Rhesus Macaques Develop Metabolic Syndrome With Reversible Vascular Dysfunction Responsive to Pioglitazone

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Background—The metabolic syndrome (MetS) is a constellation of clinical features that include central obesity, hypertension, atherogenic dyslipidemia, and insulin resistance. However, the concept remains controversial; it has been debated whether MetS represents nothing more than simultaneous co-occurrence of individual risk factors or whether there are common shared pathophysiological mechanisms that link the individual components.

Methods and Results—To investigate the emergence of metabolic and cardiovascular components during the development of MetS, we identified MetS-predisposed animals (n = 35) in a large population of rhesus macaques (Macaca mulatta, 12.7 ± 2.9 years old, n = 408), acclimated them to standardized conditions, and monitored the progression of individual component features over 18 months. In 18 MetS animals with recently developed fasting hyperinsulinemia, central obesity, hypertension, and atherogenic dyslipidemia, we found that individual metabolic and cardiovascular components track together during the transition from pre-MetS to onset of MetS; MetS was associated with a 60% impairment of flow-mediated dilation, establishing the mechanistic link with vascular dysfunction. Pioglitazone treatment (3 mg/kg body weight/d for 6 weeks), a peroxisome proliferator–activated receptor γ agonist, reversibly improved atherogenic dyslipidemia and insulin resistance and fully restored flow-mediated dilation with persistent benefits.

Conclusions—Coemergence of metabolic and cardiovascular components during MetS progression and complete normalization of vascular dysfunction with peroxisome proliferator–activated receptor γ agonists suggest shared underlying mechanisms rather than separate processes, arguing for the benefit of early intervention of MetS components. Predictive nonhuman primate (NHP) models of MetS should be highly valuable in mechanistic and translational studies on the pathogenesis of MetS in relation to cardiovascular disease and diabetes mellitus. (Circulation. 2011;124:77-86.)

Key Words: metabolic X syndrome ■ primates ■ cardiovascular diseases ■ pathogenesis ■ peroxisome proliferator-activated receptors ■ agonists

The metabolic syndrome (MetS) is a constellation of clinical features that include central obesity, hypertension, atherogenic dyslipidemia, and insulin resistance (IR), as well as systemic inflammation and hypercoagulability. The prevalence of MetS is increasing dramatically worldwide. By age 60, up to 40% of the US population has MetS, and the prevalence in developing countries like China and India is catching up rapidly. MetS increases the risk of developing cardiovascular disease 2 fold, and the risk of type 2 diabetes mellitus (T2D) 5 fold. Most definitions of MetS require the presence of ≥3 of the 5 component features: central obesity, hypertension, low high-density lipoprotein levels, high triglyceride levels, and hyperglycemia. A recent joint statement from the International Diabetes Federation, National Heart, Lung, and Blood Institute, the American Heart Association, the World Health Organization, the International Atherosclerosis Society, and the International Association for the Study of Obesity has harmonized the various definitions, addressing concerns about multiple definitions and taking into account racial and ethnic differences in body habitus.
The concept of the MetS has been debated since its inception, and continues to be controversial.6,12–15 On the one hand, the MetS concept is important because it identifies a subgroup of patients at increased risk of cardiovascular disease who may benefit from intensive medical and lifestyle interventions to lower such risk.1,5,14–17 Furthermore, it is generally accepted that each individual component of MetS is mechanistically linked to the development of atherosclerotic cardiovascular disease.18 On the other hand, there are several arguments that the MetS concept has limited utility from the perspective of diabetes mellitus.13,15 First, the risk of cardiovascular disease is no greater with the simultaneous clustering of components of MetS (hypertension, dyslipidemia, and glucose intolerance) than the sum of each risk. Thus, the question arises of what additional information MetS provides for predicting or managing cardiovascular risk over considering each component risk separately. Second, it is not known whether MetS patients all share common pathophysiological mechanisms. Such unifying underlying mechanisms would have important implications for clinical care. For example, it is not known whether pharmacological therapy to reduce IR would be beneficial to reduce cardiovascular disease in MetS. Third, a better understanding of the pathogenesis of MetS and its development and progression is needed.

