Pharmacokinetic modeling of the subcutaneous absorption of therapeutic proteins

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Abbreviations:

AUC – area under the plasma (or serum) concentration-time curve; BMI – body mass index; CHO – Chinese hamster ovary; CL – clearance; F – absolute bioavailability; FcRn – Fc receptor of neonates; GCSF - granulocyte colony stimulating factor; GH – growth hormone; IFN – interferon; IL – interleukin; IU – international units; IV – intravenous; mAbs - monoclonal antibodies; MIU – million international units; MM – Michaelis-Menten; NPH - neutral protamine Hagedorn; rh – recombinant human; PEG – pegylated; SC – subcutaneous; TMDD – target-mediated drug disposition; TNF – tumor necrosis factor; Vₐ – volume of distribution; VEGF – vascular endothelial growth factor.
Abstract

Subcutaneous (SC) injection is an important route of administration for therapeutic proteins that provides several advantages over other modes of parenteral delivery. Despite extensive clinical use, the exact mechanism underlying SC absorption of proteins is not completely understood and the accuracy of prediction of absorption of biotherapeutics in humans remains unsatisfactory. This review summarizes a variety of models that have been developed for describing the pharmacokinetics of therapeutic proteins administered by SC injection, including single- and dual-pathway absorption models. Modeling of the lymphatic uptake and redistribution, absorption of monoclonal antibodies and insulin, and population analysis of protein absorption are discussed. The review also addresses interspecies modeling and prediction of absorption in humans, highlights important factors affecting the absorption processes, and suggests approaches for future development of mechanism-based absorption models.
Introduction

Biotherapeutics is a rapidly growing class of drugs with more than 200 biopharmaceuticals already approved, including vaccines, hormones, cytokines, and monoclonal antibodies (mAb) (Walsh, 2010; Janowitz, 2011; Reichert, 2012). Several hundred new products are under development, such as a variety of mAbs, Fc-fusion proteins, antibody-drug conjugates, and alternative protein scaffolds (Beck et al., 2010; Dostalek et al., 2013). Parenteral administration is a major limitation for treatment with biotherapeutics. The subcutaneous (SC) route may be advantageous over intravenous (IV) or intramuscular administration, especially for improving patients’ convenience and reducing treatment costs (McDonald et al., 2010; Dychter et al., 2012; Richter et al., 2012). SC injection has been used clinically for decades for administration of insulin, erythropoietin, growth hormone, etc. Several mAbs and Fc-fusion proteins are currently approved for SC administration, and the interest in SC delivery of other antibody-based treatments is growing (Misbah et al., 2009; Aue et al., 2010; Bittner et al., 2012; Bittner and Schmidt, 2012; Davies et al., 2014; Tobinai, 2014). Variable and incomplete bioavailability is a major concern for SC delivery; and the mechanisms of SC absorption of protein therapeutics are not fully understood and require further investigation. Furthermore, current methods for predicting absorption of biotherapeutics in humans are inadequate (McDonald et al., 2010; Richter et al., 2012). Pharmacokinetics of protein therapeutics is often nonlinear, and extensive mathematical modeling may be required for resolving complexities associated with their absorption and disposition.

This review summarizes a variety of existing models for describing the pharmacokinetics of therapeutic proteins administered by SC injection, highlights important factors affecting the absorption process, and suggests approaches for future development of mechanism-based absorption models.

Background

SC injection delivers drugs into the hypodermis, the interstitial space located below the dermis. The structure and physiology of the interstitial space have been reviewed extensively (Porter and Charman, 2000; Swartz, 2001; Lin, 2009; Richter et al., 2012). Briefly, collagen, glycosaminoglycans, and elastin...
are the main structural units of the interstitium that provide strength and elasticity, while maintaining the volume of the matrix. The extracellular matrix has an overall negative charge, and transport of macromolecules through the interstitium is likely dependent on their physical and electrostatic interaction with the components of the matrix. It has been suggested that the interstitial transport is the rate-limiting step in absorption of macromolecules (Porter and Charman, 2000; Swartz, 2001). Following SC injection, protein therapeutics can reach the systemic circulation through blood and lymphatic capillaries. Blood capillaries of the SC space are continuous in nature, characterized by tight interendothelial junctions and an uninterrupted basement membrane, which provides a selective barrier for molecular permeation. Lymphatic capillaries are built from a single layer of overlapping endothelial cells, the basement membrane is incomplete, and the tight junctions are absent (Swartz, 2001). The structure of the lymphatic system allows for unidirectional flow; and the lymph is formed as the interstitial fluid enters the lymphatic capillaries, driven by the gradient in the hydrostatic and osmotic pressure. The role of the lymphatic system in SC absorption of therapeutic proteins and particulate formulations has been investigated in several species (Porter et al., 2001; McLennan et al., 2005b; Gershkovich et al., 2007; Wang et al., 2012).

Factors affecting the absorption of biotherapeutics after SC injection can be divided into species-, subject-, and drug-dependent. Differences in skin morphology, e.g., presence of panniculus carnosus muscle in lower species and the greater mobility of skin in scruff animals, may contribute to the lack of correlation between the bioavailability of biotherapeutics in animals and humans (McDonald et al., 2010; Richter et al., 2012). Differences in binding affinity between FcRn and IgG among species may complicate interspecies modeling of SC absorption of mAbs (Ober et al., 2001). Although pig skin is similar to human skin, the rate of SC absorption of several mAbs was 2- to 5-fold greater in minipigs as compared to humans (Zheng et al., 2012). The bioavailability of mAbs in humans is often overestimated based on non-human primate data (Richter et al., 2012). The structure of the SC tissue in animals and humans varies depending on the anatomical location in the body, which may result in different absorption behavior and increase the pharmacokinetic variability (Beshyah et al., 1991; Macdougall et al., 1991; Ter Braak et al., 1996; Kota et al., 2007; Kagan et al., 2012). In humans, hypodermis thickness increases
with body weight, decreases with age, and is dependent on gender (Gibney et al., 2010). For example, the distance between the skin and the gluteal muscle was 1.6 cm in normal women (BMI 18.5-24.9 kg/m²) as compared to 3.6 cm in obese women (BMI 30-40 kg/m²) (Shah et al., 2014). Other factors controlling the rate and the extent of absorption include the molecular size of the drug, formulation excipients, restraint status or anesthesia, and application of heat or pressure (Porter and Charman, 2000; Porter et al., 2001; McLennan et al., 2005b). For example, the rate of the thoracic lymph flow was higher in freely moving animals as compared to anesthetized ones (Lindena et al., 1986), and the rate of absorption of soluble insulin was positively correlated with the SC blood flow (Vora et al., 1992).

Modeling of the SC absorption of therapeutics proteins

Pharmacokinetic and pharmacodynamic relationships for therapeutic proteins tend to be more complex than for small molecule drugs. High affinity binding to pharmacological targets (soluble and tissue-bound) often leads to nonlinear distribution and elimination patterns, which are dependent on the drug dose and the underlying dynamics of the target. The biodisposition of mAbs is affected by multiple binding interactions specific to the particular drug (through the Fab region) or drug class (through the Fc region). Noncompartmental methods of analysis, including calculation of the bioavailability and interspecies scaling, can be unreliable for biotherapeutics due to dose-dependent pharmacokinetics and slow equilibration between the plasma and the sites of elimination (which violates the underlying assumptions of this approach). Therefore, advanced mechanism-based pharmacokinetic/pharmacodynamic models may be required for describing absorption, biodisposition, and efficacy in preclinical and clinical studies. Significant progress has been made in understanding the complexities of the systemic pharmacokinetics of protein therapeutics in recent years. Target-mediated (or receptor-mediated) drug disposition (TMDD) models are often applied to mechanistically describe the systemic disposition of biotherapeutics (Levy, 1994; Mager and Jusko, 2001). Binding of mAbs to FcRn and the role of this interaction in protecting mAbs from degradation and extending their biological half-life
is well established (Ghetie et al., 1996; Junghans and Anderson, 1996), and an endosomal recycling model was proposed to describe this phenomenon (Hansen and Balthasar, 2003).

