Mechanism-Based Modeling of Rebound Tachycardia after Chronic \(l\)-Propranolol Infusion in Spontaneous Hypertensive Rats\(^1\)

LENNA BRYNNE, LENNART K. PAALZOW, and MATS O. KARLSSON

Department of Pharmacy, Division of Biopharmaceutics and Pharmacokinetics, Uppsala University, Uppsala, Sweden

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ABSTRACT

The aims of the study were to characterize the rate and extent of the rebound effect after abrupt cessation of a chronic exposure of \(l\)-propranolol in spontaneous hypertensive rats, using exercise-induced tachycardia as a pharmacodynamic endpoint. Thirty-two spontaneous hypertensive rats were randomized to receive either placebo or 4 or 8 mg/kg/day s.c. infusion of \(l\)-propranolol for 11 days using osmotic minipumps. The heart rate was measured after standardized physical exercise before and during drug exposure and over 12 days after cessation, using a computerized tail-cuff method. Blood samples were collected after each effect measurement during the infusion. A similar reduction in exercise tachycardia was registered for the two doses. No apparent tolerance development was found, but both doses showed a clear rebound effect of similar extent and intensity. The maximal rebound effect was observed on the second day after cessation and was found to have a duration of about 6 days. A mechanism-based model was developed to describe the rate and extent of changes in \(\beta\)-adrenoceptor up- and down-regulation with increased sensitivity of the transducer complex. The half-life of disappearance of up-regulated \(\beta\)-adrenoceptors was estimated to be 2.0 days (1.0–3.9 days). The effect-versus-time data was analyzed by nonlinear mixed-effect modeling with the program NONMEM. A dose-dependent reduction in the growth of body weight was observed during drug treatment, which was reversible. A dose- and time-dependent increase in the \(\alpha_1\)-acid glycoprotein concentration was also observed.

Rebound effects may occur on abrupt withdrawal of chronic \(\beta\)-antagonist therapy and are a known phenomenon in patients with coronary heart disease. This phenomenon has been described for patients using propranolol, a nonselective \(\beta\)-antagonist, but also for patients using other \(\beta\)-antagonists (see Houston and Hodge, 1988, for a review). The symptoms seem to be related to the severity of the disease (Miller et al., 1975), where a worsening of angina, myocardial infarction, ventricular dysrhythmias, and sudden death have been observed after discontinuation of propranolol in patients with angina pectoris. Hypertensive patients have experienced transient symptoms of palpitations, tremors, headache, malaise, and sweating after drug cessation (Nattel et al., 1979). Several different mechanisms have been proposed for these symptoms, but the hypothesis that has received the most interest is that a transient hyperadrenergic state follows drug cessation. This hypothesis has been confirmed in many but not all studies in humans (Boudoulas et al., 1977; Nattel et al., 1979) and in animals (Faulkner et al., 1973; Aarons et al., 1980; Cramb et al., 1984; Ebii et al., 1991). The hypersensitivity may relate in part to an increased \(\beta\)-adrenoceptor density during chronic treatment (Aarons et al., 1980; Motulsky and Insel, 1982; Brodde et al., 1986). However, it could also be that there are changes in the efficiency of the coupling between the receptor and the second messenger system and/or an increased concentration of endogenous mediators such as catecholamines. Other explanations include the progression of underlying coronary artery disease, enhanced platelet aggregation or tri-iodothyronine levels, alteration in plasma renin activity, or continued high levels of physical activity despite withdrawal of propranolol (for reviews, see Prichard et al., 1983; Frishman, 1987).

The cardiovascular effects of \(\beta\)-antagonists result in alterations in myocardial contractility and rate, which is the primary mechanism by which most \(\beta\)-antagonists produce their therapeutic effects in conditions like angina and hypertension. Reduction in exercise-induced tachycardia is the most widely used method to measure the pharmacodynamic effect of \(\beta\)-antagonists in humans. Exercise-induced tachycardia is

ABBREVIATION: AGP, \(\alpha_1\)-acid glycoprotein.
also a reliable pharmacodynamic endpoint in spontaneous hypertensive rats when studying β-antagonists (Bryrne et al., 1998). Changes in the response were detectable at low concentrations of the β-antagonists, and the effect was studied over a wide range of concentrations.

