal subjects. Values are presented as mean SE [20].

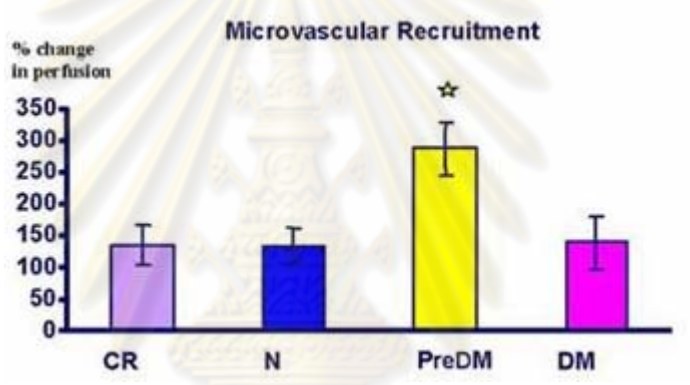


Fig. 7 Skin microvascular flow rate in response to thermal stimulation is enhanced among prediabetic subjects compared to normal subjects, a finding which is significantly correlated with the elevated levels of hsCRP. Values are presented as mean SE [20].

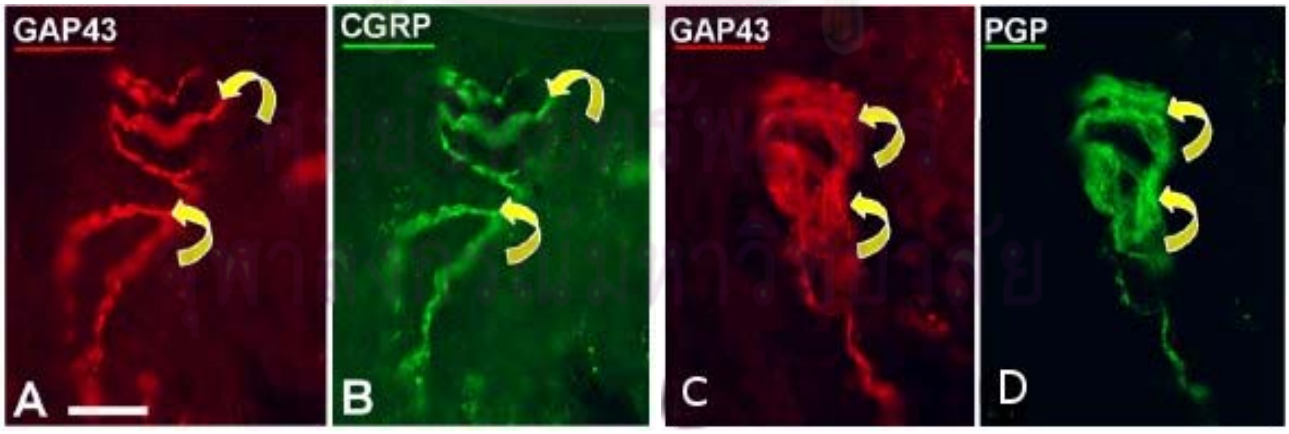


Fig. 8 Diabetes-related changes in the circumferentially oriented swirl endings that occasionally appear in dermal papilla. **A** and **B**: Separate color channels of a dermal papilla from a young (4 year old) nondiabetic monkey demonstrating that most of the swirl innervation labels with both CGRP and GAP43 (yellow curved arrows). **C** and **D**: Separate color channels of a dermal papilla from a 23 year-old monkey with short-term diabetes, exhibiting abnormal expansion of numerous fine-caliber processes in the swirl endings that over-express GAP43 and PGP (yellow curved arrows). Scale bar = 25 µm. Modified from [21].