This review is focused on the absorption component of the pharmacokinetic models developed for therapeutic proteins. In the majority of the discussed studies, modeling of systemic disposition was supported by data obtained after IV administration of compounds. It should be noted, that the selection of the systemic structural model (especially in the case of nonlinear disposition) might have a substantial impact on the fit of the absorption phase of pharmacokinetic profiles. Analysis of such interactions and their impact on the selection of appropriate absorption models is beyond the scope of this review. To facilitate the discussion, equations describing the systemic disposition were replaced by a (±disposition) term. For the purposes of this review, absorption models were divided into single- and dual-pathway categories. The later was subdivided into empirical models (based on studies measuring plasma/serum concentrations only) and mechanistic models describing the plasma pharmacokinetic profiles and the amount of drugs in the lymph simultaneously. Absorption models for mAbs and insulin are reviewed separately.

Single-pathway SC absorption models

Although SC absorption of therapeutic proteins is controlled by multiple factors, a rate-limiting step in the absorption pathway (such as, transport through the interstitium, penetration of blood capillaries or lymph flow rate) may limit our ability to identify these complexities. The simplest structural model being evaluated to describe the SC absorption includes a first-order absorption rate constant (\(k_a\)) and a bioavailability term (\(F\)) (Figure 1A). This model has been successfully applied for describing the SC pharmacokinetics of many proteins (e.g., human IFN-\(\alpha\)2b in healthy human volunteers (Radwanski et al., 1987) and rhGH in rhesus monkeys (Sun et al., 1999)). Selection of this simple model is often dictated by limited data (e.g., single dose level or sparse sampling during the absorption phase). The number the model parameters can be further reduced by estimating the apparent volume of distribution (\(V_d/F\)) and clearance (\(CL/F\)) terms if the IV administration is not evaluated and \textit{a priori} knowledge of the systemic bioavailability is unavailable. Absorption rate-limited elimination (flip-flop kinetics) should be considered
in this case and physiological interpretation of the modeling results may be problematic. When the rates of absorption and elimination appear similar, the corresponding rate constants can be constrained to be equal. This approach was applied to describe the time-course of IFN-α2a and PEG-IFN-α2a concentrations after SC administration of a range of doses (3-18 MIU) to healthy volunteers (Nieforth et al., 1996) and PEG-IFN-β1a (0.3-3 MIU/kg) to cynomolgus monkeys (Mager et al., 2005).

A Michaelis-Menten model ($V_{\text{max}}$ and $K_m$) can be implemented if saturation of the absorption kinetics with the increasing dose level is observed. This mechanism was used to describe the absorption of rhGH in chronic dialysis patients and healthy subjects (Klitgaard et al., 2009) and to support dose selection for exenatide in clinical studies (Fineman et al., 2007). Carrier-mediated transport or precipitation at the injection site are commonly postulated; however, the nature of this saturable absorption for proteins is generally unknown.

A model with a single transit compartment between the injection site and the central compartment of the systemic disposition model was proposed to describe a distinct delay in SC absorption of some proteins (Figure 1B) (Mager and Jusko, 2002; Mager et al., 2003; Segrave et al., 2004). The transit compartment was sometimes attributed to the lymphatic system, although no direct assessment of the lymphatic component was performed. Parameterization of the model includes the bioavailability ($F$) and two first-order rate constants ($k_{a1}$ and $k_{a2}$), which cannot be uniquely identified.

Biotherapeutics often exhibit nonlinear SC absorption, and dose-depended parameters are commonly utilized to facilitate model fitting. While this approach may be appropriate in fit-for-purpose scenarios, it provides little insight into the mechanisms of absorption for a specific drug, which is essential for accurate assessment of the bioavailability, optimizing delivery, and predicting absorption in humans, including the effects of population covariates. For example, a dose-dependent absorption rate constant ($k_{a2}$, increasing with the dose level) was required for describing the absorption of leukemia inhibitory factor administered to sheep (Segrave et al., 2004). Evaluation of other absorption models, e.g., zero-order and a combination of the first- and zero-order, did not provide satisfactory results. Dose-dependent bioavailability (increasing with the dose level) combined with target-mediated systemic disposition was used to capture the pharmacokinetics of IFN-β1a in cynomolgus monkeys (Mager et al., 2005).
Saturation of the injection site-specific metabolism was hypothesized to be the source of this phenomenon; however, incorporation of the Michaelis-Menten mechanism did not improve the fit (Mager et al., 2003).

**Empirical dual-pathways SC absorption models**

The existence of two absorption pathways (through the blood and lymph capillaries) for large molecules following injection into the interstitial space has been recognized for many years. Deconvolution analysis of plasma concentration-time profiles after SC administration frequently demonstrated several absorption phases for therapeutic proteins (Radwanski et al., 1998; Ramakrishnan et al., 2004). A model with two parallel absorption processes was suggested, although a direct assessment of the contribution of the lymphatic system was not performed experimentally. A general schematic of the model is shown in Figure 1C, and several variations of this model have been used to describe the absorption of proteins in animals and humans (Table 1). The model utilizes a combination of a zero- and first-order processes originating from the SC space and leading to the central distribution compartment. The zero-order process is limited in duration from the time of injection to time \( \tau \), and is usually described as absorption through the blood capillaries. The first-order process \( (k_a) \) is usually attributed to lymphatic absorption that occurs after a delay \( (T_{lag}) \). The fractions delivered by the first- and zero-order processes are modeled as parameters \( F_{rc} \) and \( (1-F_{rc}) \). In a general form the resulting input function can be represented by the following equation:

\[
\text{Input} = \frac{F \cdot \text{Dose} \cdot (1-F_{rc})}{\tau} \cdot \Theta(\tau - t) + \\
+ k_a \cdot F \cdot \text{Dose} \cdot F_{rc} \cdot e^{-k_a(t-T_{lag})} \cdot \Theta(t - T_{lag})
\]

Eq. 1

where \( F \) is the absolute bioavailability, and \( \Theta(x) \) is the step function that equals to 1 for \( x>0 \) and 0 otherwise. To further delay the absorption, a single transit compartment is sometimes added to either one of the two pathways with sequential first-order uptake into the central compartment \( (k_{a2} \text{ or } k_{a3}, \text{ dashed line compartments in Figure 1C}) \) (Bocci et al., 1986; Radwanski et al., 1998). To reduce the number of the estimated parameters, \( T_{lag} \) and \( \tau \) are often constrained to be equal to each other (Table 1).
However, this assumption essentially transforms the model into a sequential two-phase absorption, which limits its physiological interpretation. It should be noted, that by fixing the fraction absorbed of one of the processes to zero the model could be reduced to a single-pathway model (which can be useful for model evaluation).

This model was suggested to capture the pharmacokinetics of rhIFN-α2 after SC injection to rabbits (Bocci et al., 1986). The systemic disposition was described using three compartments and linear elimination. Separate sets of parameters were estimated for IFN-α2 given alone or in combination with absorption modifiers (albumin and hyaluronidase). Individual pharmacokinetic profiles of IL-10 in healthy humans after IV and SC dosing were described by a similar model (Radwanski et al., 1998). A single transit compartment with a first-order rate transfer to the systemic circulation ($k_{a2}$) was added to the linear pathway to further delay the absorption. The mean duration of the zero-order absorption ($\tau$) was estimated as 0.47±0.52 h (mean±SD), and the $T_{lag}$ for the first-order absorption was 0.23±0.52 h, which allowed for two processes to occur simultaneously. Similar to the single-pathway models, dose-specific parameters (bioavailability and first-order absorption rate constant) have been utilized to account for nonlinearities in the absorption process (Ramakrishnan et al., 2003; Ramakrishnan et al., 2004).

A wide range of values was reported for the parameters for various proteins in different species (Table 1). Although this model was originally proposed to mechanistically describe protein absorption, limited inferences of physiological relevance of the estimated parameters could be drawn at this time. For example, the fraction of the bioavailable dose absorbed through the zero-order pathway varied from 5 to 88%, and the estimated duration of the zero-order process in humans ranged from 0.47 to 60 h.