These considerations are more than semantic. They carry important clinical implications for patient care. They are also relevant to the current regulatory landscape for diabetes drugs; although cardiovascular disease is the major cause of death in patients with diabetes mellitus, meta-analysis reveals that treatment of diabetes mellitus with rosiglitazone is associated with higher cardiovascular mortality.19 Furthermore, several large clinical outcomes studies fail to demonstrate a relationship between glycemic control and cardiovascular disease or death.20,21 These findings have led to the current US Food and Drug Administration position that diabetes drugs must be evaluated for their effects on cardiovascular end points and not simply for efficacy in glycemic control.22,23

In this setting, we developed a nonhuman primate (NHP) model of MetS. Phylogenetically, NHPs are more similar to humans than other models in terms of lipoprotein profiles, pathogenesis of atherosclerotic disease, and genetic makeup.24–27 In their pioneering work, Hansen et al demonstrated that, along with aging, some rhesus monkeys spontaneously develop obesity, followed by IR and T2D.28–31 In the present study, we established a rhesus monkey model of spontaneous MetS using population screening approaches. By directly observing the development of MetS in these rhesus monkeys, we were in a position to gain unique insights into the early pathogenesis of MetS and vascular complications. In addition, we investigated the metabolic and vascular responses of this model to an established pharmacological treatment for diabetes mellitus.

**Methods**

**Screening Design**

The present study was approved by the Institutional Animal Care and Use Committee of Peking University and was in accordance with the
follow-Up Tests

Monkey Husbandry

Sixty selected monkeys from the screening were brought to the Association for Assessment and Accreditation of Laboratory Animal Care International–accredited animal facility at Peking University where they were housed individually in cages. The monkeys had free access to water and were fed ad libitum with pellet monkey chow (Beijing HFK Bio-Technology Co Ltd, China), which contains 7% to 10% crude fat, 16% to 20% crude protein, and 55% to 65% crude carbohydrate.

Blood Sampling and Biochemical Tests

The blood samples were taken from a vein after 14 to 16 hours fasting and anesthesia with ketamine at 10 mg/kg BW. All measurements of plasma lipids and glucose were performed at the Department of Clinical Biochemistry of 301 Hospital, Beijing, China, using kits from Roche. Insulin was measured by Roche Modular Analytics E170 Combinations (Cobas 12017547 122). C-reactive protein was measured by Roche Modular PE (Roche 03002012 122). C-peptide and proinsulin were measured by ELISA (Mercodia 10 to 1118-01, Mercodia 10 to 1136-01), and other measurements were performed by a HITACHI 7600-110 auto-analyzer.

Intravenous Glucose Tolerance Test

An intravenous glucose tolerance test (IVGTT) was performed as described previously. Insulin resistance was calculated by means of the homeostasis model assessment of insulin resistance (HOMA-IR): FPG (mmol/L)/22.5. For glucose disappearance constant (KG), KG=[ln(glucose level at 5 minutes)–fasting insulin (mU/L)]/[ln(glucose level at 20 minutes)]/15 minutes×100%, where ln refers to natural logarithm.

Brachial Artery Flow-Mediated Dilation Measurement

Flow-mediated dilation (FMD) was performed using a protocol modified from that for humans. In brief, monkeys were anesthetized by ketamine at 14 mg/kg BW (a higher dose to reduce motion artifacts during measurement) after overnight fasting. Brachial artery images were acquired at baseline, 1 minute after occlusion, and also 1, 2, 3, 4, and 5 minutes after cuff deflation. Image was analyzed using custom-developed edge detection software. FMD was calculated as (Dmax–DBL)/DBL×100%, where DBL is the baseline brachial artery diameter and Dmax is the maximum diameter after cuff release.

Validation Study With Pioglitazone

Twelve obese MetS rhesus monkeys that were cooperative for oral drug administration underwent a baseline phase with vehicle administration for 2 weeks followed by 6 weeks of treatment with pioglitazone at an oral dose of 3 mg·kg⁻¹·d⁻¹, followed by a 6-week washout period. Metabolic anthropometric parameters and BP, IVGTT, and FMD were measured at baseline, after 3 and 6 weeks of pioglitazone treatment, and at the end of washout.

Statistical Analysis

All data presented here are expressed as mean±SEM unless specified otherwise. Student t test and 2-way ANOVA with repeated measures followed by the Bonferroni post hoc correction were used to compare the differences between groups when appropriate. A P value <0.05 was considered statistically significant.