Another cytokine implicated in obesity-related insulin resistance, resistin, which in rodents is primarily secreted by adipose tissues, has also been examined. Based on plasma resistin levels from a subset of 41 diabetic and nondiabetic monkeys, we failed to find any significant correlation between resistin levels and indices of insulin resistance, such as the Fasting Glucose/Fasting Insulin ratio, the QUICKI-insulin sensitivity index and the HOMA-Insulin Resistance index. Furthermore, resistin was also not associated with any of the characteristic lipid parameters of insulin resistance. Thus, the role of this cytokine in insulin resistance and diabetes progression in humans and nonhuman primates remains equivocal. Similarly, Serum Amyloid A protein (SAA), an acute-phase reactant synthesized by the liver during inflammation, has been suggested to play a causal role or be associated with development of diabetes. In our study of 67 monkeys aged 5-30 years, comprised of healthy, metabolic syndrome and spontaneously diabetic subjects, circulating SAA, determined using ELISA techniques, although present in high quantities, was not found to be associated with any of the insulin resistance indicators nor with body fat. SAA values did not even differ significantly among the three groups of monkeys (Data presented at the Endocrine Sociaty Annual meeting, 2007). This finding argues against a causal role for SAA in the pathophysiology of diabetes in primates, and highlights that findings in rodents do not necessarily translate to primates, including humans.

Distinguishing normal aging from diabetes-induced changes

Diabetes is an age-related disease. Therefore, it is important to be able to distinguish which changes in selected variables are a natural consequence of aging, and which are due to disease. Longitudinal assessments taken of the entire colony [14] as well as of two individual monkeys followed through time clearly elucidate not only the natural history of type 2 diabetes in this colony of monkeys, but also separates the effects of disease from natural aging. While onset of diabetes may vary from one monkey to another, the trajectory towards developing the disease is clearly defined. **Figure 9** presents the change in both fasting glucose and insulin with aging among normoglycemic subjects who never developed diabetes in their lifetime, and among the monkeys who eventually converted to type 2 diabetes. For this analysis, longitudinal data from 153 monkeys was used. Because we are able to clearly define symptoms of the metabolic syndrome or prediabetes, prodromal monkeys were not included in the analysis as they would not be truly "normal" from the perspective of glucose homeostasis. As can be seen, among the subjects who never developed diabetes in their lifetime, fasting plasma glucose

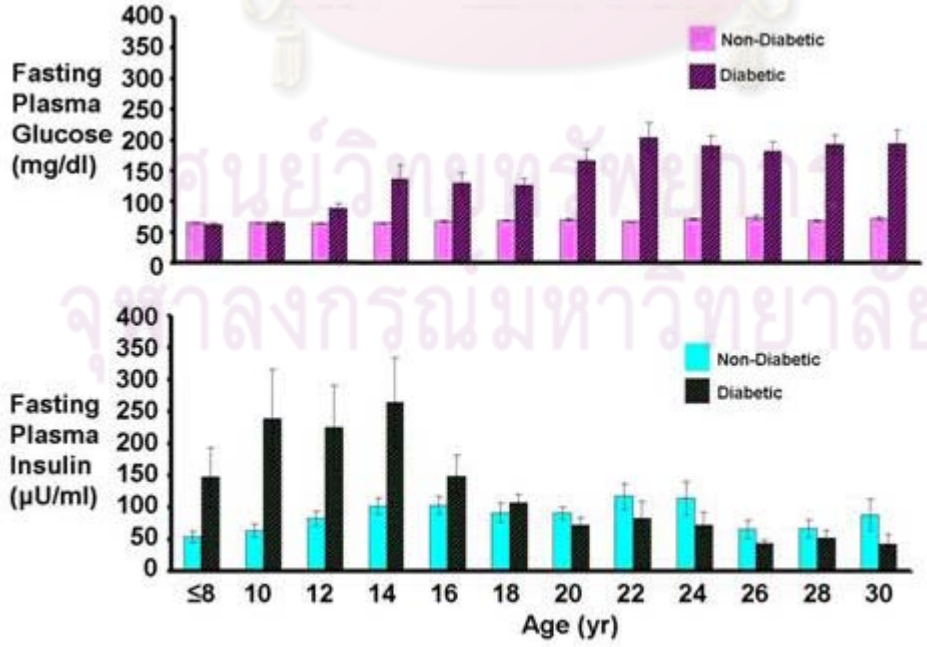


Fig. 9 Changes in fasting insulin and glucose with aging, based on lifetime longitudinal data from the colony (n=53). Values are presented as mean SE [14].

changed very little between ages 10 and 30 years old, whereas plasma glucose was already remarkably elevated by age 14 among those destined to become diabetic. Hyperinsulinemia, on the other hand, was already quite prominent at an early age among those who became diabetic, with fasting insulin values starting to deteriorate between ages 14- 20, the age at which many of the monkeys enter into the prediabetic phase.