Mechanistic dual-pathway SC absorption models (Models of lymphatic absorption)

Following an early work that found a direct linear correlation between the molecular weight of compounds and the percent of the SC dose recovered in peripheral lymph (Supersaxo et al., 1990), other investigators evaluated the contribution of the lymphatic pathway to the SC bioavailability of protein drugs in a sheep model. These studies provided most of the data for development of the lymphatic absorption models (McLennan et al., 2003; McLennan et al., 2005a; McLennan et al., 2006; Kota et al.,
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2007). The first generation of this model included two pathways connecting the injection site and the central distribution compartment in non-lymph-cannulated animals. In contrast to empirical dual-pathway models, the absorption was modeled through simultaneous analysis of the plasma (or serum) pharmacokinetic profiles in control (non-cannulated) and lymph-cannulated animals and the time-course of the drug recovery in the lymph of lymph-cannulated animals. This direct experimental assessment of the lymphatic component (although technically challenging) results in more meaningful parameter estimates as compared to empirical dual-pathway absorption models. In lymph-cannulated animals, only blood vessels deliver the drug to the central compartment, and the drug transported through the lymphatics is continuously collected. Two variations of this general concept were developed to capture the pharmacokinetics of different proteins in the sheep model (Figure 2), and two ways to parameterize the model were reported: 1) using the rate constants and 2) using the fraction terms. The corresponding parameters can be interconverted as follows:

\[ F = \frac{k_{\text{blood}} + k_{\text{lymph}}}{k_{\text{blood}} + k_{\text{lymph}} + k_{\text{loss}}} \]  
\[ F_{\text{lymph}} = \frac{k_{\text{lymph}}}{k_{\text{blood}} + k_{\text{lymph}}} \]  
\[ F_{\text{blood}} = \frac{k_{\text{blood}}}{k_{\text{blood}} + k_{\text{lymph}}} \]

where \( k_{\text{blood}}, k_{\text{lymph}}, \) and \( k_{\text{loss}} \) are the first-order rate constants for uptake by blood and lymph capillaries, and degradation, \( F \) is the absolute bioavailability, and \( F_{\text{blood}} \) and \( F_{\text{lymph}} \) represent the fraction of bioavailable drug absorbed by the corresponding pathway.

Recombinant methionyl human leptin was administered SC (0.15 mg/kg) into the interdigital space of the hind leg to control (non-lymph-cannulated) and lymph-cannulated (efferent popliteal duct) sheep (McLennan et al., 2003). The cumulative amount of leptin in the peripheral lymph was 34.4±9.7% of the dose, and the plasma AUC in the lymph-cannulated group was 38% of the AUC of the non-cannulated group. Systemic pharmacokinetics was described using a two-compartment model with linear elimination.
(based on 0.1 mg/kg IV dose), and the corresponding disposition parameters were fixed for analysis of SC groups. Absorption was modeled as two first-order processes originating from the injection space \( (k_{\text{blood}} \text{ and } k_{\text{lymph}} \text{ Figure 2A}) \). Individual animal data were described using the following equations:

\[
\frac{dSC}{dt} = -k_a \cdot SC \quad \text{Eq. 5}
\]

For the control group:

\[
\frac{dC_{\text{noncan}}}{dt} = \frac{k_a \cdot SC \cdot F}{V_c} \pm \text{disposition} \quad \text{Eq. 6}
\]

For the lymph-cannulated group:

\[
\frac{dC_{\text{can}}}{dt} = \frac{k_a \cdot SC \cdot F \cdot (1 - F_{\text{lymph}})}{V_c} \pm \text{disposition} \quad \text{Eq. 7}
\]

\[
\frac{dL}{dt} = k_a \cdot SC \cdot F_{\text{abs}} \cdot F_{\text{lymph}} \quad \text{Eq. 8}
\]

where \( SC \) is amount of the drug at the injection site, \( C \) is the concentration in the central compartment (with the volume of \( V_c \)), \( L \) is the amount of the drug in the lymph of the cannulated group, and the absorption rate constant \( (k_a = k_{\text{blood}} + k_{\text{lymph}}) \) was allowed to vary between lymph-cannulated and control animals.

The model was also applied to describe the absorption of darbepoetin, a hyperglycosylated analogue of recombinant human erythropoietin alpha, in sheep after SC administration of 2 \( \mu \)g/kg (at the interdigital space of hind leg) to control and peripheral lymph-cannulated animals (McLennan et al., 2006). Mean data from all groups were fitted simultaneously, and the absorption parameters were shared between SC groups; however, a separate elimination rate constant was estimated for IV and each of the SC groups.

A model with a separate lymphatic compartment (Figure 2B) was used to describe the absorption of recombinant human erythropoietin alpha after SC injection to sheep (McLennan et al., 2005a). Nonlinearity in systemic disposition (10-1000 IU/kg IV dose) was described using a combination of a linear and Michaelis-Menten elimination mechanisms. A single SC dose (400 IU/kg, interdigital space of
hind leg) was administered to control, peripheral lymph-cannulated (popliteal duct), and central lymph-cannulated (thoracic duct) animals. Mean data from all groups were fitted simultaneously, and all parameters were estimated with good precision. The rates of absorption through the blood capillaries and the rate of presystemic degradation were described using the first-order rate constants ($k_{\text{blood}}$ and $k_{\text{loss}}$). The rate of lymphatic uptake ($k_{\text{lymph}}$) was shared between all groups; however, a lag time parameter (0.52 h) was estimated to account for a delay in erythropoietin appearance in the central lymph. It is unclear whether the lag time was included for the SC control group. Another first-order rate constant was estimated to describe the drug transfer from the lymph compartment to the central compartment ($k_{\text{input}}$) (Figure 2B):

$$\frac{dSC}{dt} = -\left( k_{\text{blood}} + k_{\text{lymph}} + k_{\text{loss}} \right) \cdot SC$$  \hspace{1cm} \text{Eq. 9}$$

For the control group:

$$\frac{dC_{\text{noncan}}}{dt} = \frac{k_{\text{blood}} \cdot SC}{V_c} + \frac{k_{\text{input}} \cdot L}{V_c} \pm \text{disposition}$$  \hspace{1cm} \text{Eq. 10}$$

$$\frac{dL_{\text{noncan}}}{dt} = k_{\text{lymph}} \cdot SC - k_{\text{input}} \cdot L$$  \hspace{1cm} \text{Eq. 11}$$

For the lymph-cannulated group:

$$\frac{dC_{\text{can}}}{dt} = \frac{k_{\text{blood}} \cdot SC}{V_c} \pm \text{disposition}$$  \hspace{1cm} \text{Eq. 12}$$

$$\frac{dL_{\text{can}}}{dt} = k_{\text{lymph}} \cdot SC$$  \hspace{1cm} \text{Eq. 13}$$

A similar absorption model structure was used to describe pharmacokinetics of darbepoetin in another study that compared lymphatic uptake from different anatomical locations (Kota et al., 2007). Central lymph was collected in all cannulated groups. Parameters for the systemic disposition were shared among all groups. The absorption model for the injection at the interdigital space followed the model for erythropoietin (Figure 2B, Eq. 9-13). Absorption from the abdominal injection site followed a biphasic pattern that was captured by two first-order inputs for both lymph and blood pathways. Specific
equations utilized by the authors were not provided, and it is unclear whether the second rate constant was added to or replaced the first rate constant after the lag time. The presystemic degradation rate constant ($k_{loss}$) was constrained to be equal between non-cannulated and lymph-cannulated groups.

A common limitation of the previously discussed lymphatic absorption models is the inability to account for redistribution of systemically available protein to the lymphatic system. The drug collected in the lymphatic fluid after SC injection comes from two sources: 1) drug entering the lymphatics at the site of injection and 2) systemically available drug that extravasated and was drained from the tissues by the lymphatic system (Figure 3A) (Kagan et al., 2007). The role of the lymphatic system in the recovery of macromolecules from the interstitial space in tissues and return to the systemic circulation is well established. Several decades ago, this mechanism was incorporated into the physiologically-based models describing the pharmacokinetics of antibodies and antibody fragments (Covell et al., 1986; Jain and Baxter, 1988; Baxter et al., 1994; Baxter et al., 1995). Quantitative information on the lymphatic recovery of macromolecules following redistribution of the systemically available drug is limited. Collection of the lymph following the IV administration of compounds can be used to obtain such data. For example, plasma and central lymph concentration-time profiles after IV administration of TNF, IFN-β, IFN-α2, and IL-2 to rabbits (Bocci et al., 1987; Bocci et al., 1988a; Bocci et al., 1988b; Bocci et al., 1990), and PEG40-erythropoietin to a dog (Wang et al., 2012) have been reported. The recovery of IFN-α2a in peripheral lymph was 0.001-0.003% of the IV dose in sheep (Supersaxo et al., 1988). Similar concentrations of erythropoietin were found in the plasma and thoracic lymph 1.5 h after IV injection to rats (Kagan et al., 2007). Up to 20% of the dose of a radiolabeled peptide ([3H]MRL-1) was recovered in thoracic lymph over 3 days after IV injection to dogs (Zou et al., 2013), and 44% of trastuzumab IV dose was collected over 30 h in the thoracic lymph of rats (Dahlberg et al., 2014).