In contrast to the abundance of reports on rebound effects after the abrupt withdrawal of β-antagonists, only limited attention has been given to the quantitative aspects of the rebound effect despite the extensive use of β-antagonist in the clinical setting. Because of the complexity in tolerance and rebound development, physiological models have been difficult to obtain, although attempts have been made using the effect of propranolol and increased β-adrenoceptor density (Lima et al., 1989).

Because of the large difference in potency between the two enantiomers of propranolol (Walle et al., 1988), the l-enantiomer was selected for this study. The binding of l-propranolol to plasma proteins, primarily to α1-acid glycoprotein (AGP), is high in the rat and saturable at high concentrations (Bryrne et al., 1998). Thus, changes in the AGP level may affect the pharmacokinetics of propranolol (Yasuhara et al., 1983) and could be a major determinant of the variability in the response.

The aim of this study was to characterize the rate and extent of rebound effect after withdrawal of a chronic infusion of l-propranolol in spontaneous hypertensive rats using exercise-induced tachycardia as the pharmacodynamic endpoint. Due to the mechanism of drug action, we propose a mechanism-based pharmacodynamic model that characterizes the rate and extent of changes in β-adrenoceptor density with increased sensitization of the transducer complex.

Materials and Methods

Animals. Male spontaneous hypertensive rats of 285 ± 17 (S.D.) g and 3 to 3.5 months old were obtained from Mollegaard Ejby, Denmark). They were kept in a regulated room with a 12-h light/ dark cycle (lights on 7:00 AM to 7:00 PM), a temperature of 22 ± 1°C, and humidity of 55 ± 5%. Standard diet and water were provided ad libitum, except during the effect measurement. The rats were kept individually in Macrolon cages, where they received 25 ml of a d-glucose solution (50 mg/ml; KEBO Lab, Stockholm, Sweden) in a 0.9%-saline solution and 25 ml of a d-glucose solution (50 mg/ml; KEBO Lab, Stockholm, Sweden) in a 0.9%-saline solution. The rats were familiarized with the environment and the equipment. Exercise-induced tachycardia was obtained by making the animals run in a motorized wheel for 10 min (6 m/min). Then they were transferred to a Macrolon cage with a thermostat-controlled warm pad (37°C) for 10 min. The heart rate was measured over 3 consecutive days before the start of infusion and then on days 1, 4, 5, 7, 8, and 11 after the start of infusion. Six hours after surgical removal of the minipumps, an effect measurement was performed and continued on days 12, 13, 14, 15, 16, 18, 19, 21, and 22. The measurements were performed every day at the same time.

Chemicals for Analysis. Crystalline l-propranolol hydrochloride (99%), dl-metoprolol tartrate, l-6-antagonists (Brynne et al., 1998). Thus, changes in the AGP level may affect the pharmacokinetics of propranolol (Yasuhara et al., 1983) and could be a major determinant of the variability in the response.

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tions in the pharmacodynamic analysis. In a pilot study, steady-state plasma concentrations were obtained 3 h after the start of infusion with osmotic minipumps. In the present study, the first blood samples were not drawn until 24 h after the start of infusion, and therefore, the individual 24-h plasma concentration values were used after 3 h as well as after 24 h to obtain a more accurate plasma concentration-time profile. Individual terminal half-lives were calculated using standard procedures (Gibaldi and Perrier, 1982).

A mechanism-based model was developed (Fig. 1) in which l-propranolol competitively inhibits norepinephrine to activate the β-adrenoceptors and the transducer complex. This competitive antagonism results in an increased β-adrenoceptor density and a reduced heart rate. The classic equation for competitive antagonism (Gaddum, 1937)

\[ R^* = \frac{C_{NE}/K_{D,NE}}{1 + C_{prop}/K_{D,prop} + C_{NE}/K_{D,NE}} \]