Figure 10 displays the data from two monkeys studied prospectively over 20 years. Similar to the findings from the colony data, fasting plasma glucose remains virtually unchanged in the nondiabetic subject, whereas a steep upward trajectory is observed in the diabetic subject sometime during monkey middle-age. In strong agreement with the findings from the combined colony data, one observes the inverted U-shaped curve of the fasting plasma insulin values of the diabetic animal, with the downward arm coinciding with the increase in fasting glucose and eventual conversion to type 2 diabetes.

Again using the longitudinal data from the colony, one appreciates that glucose intolerance as assessed by the $K_{glucose}$ progresses dramatically among diabetic subjects (**Fig. 11**), whereas the age-matched normoglycemic control group experiences only a mild decline. Furtheremore, the decline in $K_{glucose}$ is evident as early as 12 years of age, many years before diagnosis of overt diabetes. In never-diabetic subjects, insulin sensitivity was preserved even in senescence, as can be seen from the graph.

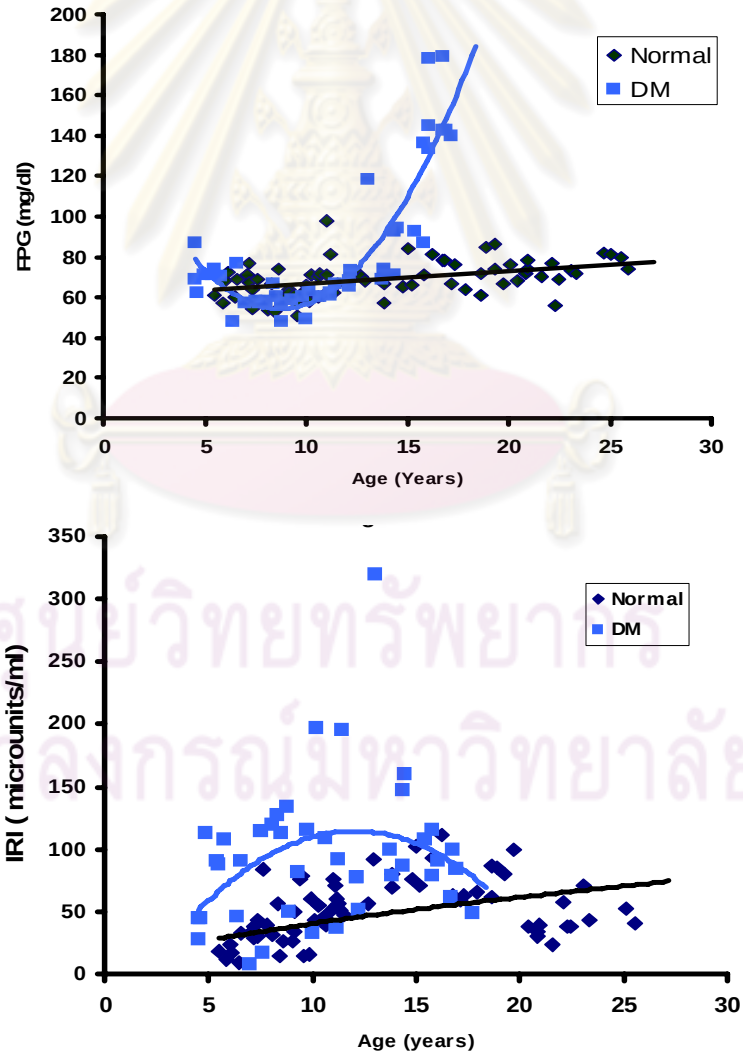


Fig. 10 Fasting plasma glucose (FPG) *vs* age (upper figure) and fasting (immunoreactive) insulin (IRI) *vs* age (lower figure) in healthy (Normal) and diabetic (DM) monkeys. These data demonstrate the changes in glucose and insulin levels with progression of the disease in the diabetic subject, compared to values in age-matched nondiabetic subject [14].