Collectively, these works indicate that redistribution of the systemically available drug to the lymph can be significant, and including this pathway(s) into the pharmacokinetic model is important. A failure to account for this phenomenon might result in an overestimation of the lymphatic absorption, especially if central lymph is collected, and the total collection period spans over a long time. Comparable concentrations measured in the plasma and thoracic lymph after SC administration of several proteins to
rats was attributed to the redistribution phenomenon, and including this pathway into the absorption model was suggested (Kagan et al., 2007); however, the estimation of the corresponding parameters was not performed. Recently, Dahlberg and coworkers extensively evaluated the pharmacokinetics of trastuzumab using IV and SC (hind leg above the ankle) delivery to lymph-cannulated and control rats (Dahlberg et al., 2014). Significant amount of trastuzumab was recovered in the lymph after both modes of administration, and a mechanistic model that included two lymphatic systems was proposed (Figure 3B). The posterior lymphatic system drained one part of the body, including the injection site; and the amount of trastuzumab in the central lymph was measured (lymph-cannulated group). The anterior lymphatic system drained the rest of the body. Each lymphatic system was represented by two sequential compartments (peripheral and central lymph). The systemic disposition of trastuzumab was described by the central and peripheral compartments, and the drug was allowed to redistribute from these compartments into the lymphatics by first-order processes ($k_{36}, k_{34}, k_{24}, k_{26}$). The absorption part of the model included first-order uptake into the posterior peripheral lymph compartment ($k_{14}$) and Michaelis-Menten transport into the central compartment ($V_{\text{max}}$ and $K_m$) (see also section on mAbs absorption models). All other processes were modeled as first-order kinetics. Due to a large number of parameters, the rate constants associated with two lymphatic systems were shared ($k_{45}=k_{67}, k_{36}=k_{34}, k_{24}=k_{26}, k_{52}=k_{72}$). A population analysis was performed and provided a good description of the data and satisfactory estimation of the structural parameters. However, large standard errors were reported for the between subject variability terms (Dahlberg et al., 2014). Simulations using the model indicated that on average each trastuzumab molecule recirculated 4-5 times through the lymphatic systems before being eliminated. These results emphasize the importance of including a redistribution mechanism into models of SC absorption for therapeutic proteins; otherwise, the relative contribution of the lymphatic system to the SC bioavailability of proteins can be overestimated. It is anticipated that the role of redistribution mechanism would be more significant for larger proteins (such as mAbs) as compared to smaller proteins and peptides.
Insulin absorption models

Insulin was the first purified protein drug to become available for human use. Since its discovery in the early 1920s, multiple forms of insulin and insulin analogs with varying absorption and action profiles have become available. The therapeutic utility of insulin has been expanded by modifying the protein sequence (recombinant DNA technology) and altering the association and release properties of the formulation (using zinc, protamine, other excipients, and pH modifications) (Beals et al., 2013). Multiple factors have been shown to affect insulin absorption, including physiological variables (such as temperature, exercise, site and depth of injection) and product-dependent factors (type of insulin, concentration, volume of injection), as reviewed in (Hoffman and Ziv, 1997; Nucci and Cobelli, 2000; Charman et al., 2001; Wong et al., 2008). An important intrinsic property of the insulin molecule that governs absorption is the ability for self-association. Most studies assumed that only insulin monomers and dimers are small enough to be effectively absorbed, and the absorption delay was attributed to the time required for the hexamers to dissociate. However, experiments with nondissociating cobalt insulin showed that it was absorbed in the form of hexamers, although the rate of absorption was slower as compared to the absorption of dimers (Brange et al., 1990). In addition, lymph collection experiments in sheep suggested that insulin was partly absorbed in the form of hexamers (Charman et al., 2001).

Simple compartmental models were used in early studies to describe insulin pharmacokinetics. For example, Kobayashi and colleagues used a single first-order rate constant to describe absorption after continuous SC infusion and SC bolus injection (Figure 1A) (Kobayashi et al., 1983). Two transit compartments (injection site and interstitium) and two first-order rate constants (Figure 1B) were used to characterize ultralente insulin absorption in diabetic patients (Puckett and Lightfoot, 1995) and in a population study in healthy volunteers (Potocka et al., 2011). A combination of zero- and first-order kinetics was selected in a rat study (Figure 1C) (Gopalakrishnan et al., 2005).

Other investigators proposed noncompartmental approaches for describing the absorption of different insulins. Berger and Rodbard used two-parameter logistic equation to describe the absorption of four types of insulin (regular, NPH, lente, and ultralente):
\[ A\% (t) = 100 \left( 1 - \frac{100 \cdot t^s}{(T_{50})^s + t^s} \right) \]

where \( A\% \) is the percent of injected insulin remaining at the absorption site, \( t \) is time after injection, \( s \) is the steepness parameter, and \( T_{50} \) is the time interval to permit 50% of the dose to be absorbed (\( T_{50} \) was expressed as a linear function of the dose) (Berger and Rodbard, 1989). Mosekilde and coworkers used three coupled partial differential equations to describe processes that were assumed to govern the absorption of soluble insulin: diffusion of free insulin, transformation between hexamers and dimers, binding of the dimers in the SC tissue, and absorption of insulin dimers (Mosekilde et al., 1989). Degradation at the injection site was considered insignificant. Equations were solved numerically by dividing the SC layer into 20 rings, centered around the injection site. The model was modified and extended to describe absorption of monomeric insulin analogs (Trajanoski et al., 1993) and glargine insulin (Tarin et al., 2005). Later, this model was incorporated into an interactive educational diabetes simulator to support clinical decisions (Lehmann et al., 2009). Although this model provides a more physiological description of the processes at the injection site (as compared to conventional compartmental models), relatively complex mathematics and intensive computation requirement might preclude its application for future PKPD analysis.

Wong and associates proposed a unifying model for six different insulins based on ordinary differential equations to simplify computation (Figure 4) (Wong et al., 2008). It was assumed that absorption of all types of insulin occurs through a common dimeric/monomeric compartment (\( X_{dim} \)). The entire dose of monomeric insulin was assumed to be injected into the dimeric/monomeric compartment. For regular insulin, the dose was distributed between the dimeric and hexameric compartments (\( X_h \)), and hexamers dissociated into dimers by a first-order process. The NPH, lente, and ultralente insulin models were similar to the model of regular insulin with an additional crystalline (\( C \)) state compartment. The NPH and lente insulin shared a common hexameric state with regular insulin, whereas ultralente insulin had a separate hexameric state with slower dissociation (\( X_{h,ulen} \)). For glargine insulin, separate precipitation (\( P_{gla} \)) and hexameric (\( X_{h,gla} \)) compartments were included, and the dissolution rate was dose-dependent.
The effect of the volume of injection was modeled by a rate of diffusive loss \( (k_d) \) (assuming a spherical depot). The delivery of insulin from the injection site into the systemic circulation occurred through a single transit compartment \( (X_{\text{int}}, \text{interstitium}) \) and loss in this compartment was included \( (k_{\text{dI}}) \). Taken together, the model was able to describe the absorption kinetics for six different insulin types, including the concentration dependency for regular insulin and dose dependency for glargine insulin (Wong et al., 2008).

**Mechanistic models for SC absorption of monoclonal antibodies**

Monoclonal antibodies are a distinct group of protein therapeutics with common structure and a high molecular weight that utilizes antibody-specific mechanisms (i.e., binding to FcRn) to reduce degradation. Therefore, it can be hypothesized that different mAbs (and Fc-fusion proteins) may exhibit similar absorption patterns. Several studies showed that binding to FcRn is an important determinant of SC absorption of mAbs. Wang and coworkers reported that the SC bioavailability of 7E3 IgG1 was 3-fold higher in wild-type mice compared to FcRn-deficient animals (Wang et al., 2008). Antibody variants with enhanced binding affinity to FcRn at pH 6.0 and 7.4 showed increased and decreased bioavailability in mice, respectively (Deng et al., 2010; Deng et al., 2012). In contrast, no definitive effect of altering FcRn affinity on SC absorption was found for a series of IgG4 variants in cynomolgus monkeys (Datta-Mannan et al., 2012).

