Chapter 7

Changes in dietary fat content rapidly alter the mouse plasma coagulation profile without affecting relative transcript levels of coagulation genes

Audrey CA Cleuren, Vicky T Blankevoort,
Janna A van Diepen, Peter J Voshol, Pieter H Reitsma,
Bart JM van Vlijmen

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Summary

Background: Obesity is associated with a hypercoagulable state and increased risk for thrombotic cardiovascular events.

Objective: Establish the onset and reversibility of the hypercoagulable state during the development and regression of nutritionally-induced obesity in mice, and its relation to transcriptional changes and clearance rates of coagulation factors as well as its relation to changes in metabolic and inflammatory parameters.

Methods: Male C57Black/6J mice were fed a low fat (10% kcal fat; LFD) or high fat diet (45% kcal; HFD) for 2, 4, 8 or 16 weeks, and *in vivo* clearance rates of human factor (F) VII, FVIII and FIX proteins were determined after 2 weeks of HFD-feeding. To study the effects of weight loss, mice were fed the HFD for 16 weeks and switched to the LFD for 1, 2 or 4 weeks. For each time point plasma and hepatic mRNA analyses were performed after overnight fasting.

Results: HFD feeding gradually increased the body and liver weight, which was accompanied by a significant increase in plasma glucose levels from 8 weeks onwards, while insulin levels were affected after 16 weeks. Plasma levels of fibrinogen, FII, FVII, FVIII, FIX, FXI and FXII were significantly higher in mice on a HFD for 2 weeks, which in general persisted throughout the 16 weeks of HFD-feeding. Remarkably, the effects on plasma levels were in general not paralleled by changes in relative hepatic transcript levels and neither by decreased clearance rates. Switching from the HFD to the LFD reversed the HFD-induced procoagulant shift, but again this did not coincide with transcriptional modulation.

Conclusions: Changes in dietary fat content rapidly alter the mouse plasma coagulation profile, thereby preceding metabolic changes. In addition, these rapid changes could not be explained by changes in relative expression levels of coagulation genes or decreased clearance rates.

Introduction

The prevalence of obesity in the Western world is rising and forms an increasing public health problem since obesity affects, amongst others, the development of cardiovascular diseases through its influence on risk factors like hyperlipidemia, hypertension, glucose intolerance and inflammation. The risk for thrombotic cardiovascular events is even further enhanced by the hypercoagulable state that is associated with obesity, as obese subjects have increased plasma levels of procoagulant factor (F) VII, VIII, XII and fibrinogen, while fibrinolysis is decreased as reflected by increased levels of plasminogen activator inhibitor-1 (PAI-1).[1-3] On the other hand, levels of the anticoagulant factors protein C and protein S are higher, and tissue plasminogen activator (tPA) levels are lower under obese conditions, which might be considered to be a compensatory response to the hypercoagulable state.[4,5]

Previous studies evaluating the effect of weight loss on hemostatic parameters showed that levels of tissue factor, FVII, PAI-1 and tPA decreased upon weight loss, resulting in a decrease in thrombin generation.[6,7] In addition, it has been suggested that almost one-third of all thrombotic events could be prevented by weight loss.[8] Taken together, these data indicate that the plasma coagulation profile and the subsequent thrombotic risk may follow both the unfavorable and favorable changes in body weight gain and weight loss, respectively.

Using an experimental animal approach, we and others have previously shown that obesity in mice also results in a hypercoagulable state, which is characterized by increased plasma levels of procoagulant factors and decreased fibrinolysis.[9,10] These results were obtained in mice being on a high fat diet for 4 to 5 months, and during this time, many other metabolic changes may have occurred influencing the coagulation profile indirectly. Therefore, the aim of the present study was to identify whether changes in coagulation stand by itself or whether it is the result of other, earlier manifesting metabolic changes. Hereto, we used mice to establish the onset and reversibility of the hypercoagulable state during the development and regression of nutritionally-induced obesity, and determined its relation to changes in hepatic transcript levels and clearance rates of coagulation factors, as well as it relation to changes in metabolic and inflammatory parameters.