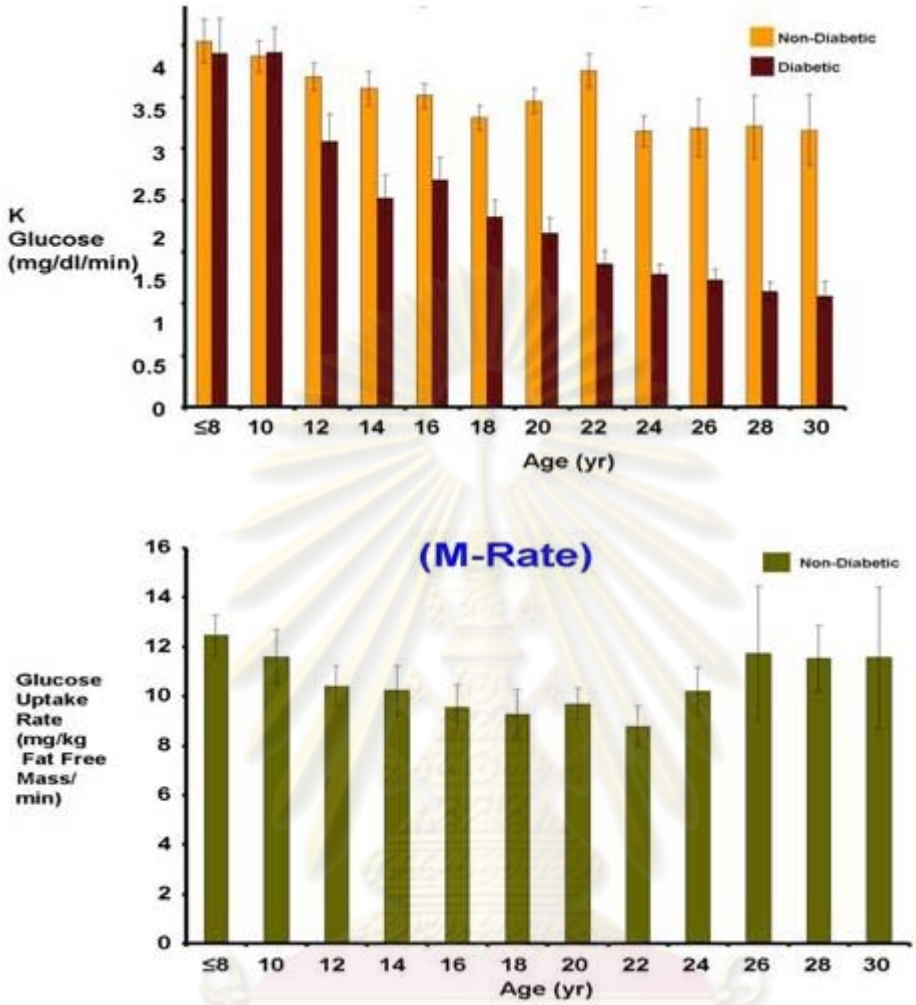


Fig. 11 Glucose tolerance, measured as $K_{glucose}$, deteriorates rapidly among diabetic monkeys, and is observable even at an early age, whereas the decay is gradual among the nondiabetic subjects. Insulin sensitivity undergoes only very gradual changes with aging, as seen from the data from the healthy monkeys. Values are presented as mean SE [14].

Very similar to the observations from the population data, the data from the two individual monkeys observed longitudinally confirm that deterioration of both glucose tolerance and insulin sensitivity are modest in the nondiabetic monkey, but very dramatic in the diabetic individual, even taking into account the aging process (**Fig. 12**).

Apart from glucose homeostasis, dysregulation of lipid homeostasis is known to occur with progression of type2 diabetes. However, it is unclear to what degree this may be due to aging, as human studies suggest circulating lipid values increase with age. Compelling evidence is seen from our longitudinal colony data, which visibly illustrates that the onset of dyslipidemia is prior to hyperglycemia (**Fig. 13**). Apart from total triglycerides, VLDL-C and VLDL-TG are elevated very early during the disease process, as early as 4-6 years before conversion to the overt diabetic state. In the light of current knowledge proposing an etiologic link between obesity and type 2 diabetes via production of adipocytokines which impair insulin sensitivity and/or interfere with insulin signaling pathways, monitoring of lipid parameters becomes increasingly important. As with glucose homeostasis, truly normal monkeys experience very little variation in lipid profile after maturation, suggesting that dyslipidemia is a consequence of an underlying pathology rather than aging per se. Recent cross-sectional analysis of circulating lipoprotein fractions also fail to substantiate any age-related elevation with aging (data presented at the American Aging Association meeting, 2007). On the other hand, glucose levels and sex, but not age, were found to be significant determinants of circulating total triglycerides, total cholesterol, HDL, LDL, IDL and VLDL, further at testing that dyslipidemia is more closely associated with disease rather than aging.

