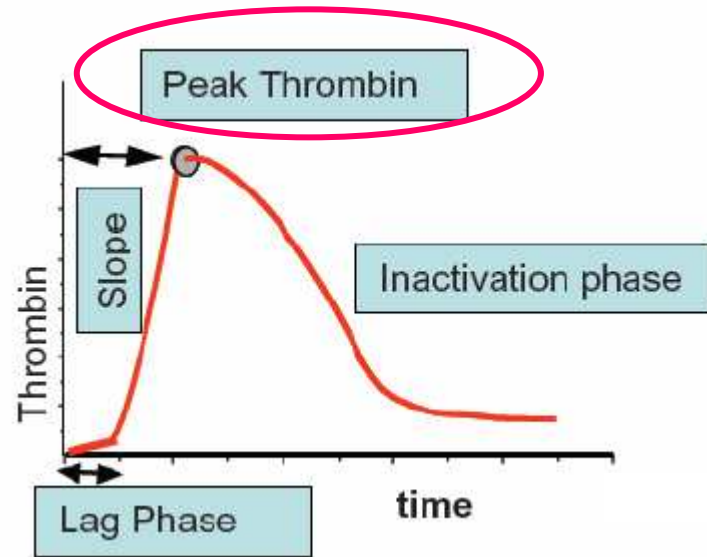


Influence of pre-analytic variables on thrombin generation

L. Wiens
Dresden, 23.02.2007

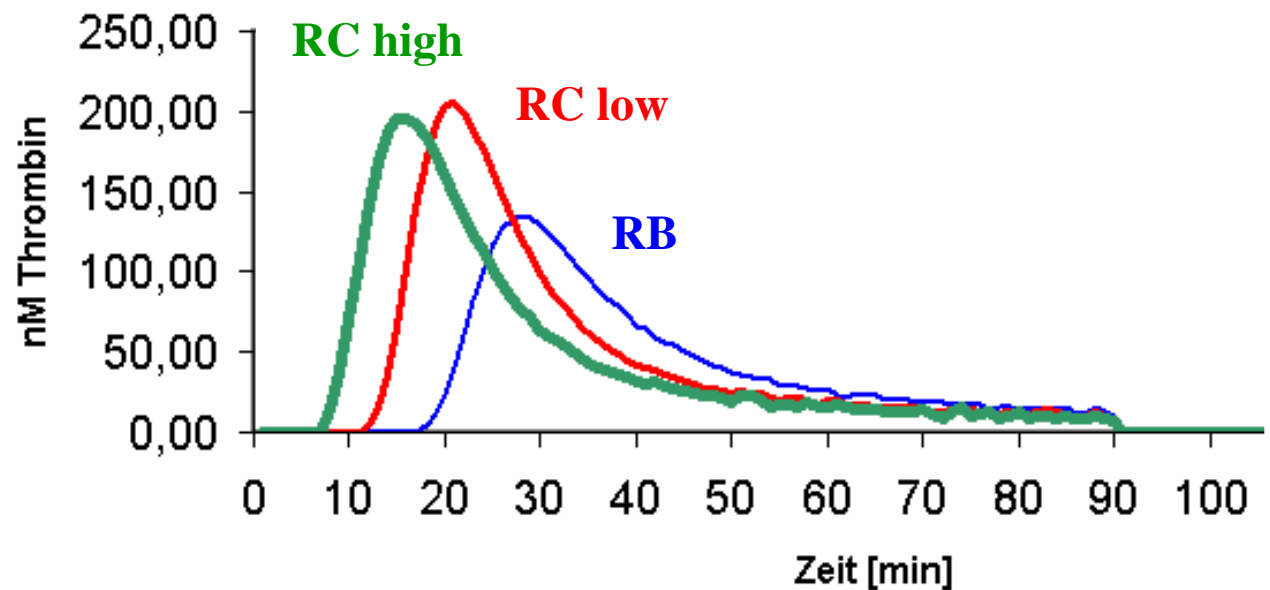
Measurement of Thrombin Generation



Test Kit: Technothrombin® TGA

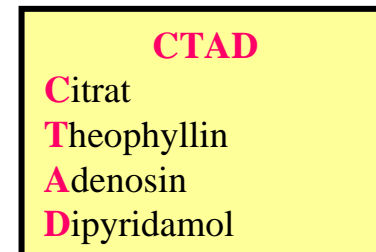
Principle: fluorescence measurement

Instrument: BioTek® FLx 800™ TC