Materials and methods

Animals

Six week old male C57Black/6J mice (Charles River) were fed a diet with 10% kcal as fat (low fat diet; Research Diets) for 4 weeks as a run-in period, after which half of the group switched to an iso-caloric diet with 45% kcal as fat (high fat diet; Research Diets), while the other group remained on the low fat diet (LFD). After 2, 4, 8 or 16 weeks mice (n=15 per group) were fasted overnight and subsequently anesthetized with a mixture of ketamine, xylazine and atropine. The abdomen was opened and a blood sample on sodium citrate (final concentration of 0.32%) was directly drawn from the inferior caval vein. Platelet-poor plasma was obtained and stored at -80°C until use. In addition, part of the left liver lobule and lungs were snap-frozen for mRNA analyses.

In order to compare nutritionally-induced obesity with genetically-induced obesity, 6 weeks old *ob/ob* mice, and their lean wild-type littermate controls (Charles River) were fed the low fat diet for 4 weeks and plasma and tissue samples were obtained for analyses after overnight fasting.

Plasma clearance of the vitamin K-dependent coagulation factors VII and IX, and FVIII were determined in a separate experiment in which 2-week HFD-fed mice received a single intravenous injection (200 µl) of either the human prothrombinase complex (Cofact) or FVIII concentrate (Aafact, both kindly provided by Dr. K. Mertens, Sanquin). Clearance rates of the human plasma-derived factors from the mouse plasma were determined by successive blood sampling via the tail vein.

To study the effects of weight loss on the obese phenotype, mice received the HFD for 16 weeks, and while half of the group remained on the HFD (n=45), the other half switched to the low fat diet. After 1, 2 or 4 weeks, mice were sacrificed after overnight fasting for plasma and tissue mRNA analyses.

All experimental animal procedures were approved by the animal welfare committee of the Leiden University.

Plasma analyses

Plasma triglyceride and insulin levels were measured using commercially available kits from Roche Diagnostics and Crystal Chem Inc., and glucose levels were determined according to the hexokinase method (Instruchemie). Plasma levels of multiple cytokines were evaluated simultaneously by using pre-coated multisport plates in an ELISA-based electrochemiluminescence assay (Meso Scale Discovery).

Coagulation factor levels were measured as previously described and pooled mouse plasma was used to generate standard curves.[11] The *in vivo* clearance of

human coagulation factors VII, VIII and IX was analyzed with home-made ELISAs specific for human proteins that were proven not to cross-react with mouse plasma proteins. Standard curves were generated by adding Cofact or Aafact to pooled mouse plasma (final concentration 20%) to calculate human antigen levels, and the level measured directly after injection (1 minute) was set as a reference (100%).

RNA isolation and real-time RT-PCR

Individual liver samples (15-20 mg) of 10 animals per group were homogenized in RNAzol (Tel-Test), and RNA isolation and cDNA synthesis was executed as previously reported.[11] Quantitative real-time PCR was performed using SybrGreen (Applied Biosystems) and gene-specific primers[11] and the comparative threshold cycle method with ß-actin as internal control was used for quantification and normalization. To evaluate the effects of weight gain on transcript levels, LFD-fed mice were set as a reference, whereas the HFD-fed mice were set as a reference to determine the effects of weight loss. The ΔC_t values of individual samples were related to the mean ΔC_t of the reference group.

Statistical analyses

Data are expressed as mean ± standard error of the mean (SEM) or as the median and range for cytokine levels. Gene expression data are presented as the mean including the minimum and maximum expression levels. Data were analyzed with the GraphPad Instat software and statistical differences between the LFD and HFD groups were evaluated using a Student's t-test, or a Mann-Whitney test in case of cytokine levels, to compare the LFD with HFD group. P-values <0.05 were considered to be statistically significant.

