ABSTRACT
Recently, we introduced a microfluidic biochip to incorporate intestinal and liver slices and demonstrated its proper functioning by comparing the metabolic capacity of slices in the biochip with those in well plates [1, 2]. We now show that interorgan interactions can successfully be studied by sequential perifusion of intestinal and liver slices in the biochip by investigating the role of the intestine in bile salt-induced regulation of Cytochrome P450 7A1 (CYP7A1) in the liver.

KEYWORDS: Liver slices, Intestinal slices, Interorgan interaction, Cytochrome P450 7A1, Fibroblast growth factor 15 (FGF15)

INTRODUCTION
The synthesis of bile acids mainly occurs in the liver, with CYP7A1 as the key enzyme. The expression of this enzyme in the liver is regulated by several mechanisms. Bile salts play a prominent role in the activation of the farnesoid X receptor (FXR)-mediated feedback down-regulation of CYP7A1 in the liver. In addition, the intestine plays an important role in the regulation of CYP7A1 [3]. Primary bile acids such as chenodeoxycholic acid (CDCA) induce the expression of fibroblast growth factor 15 (FGF15 in rodents or FGF19 in human) in the intestine. In vivo FGF15 is transported via the portal vein to the liver, where it causes a down-regulation of CYP7A1. We investigated whether this second pathway could be confirmed in vitro with intact precision-cut tissue slices of intestine and liver.

We recently developed a new system for the incubation of rat liver slices, consisting of a perfused microfluidic biochip where the liver slice is continuously perifused. (note: the term “perifusion” is used to emphasize that the medium flows around the tissue slice rather than through it, whereas the term “perfusion” applies to the medium flow through the microchamber.) The perfused biochip contains two polycarbonate membranes (10 µm thick) which form the ceiling and floor of each microchamber to realize an even distribution of medium flow around the tissue slice and to ensure that the slice is horizontally suspended in the flow (Figure 1). The chip also contains two 250-µm-thick PDMS membranes above and below each chamber to act as “breathing” membranes to keep the pH and oxygen levels stable in the incubation environment.

The investigation of interorgan effects can be achieved by coupling two microchambers, one containing an intestinal tissue slice, the other a liver slice, which can be sequentially perfused. In this way, metabolites formed by the intestine in the first chamber will be directed to the liver in the second chamber, thereby mimicking in vivo, first-pass metabolism. By sequential perifusion of intestinal and liver slices, the interplay of the two organs in the regulation of bile acid synthesis will be studied.

EXPERIMENTAL
Single liver and ileum slices were perifused for 7 hours with medium with or without 50 µM CDCA in the biochip shown in Figure 1. For sequential perfusion, the outlet of the chamber with the ileum slices was connected to the inlet of the second chamber with the liver slice (Figure 2). The mRNA expression of FGF15 in the ileum slices was measured by RT-PCR with villin as housekeeping gene. In liver slices, the expression of CYP7A1 was measured with GAPDH as housekeeping gene. The gene expression in the CDCA-treated slices was normalized to that in the slices incubated for 7 hours without CDCA. This study was performed in three rats with three slices per experiment (rat).
RESULTS AND DISCUSSION

When intestinal slices were treated with CDCA, FGF15 was highly upregulated in the intestinal slices from all three rats. As expected, no significant differences were obtained between the intestinal slices perfused in a single biochip (166 fold) and those perfused sequentially with liver slices (151 fold). The expression of CYP7A1 in the liver slices was not affected by sequential perfusion without CDCA. In contrast, when CDCA was added to the medium, CYP7A1 was reduced to 70% in the single liver biochip. More importantly, during sequential perfusion of intestine and liver, the expression was further reduced to 40% (Figure 3).

These results confirm the intestine-liver signaling pathway and support the hypothesis that FGF15 produced by the intestine plays a role in the regulation of CYP7A1 in the liver.
CONCLUSION

This biochip incorporating the sequential perifusion of slices of two different organs is the first model to show in vitro interorgan signaling using intact tissue. It represents a promising model to contribute to the reduction of animal experiments and to study these processes in man.

REFERENCES