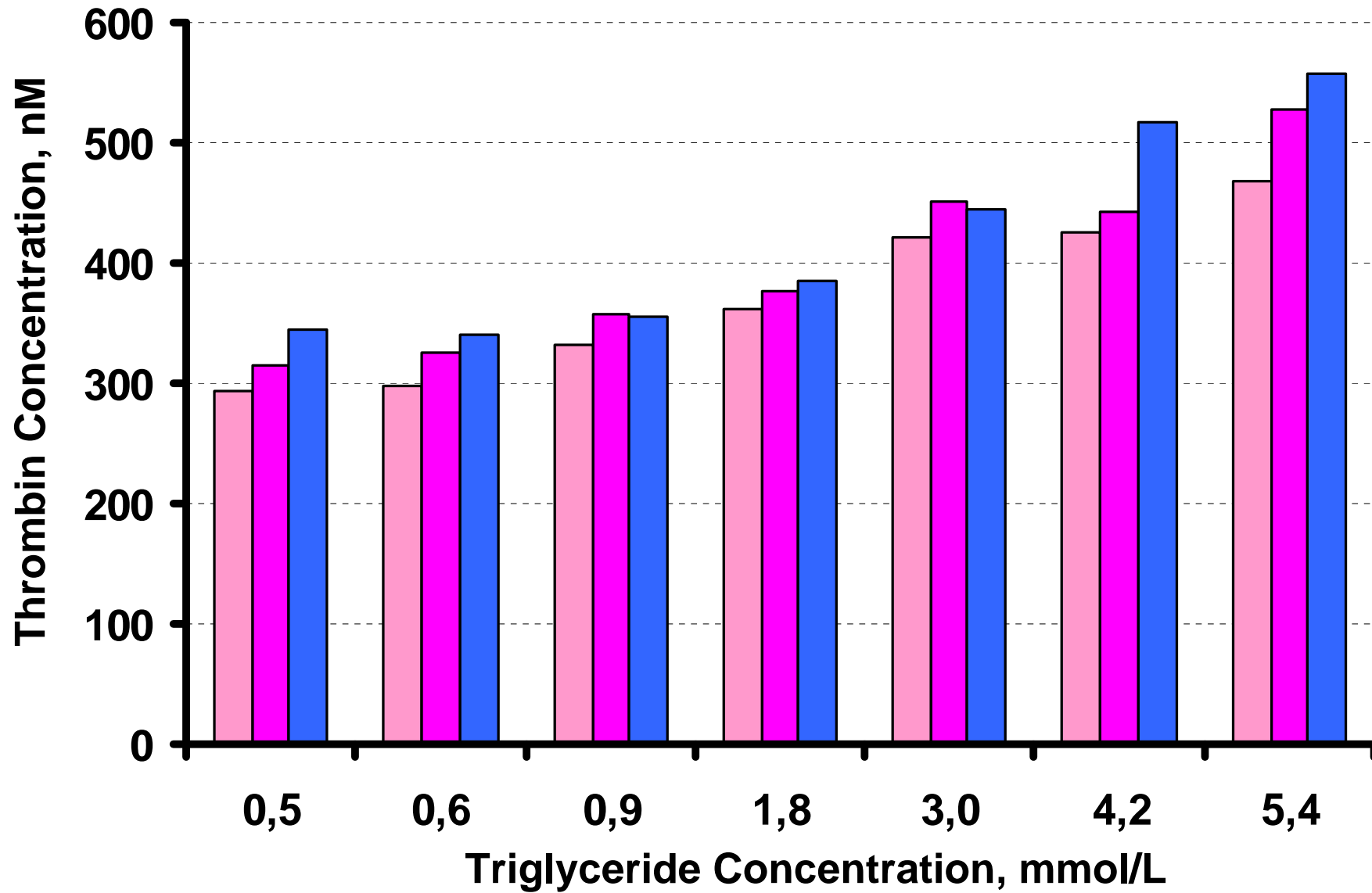


Performance of the test

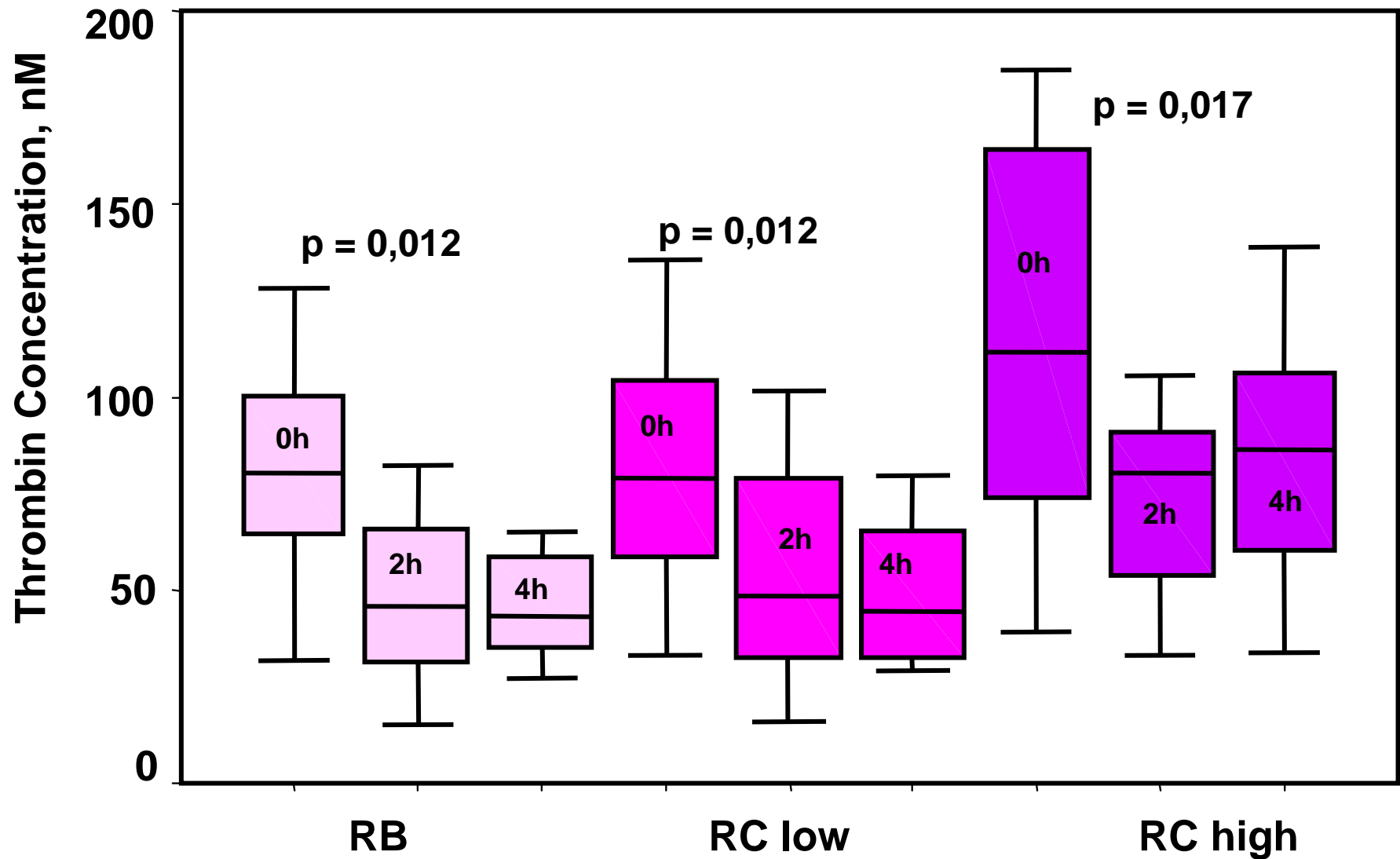
- 8 healthy subjects who did not present with any hemostatic failures
- measurement of thrombin generation in platelet free plasma using the TGA-test kit (Technoclone) with 3 reagents: RB, RC low and RC high
- pre-analytic variables:
 - triglyceride concentrations of 0.5 up to 5.4 mmol/L
 - measurement of thrombin generation immediately after collection of citrate plasma as well as after 2 and 4 hours
 - transport of whole blood samples (either citrate or CTAD) by letter shoot immediately after blood collection and 2 or 4 hours after blood collection, centrifugation after transport
 - frozen citrate plasma