Results

Induction of obesity

Two weeks of HFD-feeding resulted in a significantly increased fasted body weight as compared to the LFD-fed mice (25.0±0.6 g vs. 22.3±0.3g, p<0.001), which gradually increased further over time (table 1). From 8 week onwards, liver weights of HFD-fed mice were also significantly higher than those of LFD-fed mice.

Plasma cytokine levels showed a transient rise in interleukin (IL) 1ß and keratinocyte chemoattractant (KC) levels after 2 weeks of HFD-feeding (3.2 (0.7-20.7) pg/mL vs. 7.0 (1.1-88.9) pg/mL for IL-1β, p<0.05; 34.6 (19.1-246.8) pg/mL vs. 55.9 (28.7-233.6) pg/mL for KC, p<0.05). The levels of IL-6, IL-10, IL-12, interferon-

γ (IFN-γ) and tumor necrosis factor α (TNFα) were not affected (data not shown). Triglyceride levels were also transiently increased at the 2 week time point in mice fed the HFD (0.70±0.04 mmol/L vs. 0.86±0.06 mmol/L, $p<0.05$), and again after 16 weeks of HFD feeding (table 1). Plasma glucose levels were higher due to the high fat diet from 8 week onwards (5.7±0.2 mmol/L vs. 7.7±0.4 mmol/L, $p<0.001$), whereas insulin levels were only increased after 16 weeks (table 1).

The high fat feeding-related changes in metabolic parameters after 16 weeks largely resembled the observations in the LFD-fed *ob/ob* mice, which had a comparable weight and enlarged liver (table 1). In addition, the insulin and glucose levels were also higher as compared to their wild-type littermates. KC and IFN-γ levels were increased in *ob/ob* mice (KC 4.4 (16.5-229.1) pg/mL vs. 100.1 (17.6-268.9) pg/mL, $p<0.05$; IFN- γ 7.3 (2.0-53.9) pg/mL vs. 45.9 (1.2-125.0) pg/mL, $p<0.05$), but they displayed surprisingly lower fasted plasma triglyceride levels (table 1).

Table 1: Metabolic parameters of mice on a low fat diet (LFD) or high fat diet (HFD) for 16 weeks as compared to genetically obese *ob/ob* mice with their littermate wild-type controls after 4 weeks of LFD feeding.

	LFD (n=15)	HFD (n=15)	WT (n=15)	*ob/ob* (n=15)
Body weight (g)	27.4±0.5	41.6±0.9‡	22.1±0.6	40.3±0.6‡
Liver weight (g)	0.79±0.01	1.08±0.06‡	0.78±0.04	2.14±0.06‡
Triglycerides (mmol/L)	0.54±0.03	0.65±0.04*	0.62±0.05	0.39±0.02‡
Insulin (pg/mL)	97.4±1.0	105.0±2.8*	94.1±0.9	105.4±1.8‡
Glucose (mmol/L)	5.7±0.2	8.0±0.6‡	5.8±0.6	11.6±0.9‡

Data are expressed as mean±SEM. *$p<0.05$ and ‡$p<0.001$ as compared to LFD-fed mice or wild-type controls as appropriate.

Within 2 weeks, the high fat diet induced a clear procoagulant shift of the plasma coagulation profile, with significant increases in fibrinogen, FII, FVII, FVIII, FIX, FXI and FXII levels (fig. 1A). Continuation of the HFD resulted in sustained increased levels of fibrinogen, FII and FVII while the effects on FVIII, FIX, FXI and FXII levels became less pronounced and did not reach statistical significance after 16 weeks HFD-feeding. Factor X and antithrombin plasma levels were only significantly increased after 16 weeks of HFD-feeding (fig. 1B). Comparing the 16 week HFD-fed animals with *ob/ob* mice showed a similar procoagulant shift, although these

effects were more pronounced in *ob/ob* mice (fig. 1C). Remarkably, factor VII levels, like the triglyceride levels, were lower in *ob/ob* mice than in the wild-type controls, while plasma levels of fibrinogen and FXI were not significantly affected.