(1)
gives the fractional receptor occupancy by the agonist (R*/RT) for any given concentrations of agonist and antagonist. The total amount of receptors (RT) cannot be measured in vivo and therefore was set to start at one unity. This equation predicts that in the presence of a competitive antagonist, the fractional receptor occupancy by the agonist will be lower than that in absence of antagonist. In the equation, C<sub>NE</sub> corresponds to the plasma concentration of norepinephrine, C<sub>prop</sub> corresponds to the l-propranolol concentration, and K<sub>D,NE</sub> and K<sub>D,prop</sub> are the equilibrium dissociation constants for norepinephrine and l-propranolol, respectively. For simplicity, the ratio between the norepinephrine concentration and its K<sub>b</sub> value is estimated. When no drug is present, the fractional number of activated receptors (R*/RT) is described by the following equation:

\[ \frac{R^*}{R_T} = \frac{C_{NE}/K_{D,NE}}{1 + C_{prop}/K_{D,prop} + C_{NE}/K_{D,NE}} \]

(2)

The change in total β-adrenoceptor density (R<sub>T</sub>) over time is described by the following function:

\[ f(R_T) = \frac{dR_T}{dt} = k_{rec} \left(1 + SL \frac{R^*_0 - R^*}{R^*_0} - k_{rec} \cdot R_T \right) \]

(3)

where k<sub>rec</sub> is the first order rate constant for degradation of β-adrenoceptor density. A linear slope (SL) is used to relate the fractional change in the number of activated receptors to the change in total β-adrenoceptor density. Two models were developed according to the hypothesis of mechanisms of the rebound phenomenon. Model A—A competitive antagonist model with a change in transducer efficiency (T<sub>D</sub>) (Black and Leff, 1983) due to the increased receptor density was described as follows:

\[ T_D = \frac{E_{max} \cdot R^*_0}{K_T + R^*_0} - \frac{E_{max} \cdot R^*_0}{K_T + R^*} \]

(4)

where E<sub>max</sub> is the maximal effect in response, and K<sub>T</sub> is the fraction of activated receptors for producing 50% of maximal response.

Model B—A competitive antagonist model with both a change in receptor density (model A) and an increased sensitization of the transducer efficiency over time. The sensitization time profile is assumed to resemble the change in receptor density over time [f(R<sub>T</sub>)], which is therefore denoted k<sub>trans</sub> in Fig. 1.

\[ T_D = f(R_T) \cdot \frac{E_{max} \cdot R^*_0}{K_T + R^*_0} - \frac{E_{max} \cdot R^*_0}{K_T + R^*} \]

(5)

In this model, the estimated rate constant for degradation of the β-adrenoceptor density (k<sub>rec</sub>) from model A was used to estimate the increase in the extent of rebound tachycardia because there was a large correlation between k<sub>rec</sub> and the slope (SL).

In both models, the change in efficiency of the transducer complex was directly related to the exercise-induced tachycardia (E), according to the following relationship:

\[ E = E_0 \cdot (1 + T_D) \]

(6)

where E<sub>0</sub> is the heart rate baseline. The K<sub>T</sub> value in the transducer equations was set to 3% but subject to sensitivity analysis because it is known that the β-adrenoceptor systems have a large amount of spare receptors in rats. Only 1.5 to 3% of β-adrenoceptors have to be occupied by isoproterenol to cause 50% of maximal response (Brown et al., 1992). The effect after placebo administration and the increase in body weight were not accounted for in the pharmacokinetic/pharmacodynamic data analysis.

Both concentration- and effect-versus-time data were analyzed by nonlinear mixed-effect modeling within the program NONMEM Version V (Beal and Sheiner, 1999), using the first order approximation method and ADVAN 8 as subroutines. Mean population parameters were assessed, as well as interanimal and residual variabilities. An exponential variance model was used to describe the interanimal variability, and an additive error model was used to characterize the residual errors in the pharmacodynamic models. Individual parameter values are obtained from the Bayesian estimation. Statistical discrimination between different models was made by comparison of the objective function values (−2log likelihood) calculated by NONMEM and by visual inspection of the goodness-of-fit plots in the program Xpose (Jonsson and Karlsson, 1998) running under Splus, Version 3.3 (Statistical Sciences, 1993). The difference between the objective function values for two hierarchical models is approximately χ<sup>2</sup> distributed and may consequently be used for model selection purposes.

All data are given as mean ± S.D. or with a 95% confidence interval unless otherwise stated. For statistical comparison of stability of the drug over time, the Student’s t test for paired data was used. The differences in AGP and weight between groups were compared by using one-factor ANOVA, followed by the Fisher PLSD test (StatView; Abacus Concepts, Inc., Berkeley, CA). The significance level was set to 95%.