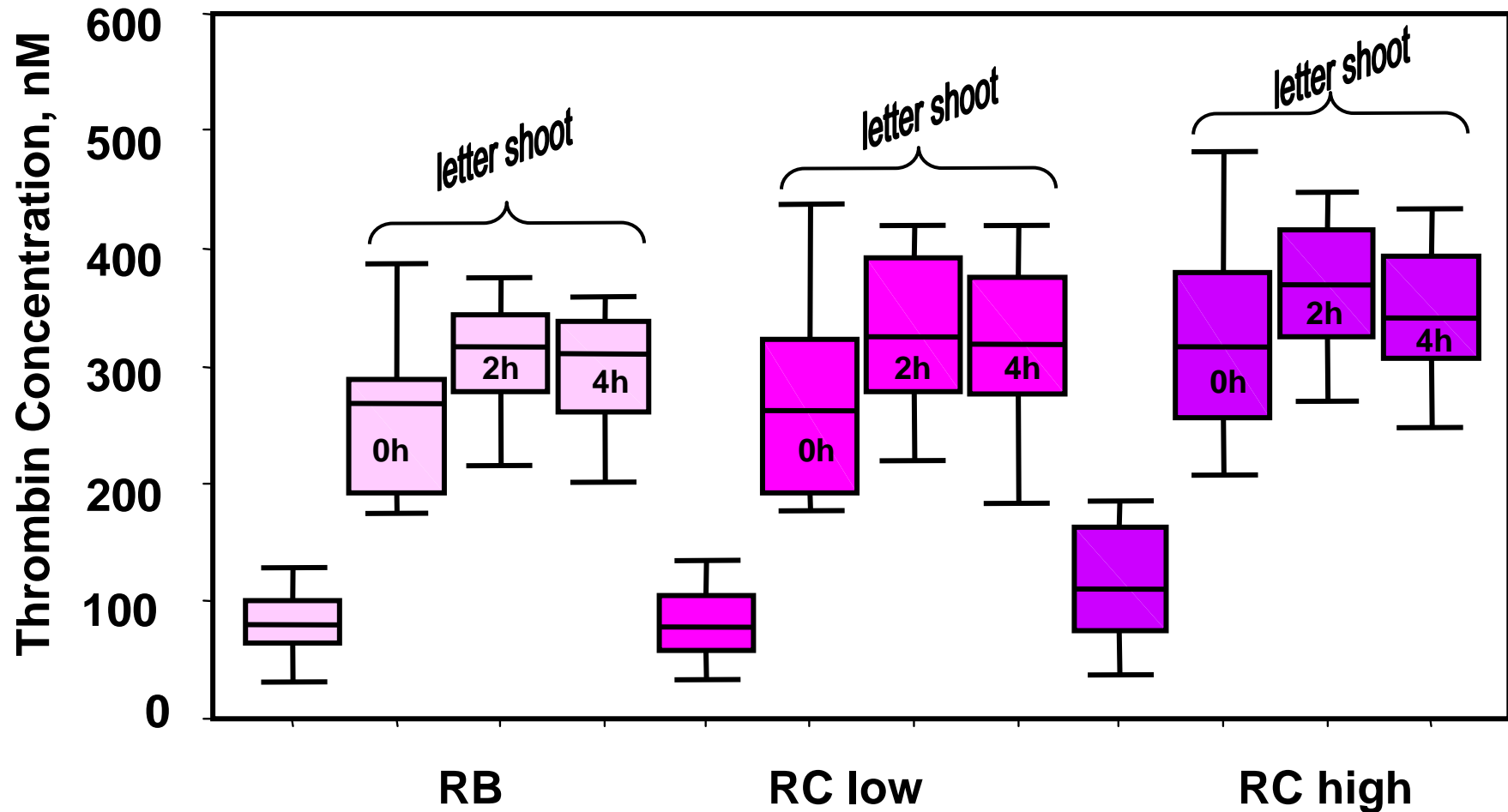
Influence of lipaemia on thrombin generation