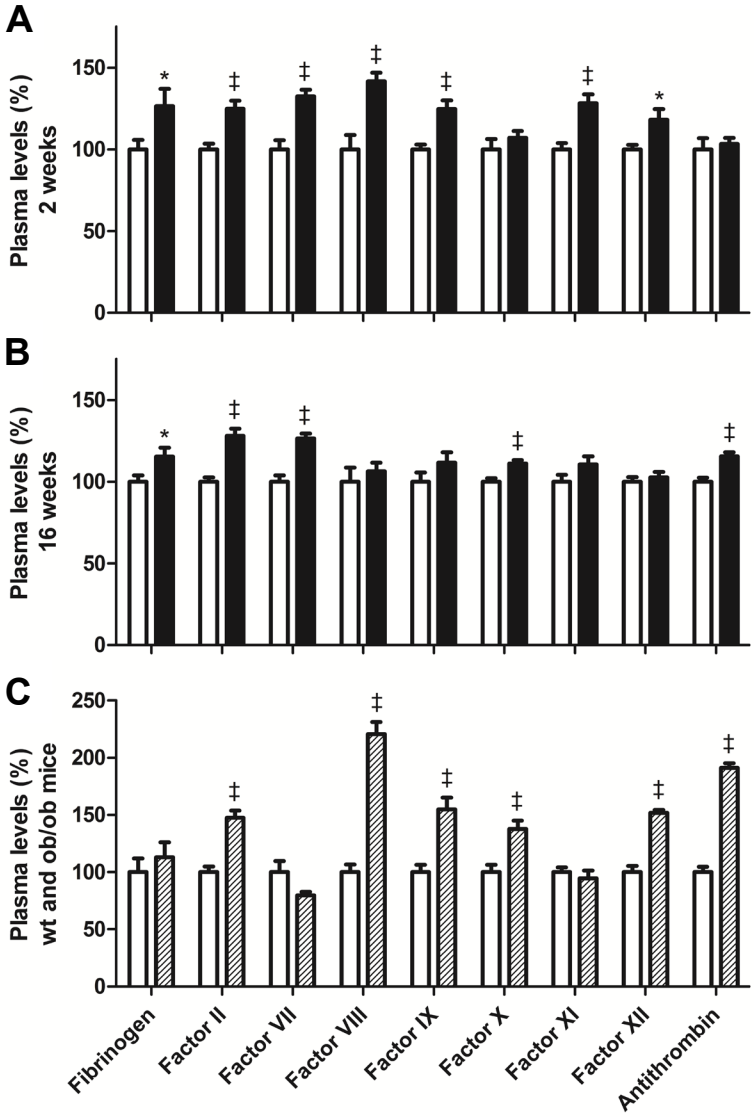

Figure 1: Effects on plasma coagulation parameters after 2 (panel A) and 16 (panel B) weeks of low fat diet (white) or high fat diet (black) feeding. Panel C shows the plasma coagulation profile of genetically obese *ob/ob* mice (striped) and their wild-type littermates (white) after 4 weeks on a low fat diet. Data are presented as mean±SEM. *$p<0.05$ and ‡$p<0.001$ as compared to the LFD-fed mice or wild-type controls as appropriate.

Since the liver is the main site of production of plasma coagulation factors, we determined whether the changes in the plasma coagulation profile due to the high fat diet were related to changes in hepatic transcript levels, as we have previously shown that changes in plasma levels can coincide with transcriptional effects.[11] Surprisingly, at the 2-week time point where the liver weight between diet treatment groups are comparable but clear increases in plasma levels are observed, relative mRNA levels of coagulation genes were not affected with the exception of FXI which was increased and FVIII which was decreased (table 2). Despite the differences in liver weight after 16 weeks of high fat-feeding, we evaluated whether prolonged exposure to dietary fat was able to affect transcription. However, HFD-feeding for 16 weeks was also not able to induce increases in relative mRNA levels of hepatically expressed coagulation factors (data not shown).