**Results**

The stability test of the drug solution showed no significant difference after 11 days of s.c. infusion. The body weight of the animals increased during the study, but a reduced rate of change was observed during l-propranolol exposure at the higher dose level (Fig. 2), an effect that was reversible when l-propranolol was withdrawn. The difference in the body weight was only significant (p < .05) for the rats that received 8 mg/kg/day l-propranolol on day 11.
Pharmacokinetics. A statistical significant increase in AGP levels was observed on day 11 at the lower L-propranolol dose and on day 5 during the higher L-propranolol dose (Fig. 3), but no changes were observed in the control group.

The mean plasma concentration time profiles of L-propranolol for the two dose groups are presented in Fig. 4. A relatively large concentration range was obtained (221–448 and 89–213 ng/ml for the higher- and lower-dose group, respectively, on day 1). The rate of infusion from the minipumps declined over time. The terminal half-lives of L-propranolol, 1.31 ± 0.24 h and 1.13 ± 0.26 h, did not deviate between the two dose groups.

Postexercise Heart Rate over Time. The mean basal exercise-induced heart rate was 386 ± 15 beats/min (n = 11) and 383 ± 17 beats/min (n = 11) for the lower- and higher-dose groups, respectively, and 388 ± 19 beats/min in the control group, before the start of infusion (Fig. 4).

Similar reductions in exercise-induced heart rate during the L-propranolol infusion were observed in both groups (Fig. 4; the individual observed and predicted heart rate values were normalized to the corresponding baseline values). The mean reductions for the lower- and higher-dose groups were similar and varied between 12 and 14%. No apparent tolerance development was observed, but a clear rebound effect was obtained after the cessation of the drug. The rebound effect was similar in the two dose groups, where the rebound effect reached its maximum 2 days after cessation and had a duration of about 6 days for both doses. The intensity of the rebound effect was also similar in the two dose groups (about 6% increase). The placebo effect showed a transient increase in exercise-induced heart rate with a maximal effect on day 5.

Pharmacodynamic Modeling. The population pharmacodynamic estimates for model A (competitive model with increased receptor density) and model B (model A with increased transducer sensitivity) are listed in Table 1. The estimated half-life of the production and disappearance of the β-adrenergic receptor was 2.0 days (1.0–3.9 days). The interanimal variability for each pharmacodynamic estimate is shown in Table 1, and the S.D. of the residual variability was 15 beats/min. Only a small rebound effect was predicted by model A, whereas model B gave a more accurate fit to data when adding a function describing increased sensitization of the transducer complex.
The present study demonstrates that rebound tachycardia occurred after abrupt cessation of a chronic s.c. infusion of l-propranolol in spontaneous hypertensive rats, using exercise-induced tachycardia as the pharmacodynamic endpoint. The rebound effect was seen without any apparent tolerance development, which might be due to the limited information that could be obtained after the initial surgery. The rebound maximum, 2 days after drug withdrawal, and the 6-day duration correspond well with studies in hypertensive patients, where the transient hypersensitivity to isoproterenol or exercise-induced tachycardia commenced 2 to 6 days after cessation and lasted for 3 to 13 days (Nattel et al., 1979). The absence of a difference in effect between the two doses could be because the concentration range was near or at the maximal effect of the drug (about 15% reduction), which is close to an earlier observed maximal effect (I\textsubscript{max} = 21%; Brynne et al., 1998). The transient increase in exercise-induced heart

![Fig. 5. Simulations of different steady-state concentrations (5, 10, 20, 50, 100, 200, and 400 ng/ml) using the population parameter estimates from model B. Top, reduction and rebound tachycardia after the different concentrations. Bottom, fractional changes in total \( \beta \)-adrenoceptor density (R\textsuperscript{t}) and the fractional number of activated \( \beta \)-adrenoceptors (R\textsuperscript{*}) over time.](image)