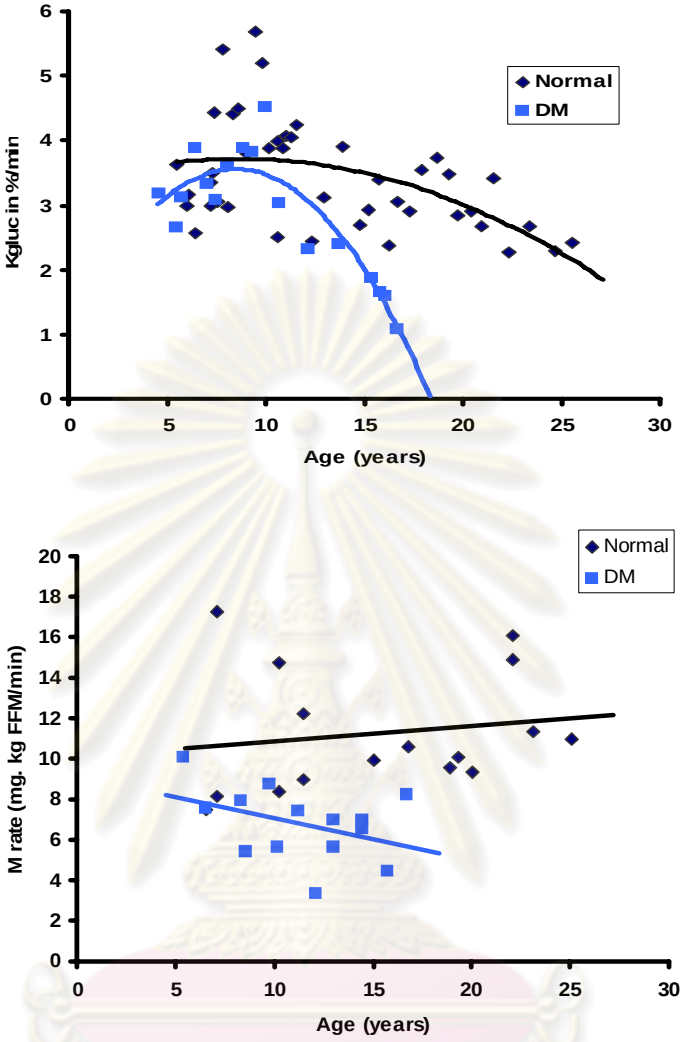
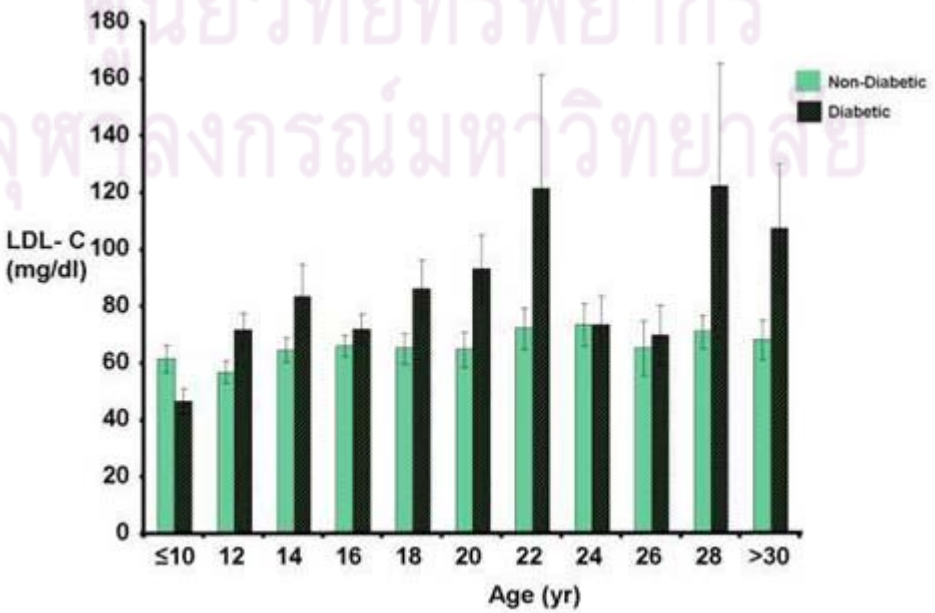


Fig. 12 $K_{glucose}$ *vs* age (upper figure) and insulin sensitivity (M rate) *vs* age (lower figure) in healthy (Normal) and diabetic (DM) monkeys. The declines in glucose tolerance and insulin sensitivity show faster deterioration in the diabetic subject with age [14].