As the changes in the plasma coagulation profile were not paralleled by changes in transcript levels, we determined whether HFD-feeding for 2 weeks affected plasma protein turnover, i.e. decreased the clearance rate. A bolus injection of either the human FVIII concentrate or prothrombin complex concentrate resulted in both the HFD and LFD group in single-phase clearance curves with comparable half-lives between LFD-fed and HFD-fed mice (FVIII 18.6±1.8 min vs. 15.3±1.7 min, FVII 112.6±7.1 min vs. 99.4±5.9 min and FIX 79.0±9.5 vs. 76.5±5.7 min).

Table 2 Hepatic mRNA levels of coagulation genes of mice on a low fat diet (LFD) or high fat diet (HFD) for 2 weeks.

	LFD (n=10)	HFD (n=10)
Fibrinogen	1 (0.93-1.08)	0.87 (0.83-0.90)
Factor II	1 (0.93-1.08)	1.05 (0.97-1.13)
Factor VII	1 (0.95-1.05)	1.07 (1.01-1.13)
Factor VIII	1 (0.94-1.06)	0.64 (0.54-0.77)*
Factor IX	1 (0.95-1.05)	1.08 (1.03-1.13)
Factor X	1 (0.96-1.04)	1.01 (0.96-1.07)
Factor XI	1 (0.92-1.09)	1.51 (1.44-1.58)‡
Factor XII	1 (0.95-1.05)	1.06 (1.01-1.12)
Antithrombin	1 (0.95-1.05)	1.05 (1.00-1.10)

Data are expressed as mean (minimum-maximum expression level). *p<0.05 and ‡p<0.001 as compared to LFD mice.

Regression of obesity

Since the dietary fat intake resulted in a rapid procoagulant shift of the plasma coagulation profile, we determined whether regression of the nutritionally-induced obesity also resulted in an altered coagulation profile. Therefore, part of the mice receiving the HFD for 16 weeks switched to the low fat diet (n=45) while the remaining mice continued the HFD (n=45). Switching to the LFD resulted in an immediate decrease in body weight, which was already significant after 1 week (37.3±1.5 g vs. 42.9±1.1 g, $p<0.01$), while effects on the liver weight were apparent after 2 weeks (1.05±0.06 g vs. 1.22±0.08 g, $p<0.05$). Fasted plasma glucose levels were also rapidly affected (6.4±0.4 mmol/L vs. 8.7±0.7 mmol/L, $p<0.01$), whereas insulin levels only differed at 4 weeks after switching to the LFD (103.5±1.0 pg/mL for the mice switched to LFD vs. 109.3±2.4 pg/mL for the HFD mice, $p<0.05$) and triglyceride levels were not affected due to the change in dietary fat content. Furthermore, KC levels were significantly increased after the first week of switching to the LFD (58.2 (36.1-168.2) pg/mL for HFD vs. 90.8 (36.3-542.6) pg/mL, $p<0.05$).

The plasma coagulation profile showed a remarkably rapid shift after switching to a low fat diet, with significantly reduced activity levels of FII, FVII, FIX, FX and FXI after only 1 week (figure 2a), and these changes persisted throughout the remaining for 4 weeks. In addition, factor VIII and antithrombin levels were altered after 2 weeks of switching diets (100±9.8% vs. 72.4±5.0%, $p<0.01$ and 100±2.3% vs. 86.6±4.4, $p<0.05$ respectively), whereas FXII levels were lower after 4 weeks (100±1.5% vs. 92.1±1.5, $p<0.01$).