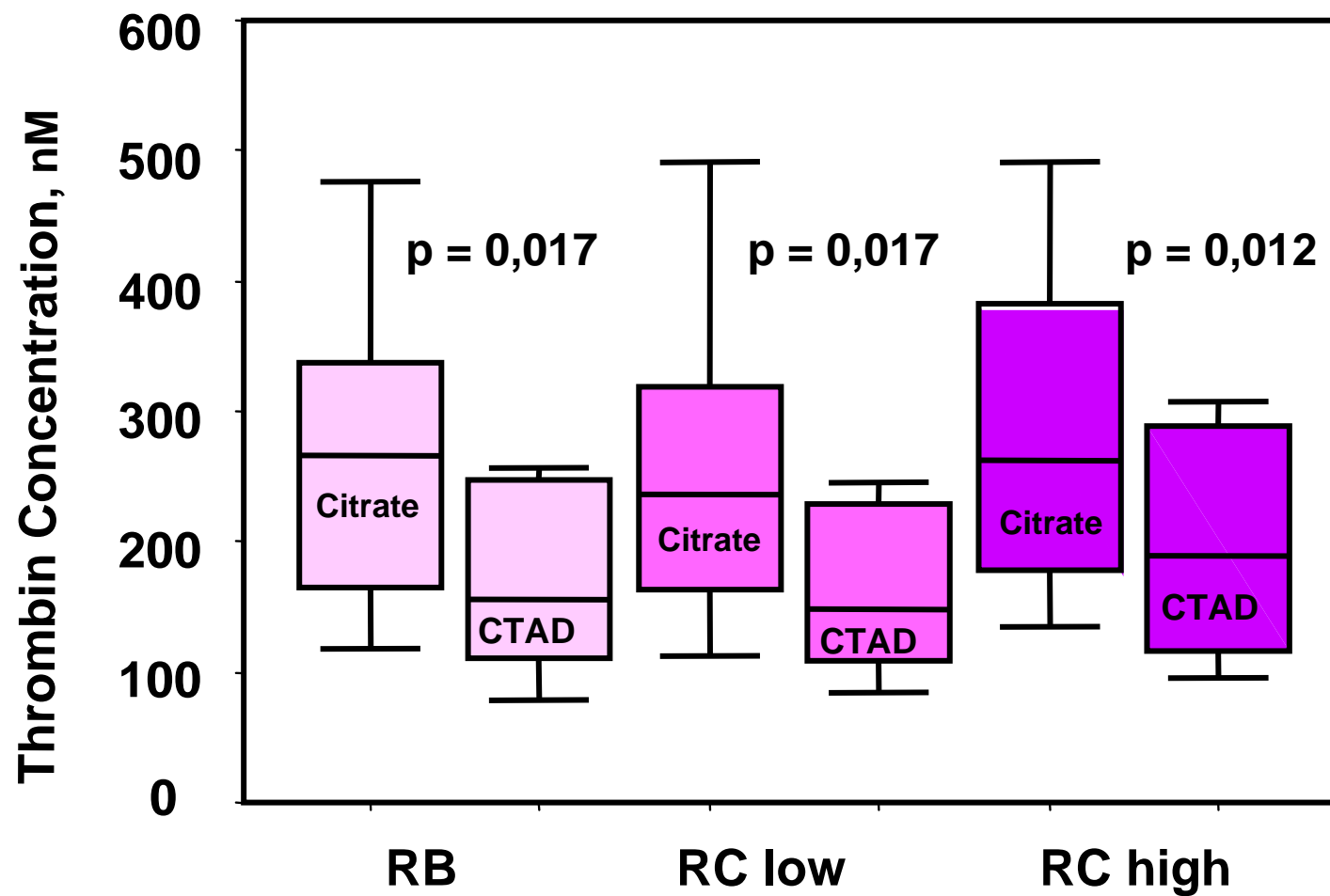
Effect of plasma samples left standing for 2 and 4 hours at room temperature before measurement of thrombin generation