Results

Overall Strategy

In order to establish a spontaneous NHP model of MetS, we first screened a large population of rhesus macaques using the 5 defining parameters of MetS (WC, BP, FPG, TG, and HDL-c). We selected animals in the top quintile of the above mentioned parameters as most predisposed to MetS and accustomed them under well controlled laboratory conditions to observe the progression of each parameter. After 7 time points in laboratory tests (T1 through T7) over 18 months, we identified MetS animals. We characterized vascular function by measuring FMD of the brachial artery. Finally, the MetS model was subjected to a clinically relevant drug treatment to validate for its predictive power and gain insight into the relationship between MetS and cardiovascular dysfunctions. Our overall procedure and the age distributions in different groups are shown in Figure 1.

Screening and Acclimation

We performed field screening of 408 male rhesus monkeys 12.7±2.9 (mean±SD) years of age. Most metabolic parameters, including TG, HDL-c, LDL-c, and insulin, were lower than previously reported in laboratory rhesus monkeys at comparable ages. This pattern closely resembled findings in monkeys on caloric restriction. Notably, none of the original monkeys screened could be classified as MetS using the criteria that were used later in this study. Table 1 and

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**Table 1. Metabolic and Anthropometric Parameters in Screened Rhesus Monkeys**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>All (n=408)</th>
<th>MetS-Predisposed (n=35)</th>
<th>Potential Control (n=25)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>12.65±0.14</td>
<td>13.22±0.50</td>
<td>13.75±0.62</td>
<td>NS</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>124.41±1.78</td>
<td>143.99±4.75</td>
<td>118.92±3.48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>79.86±1.10</td>
<td>91.29±3.24</td>
<td>79.84±2.49</td>
<td>0.007</td>
</tr>
<tr>
<td>WC, cm</td>
<td>33.65±0.22</td>
<td>38.38±0.79</td>
<td>35.49±1.14</td>
<td>0.035</td>
</tr>
<tr>
<td>BW, kg</td>
<td>9.76±0.11</td>
<td>11.98±0.37</td>
<td>10.26±0.46</td>
<td>0.005</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>0.38±0.01</td>
<td>0.40±0.03</td>
<td>0.34±0.05</td>
<td>NS</td>
</tr>
<tr>
<td>HDL-c, mmol/L</td>
<td>1.18±0.02</td>
<td>1.20±0.05</td>
<td>1.31±0.07</td>
<td>NS</td>
</tr>
<tr>
<td>LDL-c, mmol/L</td>
<td>1.17±0.02</td>
<td>1.31±0.10</td>
<td>1.20±0.07</td>
<td>NS</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>2.65±0.04</td>
<td>2.62±0.13</td>
<td>2.62±0.11</td>
<td>NS</td>
</tr>
<tr>
<td>FPG, mmol/L</td>
<td>3.44±0.04</td>
<td>3.68±0.11</td>
<td>3.30±0.13</td>
<td>0.028</td>
</tr>
<tr>
<td>Fasting insulin, μU/ml</td>
<td>15.94±0.90</td>
<td>19.00±3.14</td>
<td>19.73±3.81</td>
<td>NS</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.54±0.15</td>
<td>3.12±0.46</td>
<td>3.10±0.66</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are mean±SEM unless otherwise indicated.
MetS indicates metabolic syndrome; NS, not significant; P<0.1; SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference; BW, body weight; TG, triglyceride; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; TC, total cholesterol; FPG, fasting plasma glucose; and HOMA-IR, homeostasis model assessment for insulin resistance. P values are for MetS-predisposed versus potential control.
Table 2. Metabolic, Anthropometric, and Inflammatory Parameters in MetS and Control Monkeys