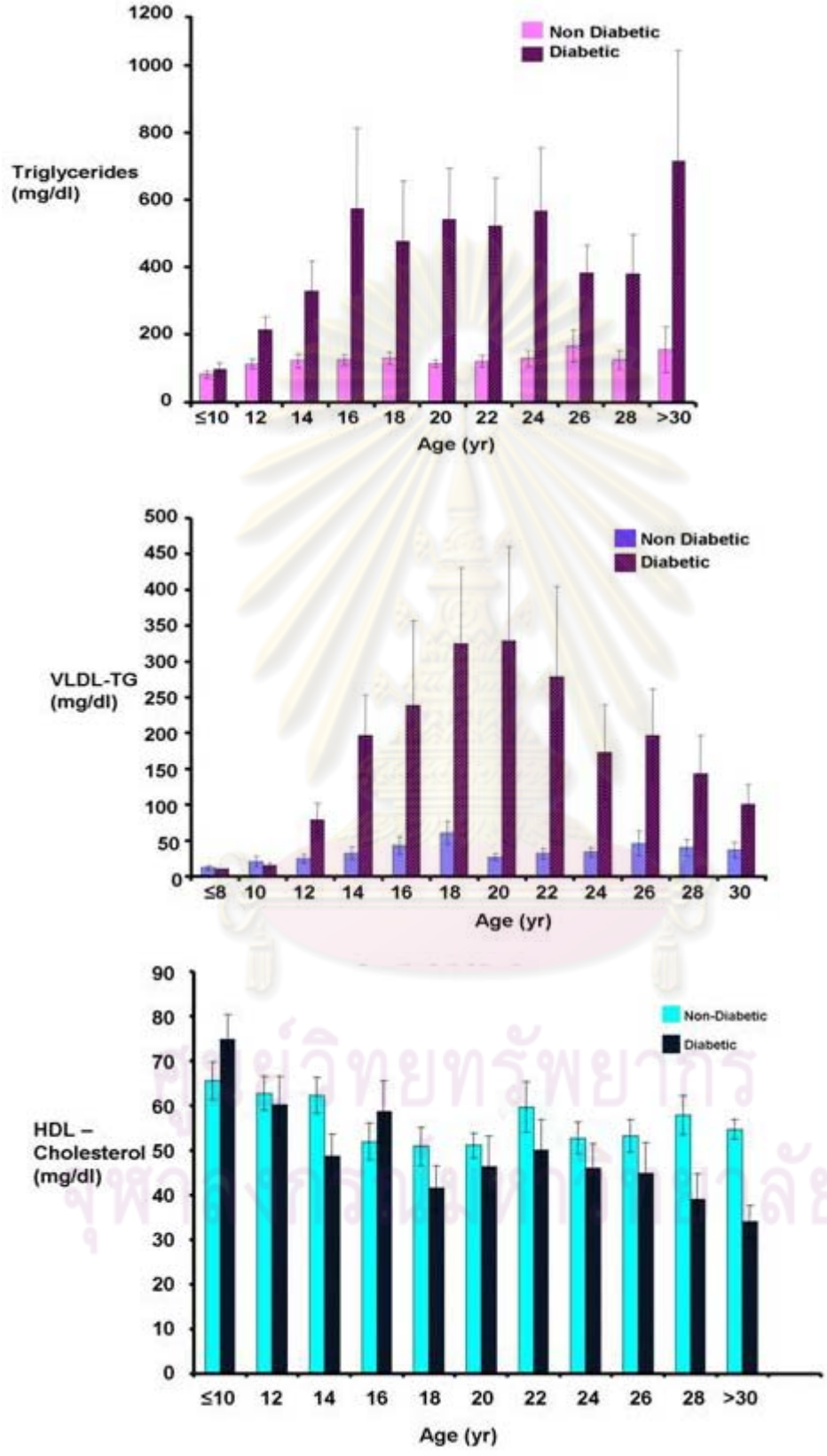


Fig. 13 The lipid profile, based on prospective data from the nondiabetic and diabetic monkeys in the colony (n=153), confirm the presence of dyslipidemia preceding conversion to type2 diabetes. Values are presented as mean SE [14].