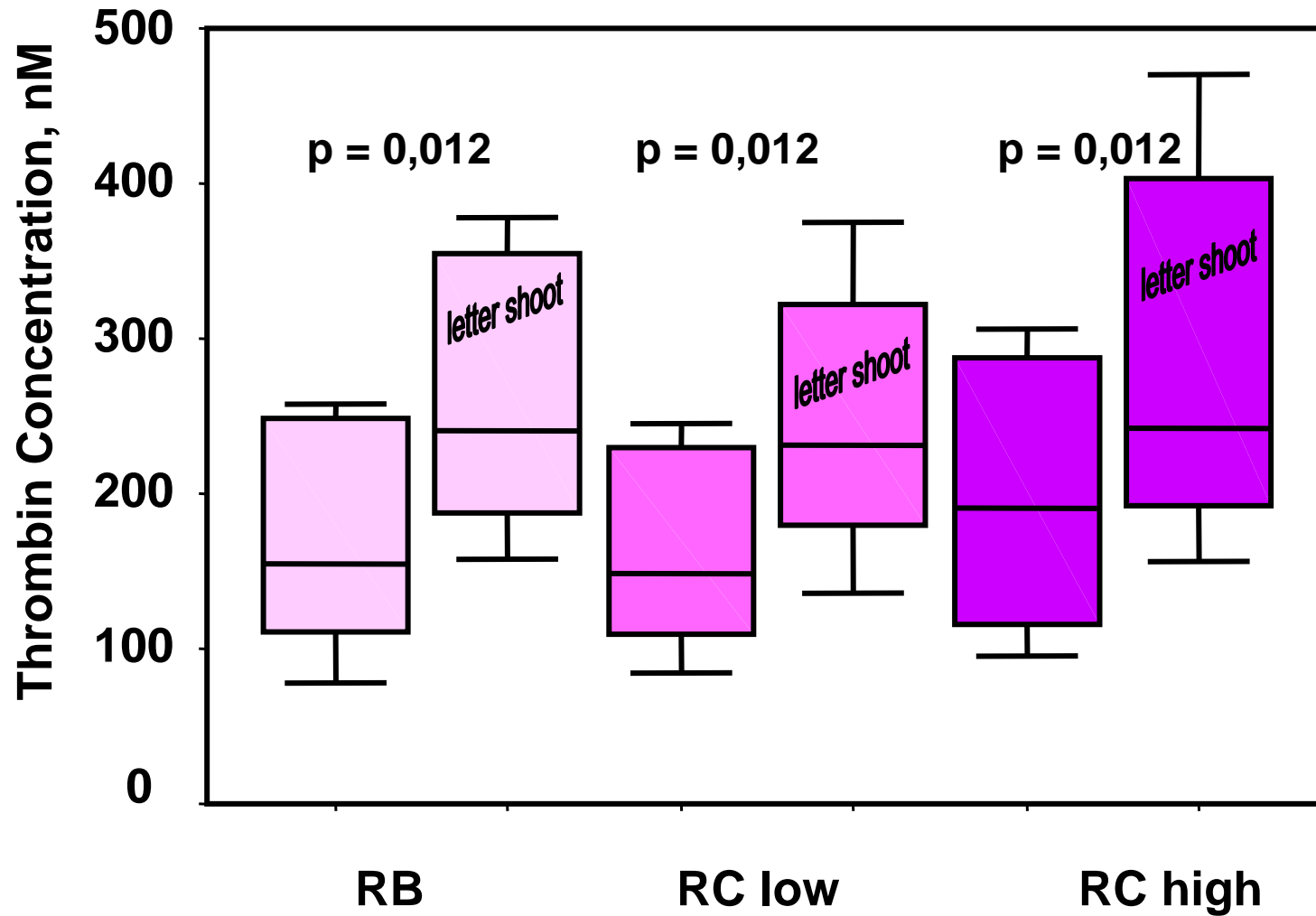
Effect of whole blood sample transport by letter shoot in combination with blood samples left standing at 2 and 4 hours at room temperature