As one week after switching diets was able to alter the plasma coagulation profile, while liver weights were not significantly affected, hepatic mRNA analyses were performed to determine whether transcript levels of coagulation genes were modulated by the diet switch. The non-significant decrease in plasma fibrinogen levels coincided with a significant reduction in transcript levels, and also for FXI the decrease in plasma was paralleled by a decrease in relative mRNA levels. However, for all other coagulation factors measured in plasma, no effects on hepatic mRNA levels were observed (figure 2).

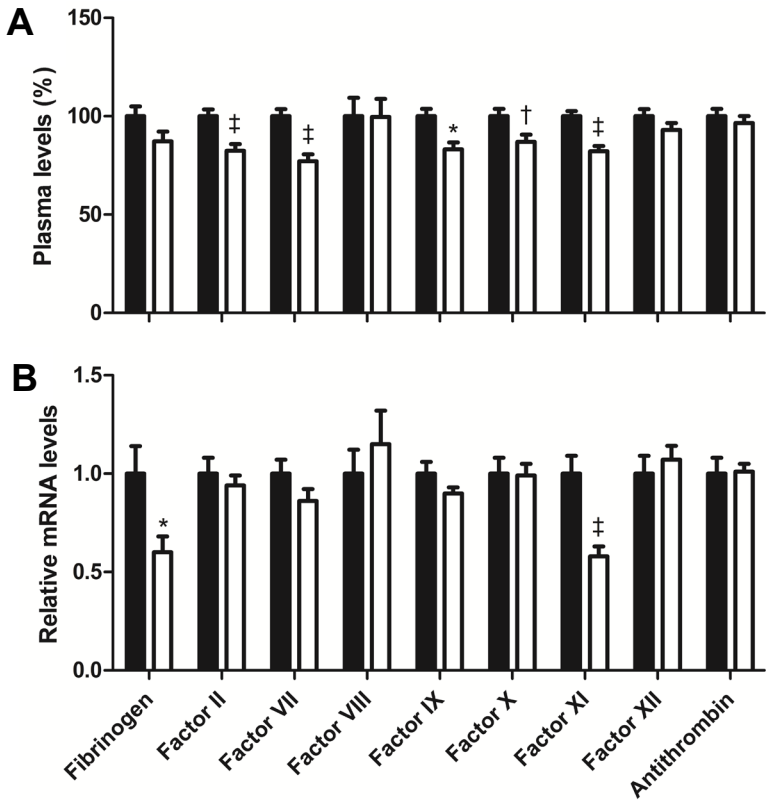

Figure 2: Plasma (A) and relative transcript levels (B) of mice on a high fat diet for 17 weeks (black) and mice that were switched after 16 weeks of HFD-feeding to the LFD for 1 week (white). Data are presented as mean±SEM for the plasma data and as mean with the error bar representing the calculated maximum expression level of n=10 mice per group for the expression levels. Relative expression levels were compared using the comparative threshold cycle method with ß-actin as internal control and the HFD-fed mice were set as a reference. *$p<0.05$, †$p<0.01$ and ‡$p<0.001$ as compared to the LFD-fed mice or wild-type controls as appropriate.

Discussion

Obesity is becoming an increasing public health problem and it is associated, amongst others, with a hypercoagulable state. In order to gain more insight in this relation between obesity and hypercoagulability we used an *in vivo* approach to study the onset and potential reversibility of the hypercoagulable state during the development and regression of nutritionally-induced obesity. In addition, we determined the mechanisms leading to this hypercoagulable state by evaluating transcription and clearance of coagulation factors, as well as determining metabolic and inflammatory parameters since they may aggravate the hypercoagulable state associated with obesity.

We have shown that that nutritionally-induced obesity coincides with an early-onset procoagulant shift of the plasma coagulation profile, which was already apparent within 2 weeks after the start of the HFD. Furthermore, these changes largely persisted during the continuation of the HFD for 16 weeks, thereby preceding the effects in metabolic parameters like glucose and insulin levels since these were only affected in overt obese mice. Switching from a high fat to a low fat diet to induce weight loss resulted in a rapid reversal of the HFD-induced procoagulant shift of the plasma coagulation profile, as evaluated 1 week after switching diets. Surprisingly, these effects on the plasma coagulation profile after switching dietary fat intake appeared to be independent of changes in relative transcript levels and clearance rates of coagulation factors.