<table>
<thead>
<tr>
<th>MetS–T7 (n=18)</th>
<th>Control–T7 (n=17)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>14.58±0.47</td>
<td>14.89±0.74</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>160.00±7.08</td>
<td>141.41±4.97</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>89.56±4.86</td>
<td>75.18±2.98</td>
</tr>
<tr>
<td>WC, cm</td>
<td>52.14±2.35</td>
<td>41.97±2.49</td>
</tr>
<tr>
<td>BW, kg</td>
<td>16.39±0.98</td>
<td>12.27±0.98</td>
</tr>
<tr>
<td>TG, mmol/L</td>
<td>1.04±0.15</td>
<td>0.58±0.05</td>
</tr>
<tr>
<td>HDL-c, mmol/L</td>
<td>1.56±0.09</td>
<td>2.13±0.12</td>
</tr>
<tr>
<td>LDL-c, mmol/L</td>
<td>1.33±0.14</td>
<td>1.11±0.07</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>3.31±0.19</td>
<td>3.50±0.16</td>
</tr>
<tr>
<td>FPG, mmol/L</td>
<td>4.46±0.21</td>
<td>3.90±0.10</td>
</tr>
<tr>
<td>Fasting insulin, μU/ml</td>
<td>58.93±15.82</td>
<td>18.54±3.57</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>12.31±3.60</td>
<td>3.18±0.58</td>
</tr>
<tr>
<td>CRP, mg/dL</td>
<td>0.17±0.03</td>
<td>0.09±0.02</td>
</tr>
</tbody>
</table>

Values are mean±SEM unless otherwise indicated. MetS indicates metabolic syndrome; T7, test 7; NS, not significant; P<0.1; SBP, systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference; BW, body weight; TG, triglyceride; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; TC, total cholesterol; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment for insulin resistance; and CRP, C-reactive protein. P values are for MetS versus control in test 7, respectively.

We sought to identify the animals most predisposed to MetS using the following criteria. Monkeys that met 3 out of the following 5 criteria: (1) BP ≥120/75 mm Hg, (2) WC ≥37 cm, (3) FPG ≥3.8 mmol/L, and (4) TG ≥1.0 mmol/L, above the 80th percentile, and (5) HDL-c ≤1.0 mmol/L, below the 20th percentile, were considered MetS predisposed. Potential control monkeys were chosen among individuals that met 3 out of the following 5 criteria: (1) BP ≤100/64 mm Hg, (2) WC ≤31 cm, (3) FPG ≤3.27 mmol/L, and (4) TG ≤0.22 mmol/L, all below the 25th percentile, and (5) HDL-c ≥1.41 mmol/L, above the 75th percentile.

Using these screening criteria, we identified a total of 35 (8.6%) MetS-predisposed monkeys, along with 25 potential controls. As expected by the way they were selected, MetS-predisposed monkeys showed systolic BP, diastolic BP, FPG, WC, and BW significantly higher than those seen in control potentials. There were no differences between the groups with respect to TG, HDL-c, LDL-c, TC, or insulin (Table 1).

After being brought to the Laboratory Animal Center at Peking University, all 60 monkeys were allowed free access to pellet monkey chow after an initial 3 months of acclimation. We then performed 7 in-laboratory tests during a 1.5-year period. Four potential control monkeys were excluded because of hypersensitivity to ketamine or chronic diarrhea.

Identification of Rhesus Monkeys With Metabolic Syndrome

We set forth the following criteria for MetS and control monkeys: (1) WC ≥40 cm and waist/hip ratio ≥0.9, (2) FPG ≥4.40 mmol/L, (3) TG ≥0.90 mmol/L, (4) HDL-c ≤1.55 mmol/L, and (5) BP ≥130/80 mm Hg. Individuals displaying ≥3 components were classified as MetS monkeys whereas those displaying none or only 1 component were classified as control monkeys. The rest, which manifested 2 MS components, were classified as at-risk monkeys.

By this set of criteria, using the values from T7, we identified 18 MetS, 17 control, and 21 at-risk monkeys. Of these, 17 out of 18 MetS monkeys (94%) were from the original MetS-predisposed group whereas 10 out of 17 control monkeys (59%) were from the original potential control group. Monkeys in all groups were of comparable age (Table 2).

Metabolic Characterization of Metabolic Syndrome in Rhesus Monkeys

Compared with controls, MetS monkeys exhibited clear symptoms of obesity, with greater BW and WC. Monkeys with MetS showed evidence of IR, with markedly higher insulin levels but comparable glucose levels, with resulting increased levels of the HOMA-IR index. Monkeys with MetS...
showed elevated TG and lower HDL-c than control animals whereas TC and LDL-c were not significantly different. These changes are summarized in Table 2.