A series of studies by our group has systematically investigated multiple factors that influence the absorption of rituximab in rodents (Kagan et al., 2012; Kagan and Mager, 2013; Kagan et al., 2014). Specifically, the following factors were evaluated: 1) the role of the anatomical location of the SC injection site (back, abdomen, and the dorsal side of the foot); 2) the volume of the injection; 3) dose level; 4) binding to FcRn; 5) diffusion through the extracellular matrix; and 6) the rate of drug delivery. Rodents do not express human CD20 antigen; therefore, rituximab pharmacokinetics is not affected by the antigen-antibody binding in these species, facilitating the analysis of the absorption kinetics. The anatomical site of injection had a major impact on the rate and the extent of SC absorption of rituximab in
mice and rats, which is consistent with other studies (Beshyah et al., 1991; Macdougall et al., 1991; Jensen et al., 1994; Ter Braak et al., 1996; Kota et al., 2007). Interestingly, injection site differences were not observed for golimumab in humans (Xu et al., 2010a). An inverse correlation was found between the dose level (1-40 mg/kg) and the SC bioavailability of rituximab in mice and rats (Kagan et al., 2012; Kagan et al., 2014). Modeling was used to distinguish between the two proposed mechanisms of dose-dependent absorption of rituximab: 1) FcRn-mediated protection from degradation at the injection site, and 2) FcRn-mediated transcytosis from the interstitial space to the blood. FcRn-mediated transport of IgG across cells has been demonstrated in-vitro (Dickinson et al., 1999; Antohe et al., 2001). Only the model that incorporated binding of rituximab to a receptor (presumably FcRn) as a part of the absorption pathway was able to capture the observed dose-dependent bioavailability of rituximab (Kagan et al., 2012). The final absorption model included three competing processes at the absorption site: degradation of free rituximab ($k_{deg}^{SC}$), absorption of free rituximab (presumably through the lymphatics, $k_{a1}$), and absorption of bound rituximab (presumably through FcRn-mediated transcytosis, $k_{a2}$) (Figure 5A). The binding process was assumed to occur rapidly at the absorption site (ABS) and was characterized by the equilibrium dissociation constant ($K_{D}^{SC}$), and the amount of binding sites was held constant ($R_{tot}^{SC}$). The following differential equations defined the absorption model:

$$\frac{dA_{inj}}{dt} = -k_{inj} \cdot A_{inj}$$

**Eq. 15**

$$\frac{dABS_{tot}}{dt} = k_{inj} \cdot A_{inj} - (k_{deg}^{SC} - k_{a1}) \cdot ABS_{free} - k_{a2} \cdot (ABS_{tot} - ABS_{free})$$

**Eq. 16**

$$ABS_{free} = 0.5 \left[ (ABS_{tot} - R_{tot}^{SC} - K_{D}^{SC}) + \sqrt{(ABS_{tot} - R_{tot}^{SC} - K_{D}^{SC})^2 + 4 \cdot K_{D}^{SC} \cdot ABS_{tot}} \right]$$

**Eq. 17**

$$\frac{dC}{dt} = \frac{k_{a1} \cdot ABS_{free}}{V_c} + \frac{k_{a2} \cdot (ABS_{tot} - ABS_{free})}{V_c} \pm \text{disposition}$$

**Eq. 18**
where $C$ is the antibody concentration in the central compartment, $ABS_{\text{free}}$ is free rituximab at the absorption site, and $(ABS_{\text{tot}} - ABS_{\text{free}})$ is bound rituximab at the absorption site. Interestingly, the majority of model parameters were shared between the back and the abdomen injection sites (with the exception of the rate of absorption for free drug - $k_{a1}$, which was attributed to the differences in the SC space structure between two anatomical locations). A separate injection site compartment ($A_{\text{inj}}$) was included to account for a delay in the absorption observed in rats; this compartment was not required for describing the mouse data. To further investigate the role of FcRn in the SC absorption of mAbs, rituximab was co-administered SC with a large amount of nonspecific IgG (500 mg/kg). The observed decrease in bioavailability was attributed to the saturation of FcRn-binding, and the model provided a good prediction of the experimental results for different rituximab dose levels and injection sites based on parameters estimated for rituximab given alone (Kagan and Mager, 2013). For simplicity, the same affinity to FcRn was assumed for rituximab and IgG, and the amount of endogenous IgG at the absorption site was considered negligible. In addition, the model captured the pharmacokinetic profiles after coadministration of rituximab with SC hyaluronidase, which resulted in an increase in bioavailability, using parameters estimated for rituximab given alone, with the exception of the absorption rate constant for the free drug (Kagan and Mager, 2013). This result was in agreement with the mode of action of hyaluronidase (i.e., disruption of glycosaminoglycan matrix) (Bookbinder et al., 2006; Frost, 2007). The model was applied for description of rituximab pharmacokinetics after IV and SC administration to mice. Several model parameters were shared between species, while others were scaled with body weight (see also section on Interspecies modeling of SC absorption) (Kagan et al., 2014). Simulations were used to help visualize the competition among the two absorption pathways and degradation kinetics (Kagan and Mager, 2013; Kagan et al., 2014). For rituximab given alone, receptor-mediated transport accounted for absorption of approximately 57, 22, and 9% of the dose for 1, 10, and 40 mg/kg dose levels (SC injection at the back to rats); this pathway was almost completely abrogated by coadministration with a large amount of nonspecific IgG (Kagan and Mager, 2013). This mechanistic model was useful for evaluating the relative contribution of two pathways to SC bioavailability of rituximab (Kagan and Mager, 2013), and may be
further applied for investigating the role of system- and drug-dependent parameters on the rate and extent of absorption of mAbs. For example, Figure 6 shows that 10-fold difference in the binding affinity or the receptor expression level may have a significant effect on SC bioavailability of rituximab.

Dahlberg and coworkers utilized a combination of the first-order lymphatic uptake and Michaelis-Menten uptake by blood capillaries in their model of trastuzumab absorption in rats (Figure 3B) (Dahlberg et al., 2014). A range of IV doses (0.02-2 mg/kg) and a single SC dose (2 mg/kg) were evaluated in non-lymph-cannulated and lymph-cannulated animals (see also section on Mechanistic dual-pathway SC absorption models). The estimated bioavailability of trastuzumab was 85.5%, which is similar to the extent of absorption reported for rituximab (70% at 1 mg/kg dose level in rats) (Kagan and Mager, 2013). The lymphatic pathway and the saturable uptake through blood capillaries accounted for 53.1% and 32.4% of the dose. Michaelis-Menten kinetics was superior to the first-order kinetics in describing the absorption, and the estimated \( K_m \) value suggested that this process was considerably saturated by the administered dose. The Michaelis-Menten kinetics as implemented in this study is similar to the receptor-mediated absorption mechanism of rituximab (as described before) (Kagan et al., 2012). A comparison of the Michaelis-Menten and receptor-binding models for describing the systemic disposition of drugs has been reported (Gibiansky et al., 2008; Yan et al., 2010).

Pharmacokinetics of anti-amyloid-β wild type and Fc-variant (I235A.H435A) mlG2a was evaluated after a single IV and SC dose (5 mg/kg) in SCID mice (Deng et al., 2012). The bioavailability for the wild type and Fc-variant antibody, lacking binding affinity to FcRn at pH 6.0 and 7.4, was 76.3 and 41.8%. A modification of the endosomal recycling model (previously used for describing the systemic disposition of mAbs by (Hansen and Balthasar, 2003)) was developed and included two separate endosomal compartments for the skin and the rest of the body (Figure 5B). Absorption of mAbs was described by a combination of the first-order absorption \( (F\cdot k_a) \) attributed to the lymphatic uptake and a first-order uptake from the skin to the endosomal compartment \( (k_u) \). The systemic disposition model was parameterized as described before (Hansen and Balthasar, 2003). Antibodies entered both endosomal spaces with a first-order rate constant \( (k_{wp}) \), where they could rapidly bind to the FcRn (with affinity \( K_D \)). Total concentration of the FcRn was set to be the same for both endosomal compartments \( (R_{T1}(\text{non-skin}) = R_{T2}(\text{skin})) \);
however, it is unclear whether the relative sizes of the endosomal spaces (whole body vs. injection site/skin) was included. Free antibodies were degraded with a first-order process \( (k_{\text{deg}}) \), and bound antibodies returned to the vascular space with a first-order process \( (k_{\text{ret}}) \), which was constrained to be equal to \( k_{\text{up}} \). The unbound fraction of IgG in the endosomal compartments was calculated (non-skin - \( f_{u1} \), skin - \( f_{u2} \)). The competition between the exogenous test antibodies and endogenous murine IgG for FcRn binding was not considered due to the low endogenous IgG concentration in SCID mice. Fraction unbound \( (f_u) \) for Fc-variant antibody was set to 1. The model was able to simultaneously capture the data for both the wild type and the Fc-variant mAbs (Deng et al., 2012).