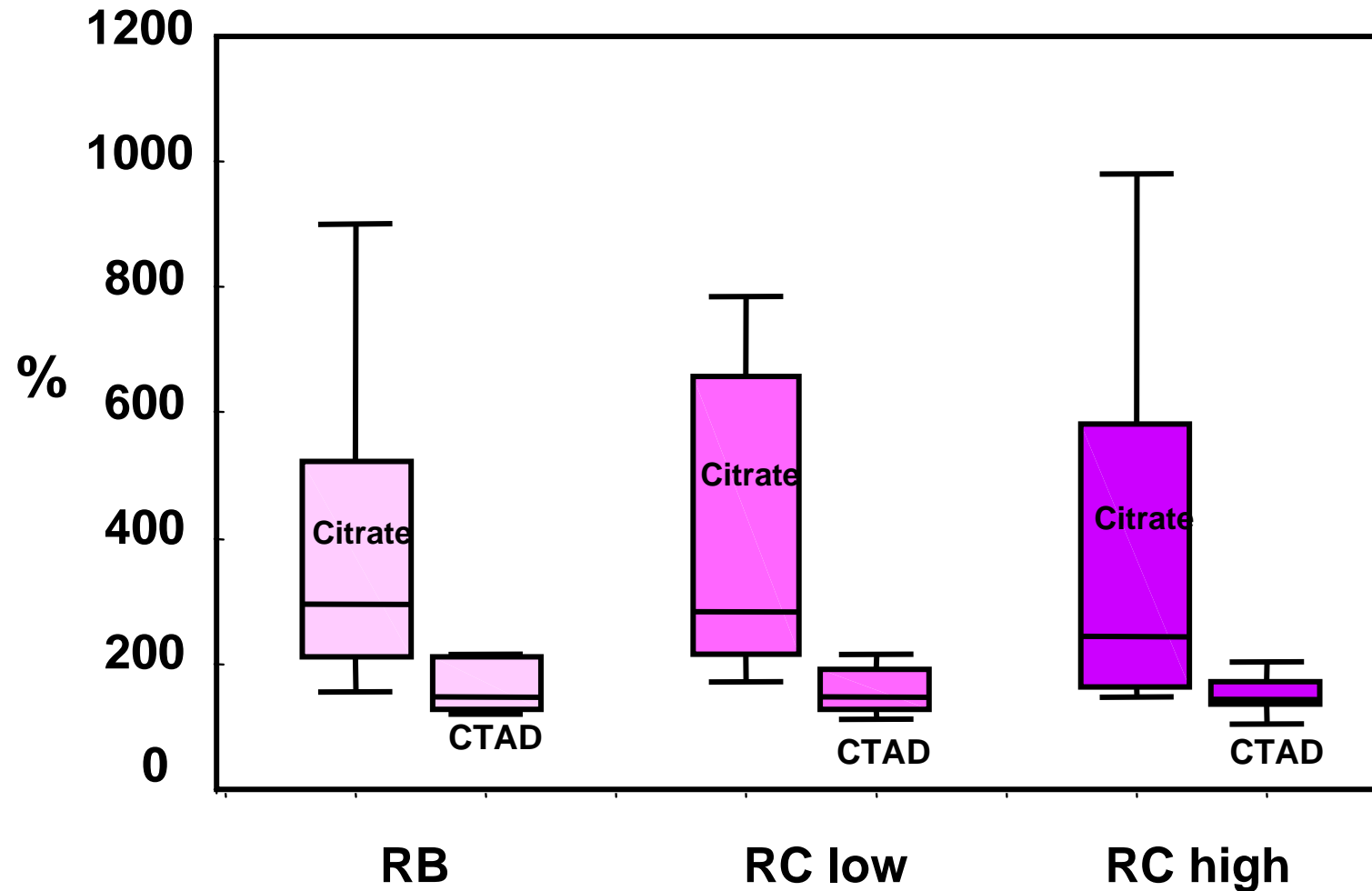
Thrombin Generation in citrate- and CTAD-Plasma (Centrifugation immediately after blood collection)