We performed IVGTT to assess the metabolic response to glucose and insulin sensitivity. As seen in Figure 2A, FPG and plasma glucose level 5 minutes after the glucose challenge were higher in the MetS group (Student t test \( P < 0.05 \)). The glucose-induced rise of insulin level showed a trend of increase in the MetS group at multiple time points (Figure 2B). The ratio between insulin and glucose at 10 minutes, an index of insulin sensitivity, was higher in the MetS group (Figure 2C), as was the area under the insulin curve (AUCinsulin) (0 to 10 minutes) (Figure 2D). These measures of insulin sensitivity parallel the HOMA-IR index (Figure 2E).

Taken together, the current NHP model of MetS reproduces virtually all salient features of MetS in humans. These results lend support to the strategy of first identifying NHP most predisposed to MetS, followed by acclimation to standardized conditions with dietary liberalization for cohort stratification. Notably, none of the MetS monkeys have FPG levels above the cutoff value of 6.99 mmol/L for T2D in rhesus monkeys, suggesting that this NHP model represents an early stage in the onset of MetS preceding the development of T2D.

**Development and Progression of Metabolic Syndrome**

Because all our MetS monkeys evolved from a non-MetS status at the entry of this study, we were in a unique position to observe the development of MetS. We analyzed data from T1 to T7 and sought patterns in the emergence of individual components, component pairs, and component triads (i.e., MetS per se).

Of our 18 MetS monkeys, 1, 4, and 13 monkeys displayed 5, 4, and 3 components of MetS, respectively. The 2 most prevalent components were greater waist size (94%) and high BP (73%). Among 10 possible component pairs, the most common combination was waist size + elevated BP (67%), followed by waist size + high FPG (56%), waist size + low HDL (50%), and waist size + high TG (50%). The most common triads were waist size + elevated BP + high FPG (7/18), waist size + elevated BP + low HDL (6/18), and waist size + elevated BP + high TG (5/18). These patterns are similar to those found in humans.

In following the progression of these parameters in the development of MetS, we made several observations. First, WC and WB were greater in MetS than control animals at all 7 time points, from T1 to T7 (Figure 3A and 3B). Systolic BP was significantly higher in the MetS group than in control animals whereas diastolic BP was less well separated (Figure 3C). Second, both TG and HDL-c showed early variability...
related to acclimation but were not distinguishable between MetS and control animals until after 9 months. Eventually, MetS monkeys showed progressive increase in TG and decrease in HDL-c. In contrast, control animals showed stable TG and a rise in HDL-c (Figure 3D). TC and LDL-c levels in MetS and control groups converged to similar levels (Figure 3E), but the ratios of TC/HDL-c and LDL-c/HDL-c were higher in MetS than in control animals at all 7 tests (Figure 3F). Third, there was a clear increase of the insulin level during progression to MetS but not in control monkeys (Figure 3G). The FPG level increased in the MetS group whereas it remained stable in the control group, to an extent that there were significant differences at later time points (Figure 3H). Although not a defining parameter, CRP exhibited early and sustained high levels in the MetS group compared with the control group (Figure 3I).

Vascular Function in Rhesus Monkeys With Metabolic Syndrome
To study how development of MetS affects vascular function, we measured FMD in the brachial artery as a noninvasive assessment of endothelial function. As shown in Figure 4, compared with control monkeys (n = 11), FMD in MetS monkeys (n = 15) showed a markedly abbreviated duration and a 58.6% lower amplitude (Figure 4B and 4C), indicating overtly impaired endothelium-dependent vasodilatation. These results are consistent with previous reports that obesity, IR, and MetS are closely associated with endothelial dysfunction in humans, and support the clinical finding that loss of endothelial reactivity is an early event in progression of MetS.