Biomarkers of aging

Individuals senesce at variable rates, so that biomarkers have been constantly sought that would represent changes that may be attributed to chronologic age alone. One such biomarker is cortisol. Elevated cortisol has been suggested to be associated with many degenerative changes in aging [22-25]. Aging and the repeated stresses of life are believed to result in defective feedback mechanisms acting on the hypothalamic-pituitary- adrenal cortex axis, resulting in either elevated levels or delayed return to baseline values [26]. We have performed both longitudinal and cross-sectional analyses of cortisol levels among monkeys in the colony. In a subset of 138 rhesus monkeys, aged 4- 40 years, for which cortisol determinations were available, cross-sectional analysis demonstrated a modest increase in levels ($r = 0.20$, $p < 0.05$) with aging [13]. However, age accounted for only 4 % of the variance in cortisol. Longitudinal analysis of data from 30 subjects for which at least 10 years of serial determinations was available showed a smaller ($r = 0.16$) but significant correlation. Again, age accounted for only 3 % of the variance. Surprisingly, calorie-restricted animals showed the strongest correlation with age ($r = 0.37$) whereas age-related increases occurred in only 33 % of the diabetic primates. Furthermore, analysis of individual monkeys also demonstrated that significant declines in cortisol levels with age occurred, but only among the diabetic monkeys.

Among candidate biomarkers, dehydropeian-drosterone sulfate (DHEAS), a hormone abundantly secreted by the adrenal cortex, and the precursor of both androgens and estrogens, is said to be robust. Purportedly, secretion of both dehydroepiandrosterone (DHEA) and its sulfate, DHEAS, decline with age in both human and non-human primates. We performed assays on 165 plasma specimens obtained longitudinally from 25 rhesus monkeys consisting of 8 normal, 6 calorie-restricted, 6 prediabetic and 5 diabetic, with ages ranging from 7.7 to 33.8 years. Using values from the entire population, DHEA decreased slightly but not significantly with age, whereas DHEAS increased significantly. When the different groups were analyzed, the normal group tended to show a significant increase of DHEA levels with aging, whereas the calorie- restricted showed a significant decline with age. Prediabetic monkeys also showed declining levels with age, whereas diabetics demonstrated a slight and non-significant increase. Individual monkeys also failed to show consistent increases or decreases with aging, thus arguing against the use of either DHEA or DHEAS as reliable biomarkers.

Both pituitary growth hormone secretion and its mediator, insulin-like growth factor 1 (IGF1), decline with age [27]. Primary IGF1 deficiency due to primary growth hormone resistance or insensitivity (Laron syndrome) demonstrates early aging characteristics, including thinning and wrinkling, obesity, osteoporosis and hyperlipidemia. Basal plasma IGF1 concentrations were also found to be strongly and negatively correlated with age in our monkeys, with an $r = - 0.66$, $P < 0.001$ [28], providing evidence that aging may be a state of IGF1 deficiency. Furthermore, IGF1 levels were also found to be positively and significantly correlated to glucose tolerance ($r = 0.59$, $P < 0.001$).

Apart from the above-mentioned biomarkers, we have found that several cytokines exhibit strong correlations with age, including C-reactive protein (CRP) and Serum Amyloid A (SAA).

Diabetic complications as observed in monkeys

Our observations on the monkeys demonstrate that all the complications of diabetes in humans are also present in the monkeys. These include such complications as retinopathy [29, 30], neuropathy [21, 31], nephropathy [32] and other microvascular derangements [20]. The high degree of resemblance between monkey and human diabetic symptoms and complications makes the rhesus model far superior to any other model, natural or induced, for type 2 diabetes, and the most appropriate one for studies related to diabetic complications.