A remarkable observation in this study is that the changes observed in plasma activity levels were not paralleled by changes in relative transcript levels of hepatically expressed coagulation genes. In addition, clearance studies, although performed with human proteins, could also not explain the increased plasma levels in HFD-fed mice. This difference between mRNA levels and clearance rates on the one hand, and plasma levels on the other, may have several reasons. Regarding the clearance studies, human coagulation factors may be differently cleared from mouse plasma than murine factors. Secondly, since high fat feeding can affect liver physiology, we were interested whether the RNA recovery in liver samples of LFD and HFD mice differed. Although the liver weights after 2 weeks of HFD feeding were comparable, the amount of RNA per mg liver weight was approximately 20% higher as compared to the RNA recovery from LFD-fed mice. One can speculate that this increased recovery might result in an overall increase in absolute transcript levels, while the relative mRNA levels are similar between diets, and therefore contribute to the increased plasma activity levels. Finally, although we have previously shown that transcript levels correlate well to plasma activity levels,[9] post-transcriptional or post-translational mechanisms may affect the activity of the resulting protein. A comparable situation, in which protein activity is increased while transcription is down-regulated under nutritionally-induced obesity, has also been reported for phosphoenolpyruvate carboxykinase (PEPCK), an enzyme associated with hepatic glycogen storage.[12]

The fact that there is a transient rise in IL-1β and KC levels when mice switch diet types, suggests that the system has to adapt to the new diet in order to maintain homeostasis. These data are in concordance with the metabolic stress response that occurs during short-term high fat feeding.[13] Furthermore, a recent genome-

wide mRNA expression study which focused on changes in hepatic gene expression during high fat feeding, also showed that exposure to dietary fat first results in inflammation which under long-term high fat feeding causes a switch to a steatotic transcriptional program.[14] Besides their roles in coagulation, fibrinogen and factor VIII also play a role in inflammation as acute phase proteins and their transcription can be induced by nuclear factor (NF)-κB, which transcriptional activity is increased under inflammatory conditions.[15,16] However, although both fibrinogen and FVIII levels in plasma are increased after 2 weeks of HFD-feeding, their transcript levels are decreased, making an increased transcriptional activity of NF-κB resulting in increased plasma coagulation factor levels less likely.

By studying coagulation during the development and regression of nutritionally-induced obesity, we were able to show that the dietary fat content plays and important role in affecting the plasma coagulation profile. We also evaluated genetically obese *ob/ob* mice, mainly as a control for determining the effects of HFD on metabolic parameters like insulin resistance. The *ob/ob* mice have been predominantly used to study metabolic disorders leading to type 2 diabetes, and although they have been used in studies focusing on tissue factor and PAI-1, in general little is known about their overall plasma coagulation profile.[17,18] Here we show that *ob/ob* mice have more pronounced increases in plasma procoagulant factor levels as compared to mice on a HFD for 16 weeks, with the exception of FVII levels since they are decreased in *ob/ob* mice. As these mice display metabolic abnormalities, this may aggravate the hypercoagulable state, for example via an increased transcriptional activity of nuclear factor (NF)-κB which in turn can induce expression of coagulation genes as previously mentioned. Because of the underlying pathologies that can potentially affect coagulation, the *ob/ob* mouse seems to be less suitable to study obesity with respect to coagulation and a (short-term) nutritionally-induced obesity model seems warranted.

In summary, this *in vivo* study shows that the plasma coagulation profile is able to rapidly respond to changes in dietary fat content, both in weight gain which is associated with a procoagulant shift, and in subsequent weight loss resulting in a reversal of the HFD-induced hypercoagulability. These changes in the plasma coagulation profile seem to be independent of changes in relative transcript levels of coagulation genes and changes in protein clearance rates. In addition, the effects on coagulation precede alterations in metabolic parameters like insulin and glucose levels. The fact that weight loss is associated with rapid beneficial effects

on coagulation, and late effects of metabolic parameters, may eventually translate in a risk reduction for thrombotic cardiovascular events.