Zhao and colleagues (Zhao et al., 2013) proposed a physiological model of SC absorption of mAbs based on the structural tissue model developed by Garg and Balthasar for mAbs disposition (Garg and Balthasar, 2007). The majority of model parameters were fixed to previously published values. Lymphatic transit time and lymphatic elimination rate constant were estimated using the pharmacokinetic profiles of omalizumab in humans. Based on the sensitivity analysis, the authors suggested that the lymphatic flow rate and lymphatic drug elimination constant were the most influential to bioavailability. However, it was mentioned that the extent of lymphatic elimination of mAbs remains unknown. Analysis of data for other mAbs administered by the SC route can be potentially utilized to further evaluate this model.

**Population modeling of absorption and covariate analysis for protein therapeutics**

Extravascular dosing commonly results in a greater variability as compared to IV administration. Quantification of the variability, in addition to the estimation of the structural parameters, is important in clinical settings for better understanding of the dosing requirements for an individual patient. At the same time, population variability may hamper identifiability of the structural parameters for complex mechanism-based models. Blood sampling during the absorption phase is often limited in clinical trials; and nonlinear disposition, frequently exhibited by protein therapeutics, may further obscure the absorption behavior. These factors may lead to oversimplification of the absorption component of the pharmacokinetic model. For example, between subject variability is often omitted for the absorption rate.
and bioavailability parameters. Covariate analysis is rarely reported for absorption parameters; however, several studies identified body size, age, and population characteristics as factors that may affect the rate of absorption (Agoram et al., 2007; Potocka et al., 2011; Naik et al., 2013).

Table 2 summarizes selected studies that utilized population modeling approach for therapeutic proteins. For example, pharmacokinetics of pegylated erythropoietin, peginesatide (a 40kDa PEG-peptide), filgrastim, and darbepoetin alfa was well described using a first-order absorption rate constant and a bioavailability term (Figure 1A) (Jolling et al., 2005; Agoram et al., 2007; Krzyzanski et al., 2010; Naik et al., 2013). Chatelut and coworkers used a dual-pathway absorption model (with sequential zero-order and first-order absorption, Figure 1C) in population pharmacokinetic analysis of IFN-α2b after a single SC injection in patients with chronic hepatitis C infection (Chatelut et al., 1999). All absorption parameters were estimated with good precision, except for the absolute bioavailability (due to lacking IV data). Covariate analysis for absorption parameters was not performed, and the between subject variability for these parameters was 33-40% (CV%). Saturable carrier-mediated membrane transport and precipitation with subsequent dissolution were discussed as potential reasons for complex absorption behavior. Other evaluated absorption models included zero-order or first-order only (with or without a lag time), and a combination of zero- and-first order processes occurring simultaneously. Models combining zero- and first-order absorption kinetics were reported for other proteins (Table 2).

A population model-based meta-analysis described the pharmacokinetics of recombinant human erythropoietin after IV and SC administration using data from 16 trials (Olsson-Gisleskog et al., 2007). Deconvolution analysis revealed complex absorption behavior, with a relatively dose-proportional first phase and more than dose-proportional second phase. The systemic disposition model included central and peripheral compartments and a combination of linear and saturable elimination. The best fit was obtained with a model that included two absorption mechanisms. The faster absorption phase was described as a sequential zero-order input into the depot compartment (with duration of 0.725 h), followed by a first-order absorption into the central compartment. The slower absorption phase started after a delay (2.72 h) and was described as a zero-order input directly into the central compartment (with duration of 37.8 h). The fraction absorbed through the first pathway was 81 and 60% for dose levels...
smaller than or greater than 300 IU/kg, respectively. The absolute bioavailability was described by a hyperbolic function based on the dose level. The model successfully captured data from all studies; however, the underlying mechanism for complex absorption remained unclear (Olsson-Gisleskog et al., 2007).

A recent review summarized a large number of human population pharmacokinetic studies for mAbs (given mostly by the IV route) (Dirks and Meibohm, 2010). Two-compartment model with linear elimination or a combination of linear and nonlinear clearances was usually used for mAbs. Body size was a commonly identified covariate for the volume of distribution and clearance. This review focuses on studies that performed population analysis for mAbs and Fc-fusion proteins given by the SC route (Table 3). First-order kinetics was selected for describing the absorption process following SC injection in all reviewed studies. Several studies reported evaluating zero-order kinetics or a combination of zero- and first-order processes. The absorption was generally slow, with the absorption rate constant ranging from 0.12 to 1.2 day\(^{-1}\). The between subject variability associated with the absorption rate constant was usually high or not estimated due to limited data during the absorption phase. The absorption rate constant was found to decrease with age for canakinumab, denosumab, and AMG317, and the exponent on the centered covariate was from -0.319 to -0.72. A decrease of the lymph flow with age was suggested before as a mechanism of this behavior (Agoram et al., 2007). In majority of studies covariate analysis for the absorption rate constant was not reported. A high shrinkage for the estimate of $k_a$ precluded further evaluation of covariate effects on this parameter in several studies. Trials that evaluated IV administration estimated the absolute bioavailability, which ranged from 7 to 74 %. For canakinumab, the bioavailability was dependent on the drug product (59-74%) (Chakraborty et al., 2012; Chakraborty et al., 2013). The estimation of between subject variability and the evaluation of covariates was generally not performed for the bioavailability parameter. Due to a lack of IV data, the apparent values for the clearance and the volume of distribution (CL/F and V/F) were estimated in several works, and body weight was frequently found to be a significant covariate. Therefore, dependence of the bioavailability on body weight and other covariates cannot be ruled out at this time.
Interspecies modeling of SC absorption for protein therapeutics

The ability to predict pharmacokinetic and pharmacodynamic properties of drug candidates in humans based on preclinical data is essential for improving the success rate and predictability of drug development. In addition, development of approaches for sharing and scaling of parameters among species may provide an efficient way for integrative analysis of data from different species. Systemic disposition of protein therapeutics in humans could be predicted reasonably well using allometric relationships:

\[ P = a \cdot BW^b \]  

Eq. 19

where \( P \) is a parameter of interest, \( BW \) is species body weight, and \( a \) and \( b \) are the coefficient and the exponent of the allometric equation. The value of the allometric exponents for clearance and the volume of distribution and the number of species required for the prediction varied among studies (Mordenti et al., 1991; Mahmood, 2004; Mahmood, 2009; Ling et al., 2009; Wang and Prueksaritanont, 2010; Deng et al., 2011). Traditional allometry cannot be applied for drugs exhibiting target-mediated disposition that results in dose-dependent pharmacokinetic parameters (Woo and Jusko, 2007; Kagan et al., 2010). We have previously combined target-mediated disposition model with allometric principles to simultaneously capture the pharmacokinetics of type I IFNs and exenatide in multiple species (Kagan et al., 2010; Chen et al., 2013).

The quantitative information on species difference in the absorption process is very limited. The first-order absorption rate constant for PEG-erythropoietin following SC injection was shown to scale with an allometric exponent of -0.147 (based on four species), and species-specific bioavailability parameter was used (Jolling et al., 2005). The absorption of erythropoietin was described by a combination of zero- and first-order kinetics in 3 species (rats, monkeys, and humans) (Woo and Jusko, 2007). The relationship between the estimated parameters and species body weight was assessed using regression analysis, and the allometric exponent for the first-order absorption rate constant was calculated as -0.349. Dose-dependent rate and extent of absorption (frequently reported for protein therapeutics) can further complicated interspecies analysis. For example, separate values for the absorption rate constant or bioavailability are sometimes utilized for each dose level (Mager et al., 2003; Ramakrishnan et al., 2003;
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Gao and Jusko, 2012). Although this approach might be useful for capturing observed data, it provides little insight into the mechanism of absorption and cannot be used effectively for interspecies prediction.