Model Validation by Pioglitazone Treatment
In obese-IR rhesus monkey and in humans with MetS, the peroxisome proliferator–activated receptor γ agonist pioglitazone improves insulin sensitivity and β-cell function and lowers plasma TG. We evaluated the effect of pioglitazone in 12 MetS monkeys. As shown in Table 3 and Figure 5, pioglitazone significantly decreased TG levels and increased HDL-c (Figure 5A). Concurrently, proinsulin and C-peptide were significantly decreased (Figure 5B). By IVGTT, pioglitazone significantly decreased plasma glucose level in the first 5 minutes after the glucose challenge and glucose-induced insulin levels at multiple time points; the glucose and insulin curves were significantly lowered by pioglitazone (2-way ANOVA with repeated measures, Bonferroni corrected P value was 1.5e-4 or 7.9e-12 for glucose or insulin curve, respectively) (Figure 5C and 5D). The HOMA-IR was significantly decreased after pioglitazone treatment (Figure 5E), and the ratio between insulin and Δglucose (at 10 minutes) was also significantly lower (Figure 5F). The AUCinsulin (0 to 10 minutes) was significantly decreased by pioglitazone (Figure 5G) whereas glucose disappearance rate was unaffected (data not shown). Thus, a 6-week pioglitazone treatment corrected dyslipidemia, normalized glucose tolerance, and restored insulin sensitivity in MetS monkeys. Moreover, data at the end of washout showed that these effects of pioglitazone were largely reversible (Figure 5, Table 3). It is noteworthy that pioglitazone didn’t normalize the BP in the MetS monkeys (Table 3), which is consistent with the findings in MetS patients. The present results in onset of MetS are in good agreement with previous observations in rhesus monkeys with overt diabetes mellitus or severe hyperglycemia (FPG >8.3 mmol/L, 3 out of 6 monkeys) and severe dyslipidemia (highest TG values >17 mmol/L), except for a lowering of BP by pioglitazone in the diabetes model.

It was of interest to determine whether and to what extent pioglitazone affects endothelial reactivity in NHPs with MetS. Pioglitazone had a profound effect on both the duration and the amplitude of FMD, such that it completely restored normal endothelial responsiveness at 6 weeks into the treatment (Figure 5H and 5I). When compared to the durability of other pioglitazone effects mentioned above, it was remarkable that the pioglitazone effect on vascular function persisted for 6 weeks after drug washout (Figure 5H and 5I).

Discussion
Metabolic syndrome is important from a clinical and public health standpoint, both because of its sheer preva-
lence and its effect on risk for cardiovascular disease and T2D.5,6 The current major controversies over the concept are centered around (1) whether MetS represents a collection of independent cardiovascular risk factors, (2) whether there is shared underlying pathophysiology between the components, and (3) a need for better understanding how MetS predisposes to cardiovascular disease and T2D. The current NHP MetS model has implications for all of these issues.

By identifying 35 MetS-predisposed animals and 25 potential controls among 408 rhesus monkeys of 12.7 years age, we were in a unique position to track and observe how the individual MetS-related parameters evolved during this period under standardized conditions of diet and physical activity. Our data suggest that the MetS NHP share common underlying processes that track together during the transition from pre-MetS to onset of MetS rather than displaying independent cardiovascular risk factors. During the first 3 months of acclimation (T1 to T3), there appeared to be a resetting of metabolic homeostasis (Figure 3) in both control and MetS groups, likely because of a change in nutritional status and activity. Over time, BW and waist size increased at a faster rate in the eventual MetS monkeys compared with control animals; BP changed similarly in both groups, but the between-group difference remained largely unchanged (Figure 3C). Retrospective analysis of entry characteristics (data from T1) revealed that the group of monkeys that went on to develop overt MetS displayed greater BW and WC, higher BP, but similar insulin, glucose, HDL-c, and TG levels and HOMA-IR compared with control animals (Figure 3). This confirms that visceral obesity and hypertension are central to predisposition to MetS and its pathogenesis, in agreement with the rationale for the definitions of MetS.11

In contrast to BW, WC, and BP, the fasting insulin level increased in the MetS group from 4 months onward (T4) and rose sharply in the MetS monkeys at 18 months (T7) whereas it remained steady in the control animals. The fasting glucose levels were also higher in the MetS monkeys than controls, particularly at later time points, but were lower than the cutoff for T2D. Thus, the MetS monkeys maintain euglycemia by hyperinsulinemia, which is very similar to the development of T2D in humans.

Previous studies have shown that when rhesus monkeys are placed on caloric restriction with 30% less food, they exhibit significantly reduced BW, body mass index, body fat, and TG than free-feeding controls. They also show lower levels of insulin and plasma glucose and increased insulin sensitivity.36–38 Thus the monkeys screened at husbandry sites might have had similar caloric restriction because they were fed with limited rations (see the online-only Data Supplement), which prevented full expression of the MetS phenotype. In laboratory conditions, the total amount of food intake was relatively high in MetS (online-only Data Supplement Figure IIA). However, when the food intake normalized, BW was actually less in the MetS animals than in control monkeys (online-only Data Supplement Figure IIB). Thus, the divergence between the MetS and control animals was not due merely to a difference in food or caloric intake. Rather, it suggests that inherent genetic and metabolic predispositions interact with environmental factors of food intake and energy expenditure to determine the spontaneous emergence of MetS.