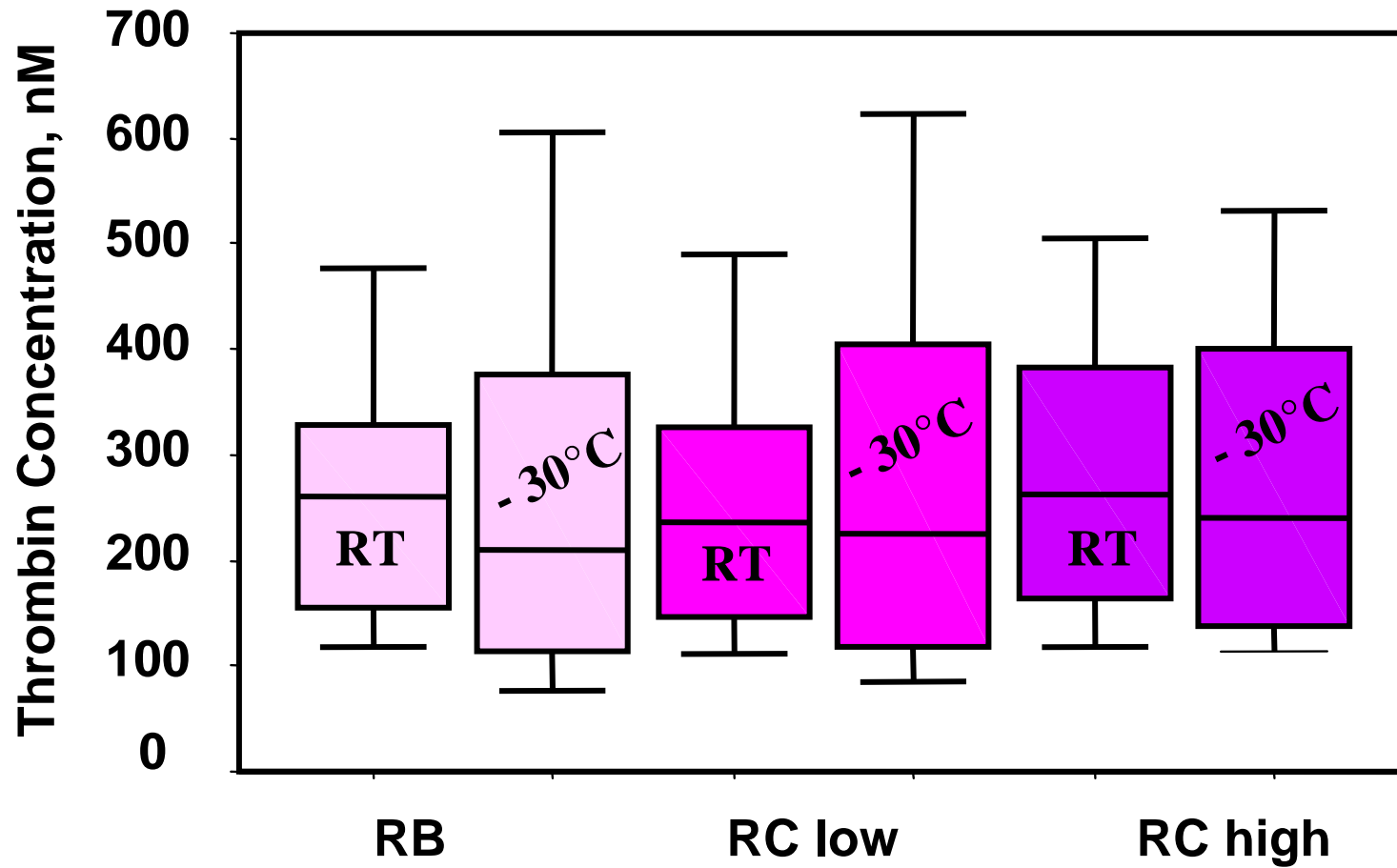
Thrombin Generation in CTAD-Plasma after transport by letter shoot



Percentaged increase in thrombin generation in citrate and CTAD-plasma after transport of whole blood samples by letter shoot



Freezing of citrate plasma – influence on thrombin generation



Summary of Results

1. Lipaemic samples were found to increase thrombin generation (triglyceride concentration > 3 mmol/L)
2. Thrombin generation is significantly decreased by a factor of 2 after leaving the citrate plasma standing for 2 to 4 hours.
3. Thrombin generation is 2 to 4-fold higher if citrate blood is immediately transported by letter shoot after collection.
4. Thrombin generation in CTAD-plasma is significantly lower than in citrate plasma.
5. Thrombin generation is significantly increased after immediate transport of CTAD blood by letter shoot.
6. Freezing citrate plasma at -30°C does not cause a significant change in thrombin generation.

Conclusions

1. The influence of a post prandial lipaemia on thrombin generation has to be taken into account.
2. Citrate or CTAD-blood has to be centrifuged immediately after blood collection and plasma has to be analysed or frozen immediately. If this is not possible standardized times for transport and leaving whole blood samples standing have to be organized.
3. Using CTAD leads to a certain degree of stabilisation of thrombin generation values after transport of samples by letter shoot, but this is not sufficient.
4. The hospital specific way of transport and staying time of the whole blood samples in the wards has to be taken into account when establishing reference ranges for thrombin generation.