Michaelis-Menten kinetics was used to successfully capture dose-dependent absorption of exenatide in multiple species; and species-specific parameters ($V_{\text{max}}$ and $K_m$) were required (Chen et al., 2013). The ability of the model to predict exenatide pharmacokinetics in humans after SC administration was evaluated by simulation using two approaches: 1) separate sets of $V_{\text{max}}$ and $K_m$ values from mice, rats, and monkeys; 2) $V_{\text{max}}$ and $K_m$ for humans were calculated using the allometric approach from three preclinical species. Interestingly, human data could not be adequately predicted using monkey absorption parameters; however, rat $V_{\text{max}}$ and $K_m$ provided results consistent with clinical data. $V_{\text{max}}$ parameter was highly correlated with body weight (allometric exponent of 0.392, $R^2=0.996$). Although $K_m$ appeared less correlated with body weight (allometric exponent of 0.605, $R^2=0.605$), the combination of $V_{\text{max}}$ and $K_m$ values obtained from the regressions provided a good prediction of human data (Chen et al., 2013).

Recently, a mechanism-based model that combines the absorption of free and bound antibody with presystemic degradation was applied to describe the pharmacokinetics of rituximab in rats and mice (Kagan and Mager, 2013; Kagan et al., 2014). SC absorption of rituximab was dose-dependent in both species; however, the absorption process was faster in mice and the magnitude of the nonlinear absorption was less pronounced in mice as compared to rats. Similar structural model was able to capture the dose-dependent SC absorption in both species (with the exception of an absorption delay for rats) (Figure 5A). The binding affinity of rituximab to the receptor (presumably FcRn), the expression level of the receptor, and the rate constant for presystemic degradation were shared between species. The rate constants for absorption of the free and bound antibody were estimated separately for mice and rats, and decreased with increasing body weight. Interestingly, the relationships between the absorption rate constants and species body weight was similar to the relationship between the distribution rate constants and body weight (Kagan et al., 2014).
Conclusions and future perspective

SC absorption of proteins is regulated by a large number of factors that can be divided into species-, subject-, and drug/formulation-specific. Predicting SC absorption in humans is a difficult task, and the best preclinical model has not been identified (McDonald et al., 2010). Species differences in skin morphology and their effect on the rate and extent of SC absorption, and approaches for integrating this information into interspecies pharmacokinetic models require further investigation. Some physiological processes that take part in the SC absorption may be scaled with body weight among species. Lindstedt and Schaeffer reported that the skin blood flow can be described using an allometric exponent of 0.741 using data for mouse, rat, dog, and human (Lindstedt and Schaeffer, 2002). Interestingly, the lymph flow rate in the thoracic duct can be scaled with body weight using an allometric exponent of 0.723-0.792 (Figure 7).

Insulin is an important paradigm that demonstrates that the mechanistic insights into the absorption process can be exploited for developing products with a wide range of properties (i.e., rapid-acting vs. long-acting insulin analogs). As described before, some of the model components were effectively shared among different insulin types (Figure 4). Similarly, it can be hypothesized that due to structural resemblance, the absorption behavior of various mAbs and Fc-fusion proteins is governed by similar factors (e.g., binding to FcRn). Therefore, some of the model parameters can be shared within this class of drugs or predicted based on in-vitro experiments (such as, measurement of binding affinity of different IgG to FcRn using surface plasmon resonance technique). Furthermore, species-differences in the affinity and in the expression levels of FcRn can be potentially utilized for interspecies prediction of the absorption kinetics.

The contribution of many drug- and formulation-dependent factors to the SC absorption of therapeutic proteins has not been sufficiently investigated (e.g., glycosylation, pegylation, the isoelectric point of protein, presence of binding proteins in the SC space). The absorption of nonglycosilated GCSF (filgrastim) was faster, and resulted in a greater AUC and C_max (by 18% and 20%) as compared to glycosylated GCSF (lenograstim) after SC injection of 5 μg/kg in normal subjects (no difference between the elimination half-lives was observed) (Watts et al., 1997). SC absorption of erythropoietin alpha in
pediatric patients was 23% faster and resulted in a 43% lower bioavailability as compared to erythropoietin delta (erythropoietin delta is produced in a human cell line and has a different carbohydrate composition from CHO cell-derived erythropoietin alfa) (Knebel et al., 2008). Lower bioavailability was reported for mAbs with a higher \( pI \) values (more than \( \sim 9.0 \)) in minipigs and humans (Zheng et al., 2012). Formulation related factors for SC injected biotherapeutics have been reviewed recently (Kinnunen and Mrsny, 2014). However, quantitative characterization of the effects of formulation excipients and absorption modifiers on the rate and extent of absorption of therapeutic proteins should be investigated in future studies.

For insulin and mAbs binding at the absorption site was proposed as a mechanism that modulates the systemic absorption (Mosekilde et al., 1989; Wang et al., 2008; Kagan et al., 2012); more information is needed regarding the effect of endogenous proteins, soluble receptors, and anti-drug antibodies in the interstitial space on absorption of exogenous proteins. Presystemic degradation processes for therapeutic proteins are poorly understood, and in-vitro incubation in SC tissue homogenates, lymph (or lymph nodes), and blood can be used for evaluating drug stability and informing the modeling process (Wang et al., 2012; Zou et al., 2013). However, in-vitro studies might not fully represent the complexity of the in-vivo conditions. Human growth hormone was stable for 6 hours in the central lymph of sheep; however, there was a significant difference in the drug recovery between the peripheral and central lymph-cannulated groups (62 vs. 8.6%) (Charman et al., 2000). Measurement of the disappearance rate of the injected protein from the SC site (in addition to plasma concentrations) can help identifying rate-limiting steps in the absorption process. A linear relationship was found between the half-life at the injection site and the molecular weight of four fluorescently labeled proteins (23 – 149 kDa) using whole-body imaging technique (Wu et al., 2012). The effect of various pathological states on SC absorption has not been sufficiently characterized (e.g., obesity). Increased adiposity was associated with a reduced SC blood flow and a slower absorption of insulin in humans (Vora et al., 1992). Wang and coworkers reported that PEG40-erythropoietin exhibited approximately 2-fold lower exposure in fat rats as compared to normal rats after body-weight normalized SC dose administration (Wang et al., 2012).
In conclusion, this review summarized current experience with modeling of the pharmacokinetics of therapeutic proteins administered by the SC route. Despite a continuing research in this area, our understanding of the quantitative aspects of SC absorption is still limited. It is anticipated, that further development of mechanism-based modeling will play a central role in resolving complexities associated with absorption of these drugs.
Authorship Contribution

Leonid Kagan wrote the manuscript.
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Footnotes

Part of this work was presented at the 2nd ADME of protein therapeutics workshop: science and application, August 11-12, 2013, Buffalo, NY.
Figure legends

**Figure 1.** General schematic of the SC absorption models for therapeutic proteins. (A) and (B) – single pathway model, (C) – empirical dual-pathway model. Systemic disposition can be described by different models depending on the drug, and for simplicity, is shown as a single grey compartment. Optional rates and compartments are shown with dashed lines. Selected studies that utilized model C are listed in Table 1. $F$ – systemic bioavailability, $k_a$ – first-order absorption rate constants, $V_{\text{max}}$ and $K_m$ – parameters for a Michaelis-Menten kinetics, $F_{\text{rc}}$ – fraction of the bioavailable dose absorbed via the first-order pathway, $T_{\text{lag}}$ – a lag time for the first-order absorption, $\tau$ – duration of the zero-order absorption.

**Figure 2.** Schematic of the first generation mechanistic dual-pathway SC absorption models. (A) – model without a lymph compartment was used for leptin (McLennan et al., 2003) and darbepoetin (McLennan et al., 2006) in sheep; (B) – model with a separate lymph compartment was used for erythropoietin (McLennan et al., 2005a) and darbepoetin (Kota et al., 2007) in sheep. The model is fitted simultaneously to the plasma (or serum) pharmacokinetic profiles in control (non-cannulated) and lymph-cannulated animals and to the cumulative recovery in peripheral or central lymph of lymph-cannulated animals. Systemic disposition can be described by different models depending on the drug, and for simplicity, is shown as a single grey compartment. $k_{\text{blood}}$, $k_{\text{lymph}}$, and $k_{\text{loss}}$ are the first-order rate constants for uptake by the blood and lymph capillaries, and degradation; $k_{\text{input}}$ – first-order rate constant for drug transfer from the lymph to the central compartment (model B - only).