<table>
<thead>
<tr>
<th>Parameter( ^a )</th>
<th>Model A</th>
<th>Model B</th>
</tr>
</thead>
<tbody>
<tr>
<td>( E_0 ) (beats/min)</td>
<td>384</td>
<td>383</td>
</tr>
<tr>
<td>( E_{\text{max}} ) (%)</td>
<td>20.2</td>
<td>17.3</td>
</tr>
<tr>
<td>( K_{D,\text{Prop}} ) (ng/ml)</td>
<td>2.15</td>
<td>1.71</td>
</tr>
<tr>
<td>( C_{\text{NE}}/K_{D,\text{NE}} )</td>
<td>0.0669</td>
<td>0.141</td>
</tr>
<tr>
<td>( SL )</td>
<td>1.1</td>
<td>0.193</td>
</tr>
<tr>
<td>( k_{\text{rec}} ) (day(^{-1} ))</td>
<td>0.35</td>
<td>0.35(^* )</td>
</tr>
</tbody>
</table>

\( ^a \) \( E_0 \), heart rate baseline; \( E_{\text{max}} \), effect of maximal response; \( K_{D,\text{Prop}} \), the equilibrium dissociation constant for l-propranolol; \( C_{\text{NE}}/K_{D,\text{NE}} \), ratio between the norepinephrine concentration and its equilibrium dissociation constant; \( SL \), linear slope that relates the fractional change in the number of activated receptors to the change in total \( \beta \)-adrenoceptor density; \( k_{\text{rec}} \), first-order rate constant for degradation of \( \beta \)-adrenoceptor density.

\( ^b \) 95% confidence interval calculated from standard errors.

\( ^c \) IAV, interanimal variability (CV%).

\( ^d \) N.E., not estimated in the model.

\( ^e \) 95% confidence interval obtained from the likelihood profile method.

\( ^f \) Fixed value from model A.
rate could be viewed as a change in the endogenous norepinephrine concentration over time; however, at maximal drug effect, the relative contribution of an increased norepinephrine concentration is negligible.

Rebound effects generally occur after withdrawal of a drug to which tolerance has been developed. This is explained by the persistence of the effect being counteracted after drug cessation, which is often linked to the process of tolerance development (Bauer and Fung, 1994). Several pharmacodynamic models that describe both tolerance development and a rebound effect have been proposed. These models differ in the driving force of the effect, which could be either the drug concentration or the drug effect (see Gårdmark et al., 1999, for a review), and they require more or less tolerance development to be able to account for the rebound effect. They also are more or less empirical with respect to the absence of knowledge of the mechanism of drug action.

The β-antagonists act by preventing catecholamines from binding to the β-adrenoceptors and thereby inhibit the activation of adenylyl cyclase, which catalyzes the intracellular production of cyclic AMP and its stimulation of protein kinases. This cascade will result in a decreased level of intracellular calcium, which activates the cardiac troponin complex, thereby lowering the heart rate. The β-adrenoceptor density and the sensitivity of the adenylyl cyclase will increase during the exposure of antagonist and slowly return to normal conditions after cessation of the drug (Aarons et al., 1980; Motulsky and Insel, 1982). We propose a mechanism-based model that includes a time-dependent increase in the β-adrenoceptor density during drug exposure and spare receptors (model A). An additional function describing a sensitization of the transducer complex was needed to better describe the extent of the rebound effect (model B). Both models described the fast reduction in exercise-induced tachycardia during drug exposure. A drawback was that none of the models were successful regarding the peak time of the rebound effect. Although efforts were made to include both magnitude and time delay in the sensitization of the transducer complex, increasing model size resulted in either unacceptably low parameter precision or numerical difficulties of the integration routine used. The rate-limiting step could be the distribution of the drug to the receptor or at the postreceptor level. Recently, Sheiner and Verotta (1995) presented a general model in which they proposed that the pharmacodynamic response consists of a cascade of dynamic and static functions. Depending on whether distributional or postreceptor events form the rate-limiting step, the general model would collapse into a direct (Sheiner et al., 1979) or an indirect (Dayneka et al., 1993) response model, respectively, and these models should be considered as submodels of the general model. The present mechanism-based model includes both prereceptor and postreceptor events and thus is related to the general model. The time course of reduction in exercise tachycardia after a single dose of propranolol is reflected by the antagonist concentration present in plasma samples at each time point, and the exercise tachycardia parallels the β₁-adrenoceptor occupancy in humans (Wellstein et al., 1985; De Mey, 1997). This means that the plasma concentrations of antagonist are representative of the concentrations in the effect compartment. Therefore, it is most likely that the sensitization of the transducer complex or other postreceptor events should be the rate-limiting step. Another rate-limiting step could be down-regulation of β-receptors, where the synthesis of the receptor could be reduced or the degradation be enhanced (Lohse, 1993).