In this study, we applied the FMD technique to assess endothelial reactivity in NHPs for the first time. MetS monkeys showed a 60% decrease of FMD compared with control monkeys, confirming the mechanistic link between MetS and vascular dysfunction.18 Pioglitazone not only normalizes metabolic parameters but also restores

<table>
<thead>
<tr>
<th>Table 3. Effects of Pioglitazone Treatment on MetS Monkeys</th>
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<tr>
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<tr>
<td>SBP, mm Hg</td>
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<tr>
<td>DBP, mm Hg</td>
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<tr>
<td>MAP, mm Hg</td>
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<tr>
<td>BW, kg</td>
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<tr>
<td>TG, mmol/L</td>
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<tr>
<td>HDL-c, mmol/L</td>
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<td>LDL-c, mmol/L</td>
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<td>TC, mmol/L</td>
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<tr>
<td>FPG, mmol/L</td>
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<tr>
<td>Fasting insulin, µU/mL</td>
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<tr>
<td>HOMA-IR</td>
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<td>HbA1c, %</td>
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Values are mean±SEM unless otherwise indicated.

MetS indicates metabolic syndrome; PIO, pioglitazone; P1, 6-week PIO versus baseline; P2, 6-week PIO versus washout; SBP, systolic blood pressure; NS, not significant, P>0.1; DBP, diastolic blood pressure; MAP, mean arterial pressure; BW, body weight; TG, triglyceride; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol; TC, total cholesterol; FPG, fasting plasma glucose; HOMA-IR, homeostasis model assessment for insulin resistance; and HbA1c, hemoglobin A1c.
normal FMD after 6 weeks of treatment. These results suggest that early intervention in lipid and glucose metabolism will not only restore insulin sensitivity but also vascular function.

Despite efficacy in improving IR and glycemic control in humans, rosiglitazone appears to increase overall cardiovascular mortality in patients with T2D by meta-analysis of large clinical studies. In addition, the 2 largest randomized clinical trials, the United Kingdom Perspective Diabetes Study and the University Group Diabetes Program, fail to show a convincing link between glycemic control and reduction in cardiovascular events. Other large clinical trials, including the ACCORD (Action to Control Cardiovascular Risk in Diabetes) study, suggest against aggressive lowering of glycemia to near-normal levels in patients at high risk for cardiovascular disease. These findings suggest that by the time T2D is established, the processes underlying atherogenesis may have proceeded to such an extent that pharmacological treatment directed to IR or glycemic control may be too late. The improvement of FMD in the MetS rhesus monkey suggests that treatment of MetS before frank T2D develops may have profound effects on vascular function and risk for atherogenesis. Of note, the MetS animals are not diabetic on the basis of FPG levels, and by serial monitoring they only recently developed hyperinsulinemia at T7. The finding of persistent improvement despite washout of drug is an unexpected finding that would be fascinating to replicate in humans.

The availability of a primate model for MetS should accelerate both basic and translational research on MetS and cardiovascular disease. Hansen et al demonstrated that with age, some rhesus monkeys spontaneously develop obesity, followed by IR and T2D. These animals undergo a sequential set of metabolic phases, starting with fasting hyperinsulinemia, impaired glucose tolerance, then fasting hyperglycemia, followed by frank diabetes mellitus. The
current MetS rhesus monkey model described here would represent an early stage in this sequence, having just recently developed fasting hyperinsulinemia in the setting of central obesity, hypertension, and other dyslipidemias. For this reason, it should be a valuable tool for further research into mechanisms of MetS.

Compared with clinical studies in humans, unique features of the MetS NHP model include drug-naive status, ability to control and modulate nutritional composition and physical activity, and freedom from confounding risk factors such as smoking. In addition, a NHP provides better availability of tissue for mechanistic studies and the ability to perform translational and clinical trials that cannot be performed in humans. Unanswered questions that could be addressed with this NHP MetS model include: comparison of rosiglitazone with pioglitazone; efficacy of metformin, thiazolidinediones, and other T2D agents in reducing cardiovascular disease in MetS before T2D occurs; mechanisms by which agents such as rosiglitazone and torcetrapib result unexpectedly in overall increases in cardiovascular mortality; and mechanisms that underlie the lack of correlation between glycemic control and cardiovascular mortality.