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References

1 Abdollahi M, Cushman M, Rosendaal FR. Obesity: risk of venous thrombosis and the interaction with coagulation factor levels and oral contraceptive use. Thromb Haemost 2003;89:493-8.
2 Mertens I, Van Gaal LF. Obesity, haemostasis and the fibrinolytic system. Obes Rev 2002;3:85-101.
3 Rosito GA, D'Agostino RB, Massaro J, L et al. Association between obesity and a prothrombotic state: the Framingham Offspring Study. Thromb Haemost 2004;91:683-9.
4 Godsland IF, Crook D, Proudler AJ, et al. Hemostatic risk factors and insulin sensitivity, regional body fat distribution, and the metabolic syndrome. J Clin Endocrinol Metab 2005;90:190-7.
5 Sola E, Navarro S, Medina P, et al. Activated protein C levels in obesity and weight loss influence. Thromb Res 2009;123:697-700.
6 Ay L, Kopp HP, Brix JM, et al. Thrombin generation in morbid obesity: significant reduction after weight loss. J Thromb Haemost 2010;8:759-65.
7 Folsom AR, Qamhieh HT, Wing RR, et al. Impact of weight loss on plasminogen activator inhibitor (PAI-1), factor VII, and other hemostatic factors in moderately overweight adults. Arterioscler Thromb 1993;13:162-9.
8 Pomp ER, le Cessie S, Rosendaal FR, et al. Risk of venous thrombosis: obesity and its joint effect with oral contraceptive use and prothrombotic mutations. Br J Haematol 2007;139:289-96.
9 Cleuren AC, Van Hoef B, Hoylaerts MF, et al. Short-term ethinyl estradiol treatment suppresses inferior caval vein thrombosis in obese mice. Thromb Haemost 2009;102:993-1000.
10 Nagai N, Hoylaerts MF, Cleuren AC, et al. Obesity promotes injury induced femoral artery thrombosis in mice. Thromb Res 2008;122:549-55.
11 Cleuren AC, Van der Linden IK, de Visser YP, et al. 17alpha-Ethinylestradiol rapidly alters transcript levels of murine coagulation genes via estrogen receptor alpha. J Thromb Haemost 2010;8:1838-46.
12 Do GM, Oh HY, Kwon EY, et al. Long-term adaptation of global transcription and metabolism in the liver of high-fat diet-fed C57BL/6J mice. Mol Nutr Food Res 2011;55:S173-S185.
13 Muller M, Kersten S. Nutrigenomics: goals and strategies. Nat Rev Genet 2003;4:315-22.
14 Radonjic M, de Haan JR, van Erk MJ, et al. Genome-wide mRNA expression analysis of hepatic adaptation to high-fat diets reveals switch from an inflammatory to steatotic transcriptional program. PLoS One 2009;4:e6646.

15 Iwasaki Y, Nishiyama M, Taguchi T, et al. Insulin exhibits short-term anti-inflammatory but long-term proinflammatory effects in vitro. Mol Cell Endocrinol 2009;298:25-32.
16 Liu SF, Malik AB. NF-kappa B activation as a pathological mechanism of septic shock and inflammation. Am J Physiol Lung Cell Mol Physiol 2006;290:L622-L645.
17 Oishi K, Ohkura N, Wakabayashi M, et al. CLOCK is involved in obesity-induced disordered fibrinolysis in ob/ob mice by regulating PAI-1 gene expression. J Thromb Haemost 2006;4:1774-80.
18 Samad F, Loskutoff DJ. Hemostatic gene expression and vascular disease in obesity: insights from studies of genetically obese mice. Thromb Haemost 1999;82:742-7.