Both models predicted rebound tachycardia, although model B did so to a larger extent, thus suggesting that a sensitization of the transducer complex occurs during the drug exposure. The steady-state concentration used in the present study resulted in similar reductions and rebound tachycardia. It would have been interesting to use a lower dose to obtain concentrations below the IC₅₀ value of I-propranolol (18.1 ng/ml; Brynne et al., 1998) to be able to register the increase in receptor density by less reduction in heart rate during steady state, according to the simulations in Fig. 5. However, registrations of small changes in heart rate (at low plasma concentrations) are difficult due to the variability in the tail-cuff technique. The influence on the rebound tachycardia due to different half-lives of the drug was also simulated, which was shown to be large. This indicates that the pharmacokinetics of the drug (longer half-life) or a slow withdrawal of the drug will reduce the extent of rebound tachycardia (see Fig. 6).

The β-adrenoceptor density was estimated to increase by 32% (model B), which is similar to values reported from human studies on β₂-adrenoceptors lymphocytes (25–51%; Fitzgerald et al., 1981; Brodde et al., 1986; van den Meiracker et al., 1989). The half-life of β-receptor degradation was 2.0 days (1.0–3.9 days) in the present study. It is little less than the mean recovery rates reported in healthy
volunteers, where the half-life was 2.7 days (Reeves et al., 1989). The large variability in the half-life of the β-receptor degradation and the linear receptor relationship in our model were probably due to a large variability in the rate and extent of rebound tachycardia between rats.

A similar mechanism-based model was presented previously by Lima et al. (1989), using literature values for computer simulations. This model uses a relationship between the parasympathetic and sympathetic nervous systems to simulate the heart rate response over time, but it does not account for spare receptors or sensitization of the transducer complex. Furthermore, this model showed a very small extent of rebound tachycardia in comparison with the reduction in isoproterenol-induced tachycardia during drug exposure (maximal effect of 100%). This model also uses a half-life of 1.5 days for the β-receptor on lymphocytes (Aarons and Molinoff, 1982), which is low in comparison with the 4.0 days reported in healthy volunteers (Reeves et al., 1989).

The dose- and time-dependent increase in the AGP concentration could be due to activation of the immune system at high propranolol concentrations. It is known that elevated sympathetic activity can modulate the immune system, and that withdrawal of this activity by the administration of drugs such as propranolol can induce the activity of the immune system (Maisel et al., 1991); although this has not been observed previously for AGP, it has been observed for other parameters of immunity. Another possible explanation for the increase in AGP level could be infection, inflammation, or stress because the AGP level increases in these situations. However, because no changes were observed in the control group, a non-drug-induced increase is unlikely. The increase in AGP was unexpected, and further investigations are needed for correct interpretation of this phenomenon. However, during both infusions, the E_{max} value was obtained, and a change in AGP will have only a marginal influence on the effect. Consequently, fluctuations in AGP concentration are likely to be important around and below the EC_{50} value, that is, when the infusions have been stopped.

The dose-dependent reduction in the growth rate observed in this study was reversible after l-propranolol withdrawal. Similar findings have been shown in Wistar rats at high propranolol doses (Paraskevopoulos et al., 1991) without any alterations in the plasma growth hormone or hypothalamic somatostatin concentrations. We observed that the rats receiving the higher dose became sedated during drug exposure. A possible explanation is that the rats that received the higher-dose level were too sedated to eat and had a more pronounced lack of appetite. The reason behind the growth retardation is unknown at present and requires further investigation.

In conclusion, similar intensities and durations of the rebound effect have been reported in human studies as in the present study, suggesting that this could be a suitable animal model in which to study rebound phenomenon, although further studies must be performed. This is an attempt to mechanistically characterize the rate and extent of the rebound effect in spontaneous hypertensive rats after l-propranolol infusion. The result suggests that additional studies on cAMP turnover and/or mRNA regulation of β-receptors are necessary for a better characterization of the rebound phenomenon.

**References**


Send reprint requests to: Dr. Lena Brynne, Department of Pharmacy, Division of Biopharmaceutics and Pharmacokinetics, Box 580, SE-751 23 Uppsala, Sweden. E-mail: lena.brynne@biof.uu.se