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Disclosures

None.

References

The metabolic syndrome (MetS) is a constellation of clinical features that include central obesity, hypertension, atherogenic dyslipidemia, and insulin resistance and is clinically important both because of its prevalence and because it increases the risk for cardiovascular disease and type 2 diabetes mellitus (T2D). However, the concept remains controversial, and there is a need for better understanding of how MetS predisposes to cardiovascular disease and T2D. Here, we devised and implemented a strategy to establish a spontaneous nonhuman primates model of MetS, investigated the emergence of MetS components of MetS emerged together, indicating common shared underlying processes rather than simultaneous occurrence of independent risk factors. Importantly, vascular dysfunction (60% impairment of flow-mediated dilation of brachial artery) tracked with development of MetS. Pioglitazone, a peroxisome proliferator–activated receptor γ agonist, reversibly improved atherogenic dyslipidemia and insulin resistance and fully restored flow-mediated dilation with persistent effect, suggesting the benefit for early treatment of MetS before frank T2D develops. This unique nonhuman primate model of MetS, as demonstrated here, should be highly valuable in mechanistic and translational studies on the pathogenesis of MetS in relation to cardiovascular disease and T2D.
Rhesus Macaques Develop Metabolic Syndrome With Reversible Vascular Dysfunction Responsive to Pioglitazone

Xiuqin Zhang, Rongli Zhang, Susanne Raab, Wen Zheng, Jue Wang, Na Liu, Tiangang Zhu, Lifang Xue, Zhentao Song, Jiaming Mao, Kaitao Li, Huiliang Zhang, Yan Zhang, Chao Han, Yi Ding, Hui Wang, Ning Hou, Yuli Liu, Shujiang Shang, Chuanyun Li, Elena Sebokova, Heping Cheng and Paul L. Huang

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http://circ.ahajournals.org/content/suppl/2011/06/15/CIRCULATIONAHA.110.990333.DC1
Supplemental Methods:

Monkey Screening:

*NHP sources and housing condition.* The screening population included 408 male adult rhesus monkeys (Macaca mulatta) from three monkey husbandry sites in China, the Academic of Military Medical Science (AMMS), Beijing (n=60); Fangshan Breeding Facility, Institute of Beijing Xieer’xin Biology Resource, Beijing (n=98); and Guangxi Grandforest Scientific Primate Co., Ltd., Guangxi, China (n=250). All available male, healthy rhesus monkeys over 10 years of age were included in screening (except for a few younger ones in the first batch of screening). They were individually housed at least 6 months before screening, and were fed twice a day with pellet primate chow containing ~20% proteins and ~5% fat at 100 -150g/monkey/day, with free access to fresh water.

*Procedures.* After overnight fasting, rhesus monkeys were anesthetized with ketamine (ketamine hydrochloride injection, 2 ml: 0.1 g, Fujian Gutian, China; ~10 mg/kg body weight). Waist circumference was measured as the circumference of the abdominal right above the iliac crest. The hip circumference was measured as the length of the circumference of the tail base by the inguina. The body weight was measured by an electronic scale. Blood pressure was measured on one of the forearms by a mercury sphygmomanometer with the monkey supine. Blood was taken from a hind limb vein through an i.v. cannula, and the plasma was used for biochemical tests. Blood samples for HbA1c analysis were collected in vacutainer tubes containing EDTA, mixed well and kept on ice until measurement. For plasma samples, blood was collected in vacutainer tubes containing EDTA, mixed and kept on ice, and centrifuged for 10 min at 1200× g at 4°C within an hour. The plasma was carefully transferred into appropriately labeled sterile microcentrifuge tubes. All fresh plasma samples were stored at 4 °C before analysis or kept at -80°C if not analyzed immediately. All measurements of plasma lipids and glucose were
performed at the Department of Clinical Biochemistry of 301 Hospital, Beijing, China, using biochemical test kits from Roche.

**Supplemental Figure Legends:**

Supplemental Figure 1. Histogram distribution of selected parameters in the screened monkeys. The curves represent nonlinear fitting by Gaussian distribution.
Supplemental Figure 2: Food intake (A) and its normalization by body weight (B) in MetS and control monkeys.