Findings in the our diabetic monkeys show that as early as two years after onset of hyperglycemia, significant reduction of motor conduction velocities, as measured from the peroneal, median and ulnar nerves, could be observed, while F-wave latencies were increased [31]. Since distal peripheral nerve endings are affected much earlier than the larger nerves, we recently did extensive immuno-flourescent labeling of distal nerve endings from skin punch biopsies at various sites of the monkeys' hands, feet and thigh areas, and using monkeys at different stages of diabetic progression. Our findings show alterations in both the density as well as neurotransmitter/ receptor types of the A-delta and C-fibers with progression of diabetes, together with significant

changes in the vascular network in the dermal layers (manuscript in preparation).

Diabetic retinopathy in our colony has been previously described [29, 30, 33]. Intraretinal hemorrhages and areas of retinal capillary nonperfusion as well as decline in numbers of photoreceptor segments have been observed using both histopathological and *in vivo* color duplex Doppler ultrasound [30, 33]. Furthermore, severity of diabetic retinopathy in the retinal vasculature was found to be associated with increased numbers of neutrophils, implicating neutrophils in the capillary loss in the diabetic retina [29].

The rhesus monkey model also closely mimics the rich complexity of diabetic nephropathy as it appears in the human condition, thus providing a very valuable model for studies relating the influences of aging, obesity, and insulin resistance on development of kidney dysfunction in overt diabetes. We have previously reported that the spontaneously diabetic monkey exhibits multiple features of human diabetic renal changes, including hyperfiltration and hypertrophy, and proteinuria [32]. Recently we have observed glomerular, tubular, and interstitial lesions with features closely overlapping those that are described for type 2 diabetic patients. These include, for example, expansion of the mesangial matrix, glomerular basement membrane thickening, tubular atrophy, and focal and global glomerulosclerosis. This animal model is clearly appropriate for investigating the complex sequence of events in the kidneys as the subject transitions from normal to obese to prediabetic/metabolic syndrome to diabetic, and provides the advantage of testing specific pathogenetic hypotheses of diabetic nephropathy.

One of these hypotheses is that an increase in renal sterol regulatory element binding protein (SREBP) expression and activity may play a pathologic role in the progression of diabetes-related renal disease, through actions on lipid synthesis, profibrotic growth factors and cytokines. Silent Information Regulator (SIR) proteins or Sirtuins, on the other hand, may attenuate diabetic kidney disease. Preliminary data from our laboratory, using renal biopsies obtained from normal, metabolic syndrome and overtly diabetic monkeys, revealed that renal mRNA expression of SIRT-1 and SIRT-3 was depressed in primates with diabetes. Furthermore, renal mRNA expression of SIRT-1 and SIRT-3 were found to be inversely and significantly correlated with fasting glucose. SIRT-3 mRNA levels were also inversely correlated with HbA1C, triglycerides and albumin excretion rate, suggesting that Sirtuins are important regulators of metabolic processes in diabetes. Since SIRT-1 and SIRT-3 levels were inversely correlated with SREBP in the kidneys of primates with diabetes, these results suggest that sirtuins may act by preventing the increased expression and activity of SREBP-1.

Future directions and opportunities for translational research

The ability to characterize the metabolic syndrome/prediabetic phase provides the advantage that interventions, including dietary modifications and therapeutic agents, can be initiated early in the course of the disease so that further progression into overt diabetes may be averted. This in fact has been confirmed through our experience whereby an intervention such as weight-stabilization through caloric restriction demonstrated an increase in average lifespan, decreased morbidity, improvement of insulin sensitivity and prevention of diabetes [16, 34, 35]. Apart from these, various studies performed in the laboratory on testing novel pharmaceutical agents have proven that the response of the nonhuman primates are highly predictive of the outcomes in humans, and in some occasions have been contrary to previous results in rodents. Many of these agents have been shown to reverse some of the symptoms and metabolic indices which are associated with worsening glucose and lipid status. Some of these agents include ligands of the Peroxisome Proliferator-Activator Receptors [19, 36-39], exendin-4 [40], and lately Fibroblast Growth Factor-21 [41]. As new agents and technologies for the treatment of diabetes emerge, we expect even greater demand for the use of the spontaneously diabetic nonhuman primate model for preclinical studies. In the very near future, we expect to make major contributions in the field of stem cell research and islet cell transplantation, in the quest for understanding diabetes and discovering its cure.

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