**Figure 3.** (A) - general schematic of the SC absorption that includes redistribution of the systemically available proteins from the tissues to the lymphatic system, as proposed by (Kagan et al., 2007); (B) – schematic of the model that was utilize for describe the plasma and lymph data after IV and SC administration of trastuzumab to rats (Dahlberg et al., 2014). Compartments and rate constants are described in the text.
**Figure 4.** Schematic of the unifying model of the SC absorption of different insulins (modified from (Wong et al., 2008)). Absorption of all types of insulin occurs through a common dimeric/monomeric compartment ($X_{dim}$). Short dashed arrows represent the distribution of the insulin dose for different types of insulin. Solid arrows represent first-order rate processes. Long dash arrows represent the diffusion loss of insulin at the absorption site ($k_d$). $X_h$ – hexameric insulin, $C$ – crystalline insulin, $P$ – precipitated insulin, $X_{int}$ – interstitial space.

**Figure 5.** (A) – schematic of the pharmacokinetic model utilized to describe the SC absorption of rituximab in mice and rats (modified from (Kagan et al., 2012; Kagan and Mager, 2013; Kagan et al., 2014)). Optional compartment is shown with dashed line. (B) - schematic of the pharmacokinetic model with two endosomal spaces used to describe the SC absorption of wild-type and Fc-variant mlgG2a in SCID mice (Deng et al., 2012). Compartments and rate constants are described in the text.

**Figure 6.** The effect of a change in the expression level of the receptor and the binding affinity on the SC bioavailability of mAb. Simulation was performed using the model developed for rituximab injected SC at the back of rats (Kagan et al., 2012), model schematic is shown in Figure 5A. Reported values of $K_D$ and $R_{tot}$ were changed 10-fold from the reported values, and all the other parameters were kept constant. The column in the middle (relative values of 1 for $K_D$ and $R_{tot}$) represent the experimentally obtained bioavailability of 69% for 1 mg/kg (panel A) and 18% for 40 mg/kg dose (panel B).

**Figure 7.** Allometric relationship between the lymph flow in the thoracic duct and species body weight. (A) – The difference in the lymphatic flow as a function of mobility state of animals. Data for mice, rats, rabbits, and dogs were extracted from (Lindena et al., 1986). (B) – mean data for multiple species as summarized by (Lindena et al., 1986) and sheep data (Porter et al., 2001).
**Table 1.** Studies that utilized a combination of zero- and first-order kinetics to describe SC absorption of therapeutic proteins

<table>
<thead>
<tr>
<th>Drug</th>
<th>Species/Population</th>
<th>Duration of the zero-order absorption</th>
<th>% of bioavailable dose absorbed by zero-order process</th>
<th>Rate constant for the first-order absorption</th>
<th>Bioavailability</th>
<th>Systemic disposition model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anakinra</td>
<td>Rat</td>
<td>2 h</td>
<td>Not reported</td>
<td>0.39 h⁻¹</td>
<td>100%</td>
<td>2CM with linear elimination</td>
<td>(Liu et al., 2011)</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Rat</td>
<td>13.5 h</td>
<td>68%</td>
<td>0.146 h⁻¹</td>
<td>59%</td>
<td>2CM with linear and MM elimination</td>
<td>(Woo et al., 2006)</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Cynomolgus monkey</td>
<td>10 h</td>
<td>35%</td>
<td>0.044-0.053 h⁻¹ dose dependent</td>
<td>27-100%</td>
<td>2CM with MM elimination</td>
<td>(Ramakrishnan et al., 2003)</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Healthy humans</td>
<td>44-60 h dose dependent</td>
<td>88%</td>
<td>0.022 h⁻¹</td>
<td>38.84 + 0.0002495×Dose</td>
<td>1CM with MM elimination</td>
<td>(Ramakrishnan et al., 2004)</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Healthy humans</td>
<td>0.38-1.39 day dose dependent</td>
<td>79.1%</td>
<td>0.7 day⁻¹</td>
<td>Not reported</td>
<td>1CM with MM elimination</td>
<td>(Krzyzanski et al., 2005)</td>
</tr>
<tr>
<td>Filgrastim</td>
<td>Healthy humans</td>
<td>6.6 h</td>
<td>58.6</td>
<td>0.403 h⁻¹</td>
<td>69.1%</td>
<td>TMDD</td>
<td>(Wiczling et al., 2009)</td>
</tr>
<tr>
<td>IL-10ᵃ</td>
<td>Healthy humans</td>
<td>0.47 h</td>
<td>5%</td>
<td>kₙ₁=0.16 h⁻¹ with Tᵢₐ₉=23h kₙ₂=0.61 h⁻¹</td>
<td>42%</td>
<td>2CM with linear elimination</td>
<td>(Radwanski et al., 1998)</td>
</tr>
<tr>
<td>IFN-α2ᵇ</td>
<td>Rabbit</td>
<td>1 h</td>
<td>Not reported</td>
<td>0.1 h⁻¹ Tᵢₐ₉=3.4 h</td>
<td>Not reported</td>
<td>3CM with linear elimination</td>
<td>(Bocci et al., 1986)</td>
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<tr>
<td>IFN-α2b</td>
<td>Human patients</td>
<td>2.5 h</td>
<td>24%</td>
<td>0.18 h⁻¹</td>
<td>Not reported</td>
<td>1CM with linear elimination</td>
<td>(Chatelut et al.,</td>
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<tr>
<td>Parathyroid hormone</td>
<td>Rat</td>
<td>5 min</td>
<td>7%</td>
<td>0.018 min⁻¹</td>
<td>Not modeled</td>
<td>Homeostasis model for endogenous hormone</td>
<td>(Abraham et al., 2009)</td>
</tr>
</tbody>
</table>

Model schematic is shown in Figure 1C

1CM, 2CM, 3CM – one-, two-, three-compartment model; MM – Michaelis-Menten; TMDD – target-mediated drug disposition

- An additional transit compartment for the first-order process
- Model parameters changed by presence of albumin or hyaluronidase
Table 2. Selected studies that evaluated SC absorption of protein therapeutics using population analysis (excluding monoclonal antibodies and Fc-fusion proteins)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Species/Population</th>
<th>Absorption model</th>
<th>Absorption parameters</th>
<th>BSV for absorption parameters, CV%</th>
<th>COV for absorption parameters</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anakinra</td>
<td>Rats</td>
<td>Sequential zero-order and first-order</td>
<td>Fraction of zero-order Duration of zero-order $k_a$</td>
<td>Yes for $k_a$</td>
<td>No</td>
<td>(Liu et al., 2011)</td>
</tr>
<tr>
<td>Darbepoetin alfa</td>
<td>Anemic patients with chronic kidney disease</td>
<td>Zero-order input into the depot compartment followed by the first-order absorption</td>
<td>$F$ Duration of input $k_a$</td>
<td>Yes</td>
<td>No</td>
<td>(Doshi et al., 2010)</td>
</tr>
<tr>
<td>Darbepoetin alfa</td>
<td>Healthy humans</td>
<td>First-order</td>
<td>$k_a$</td>
<td>Yes for $k_a$</td>
<td>No</td>
<td>(Agoram et al., 2007)</td>
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<tr>
<td>Erythropoietin delta</td>
<td>Pediatric patients with chronic kidney disease</td>
<td>First-order</td>
<td>$F$ $k_a$</td>
<td>No</td>
<td>No</td>
<td>(Knebel et al., 2008)</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td>Healthy humans (meta-analysis)</td>
<td>(1) Zero-order input into the depot compartment followed by the first-order absorption (2) Zero-order after a lag time $a$</td>
<td>$F$ - hyperbolic function of the dose; Fraction of (1) – dose dependent; For (1): $k_a$, Duration of zero-order (D1) For (2): Duration of zero-order (D2), $T_{lag}$</td>
<td>Yes for $k_a$, $D_1$, $T_{lag}$, Fraction of (1)</td>
<td>$k_a$ decreased with age and body weight; $k_a$ lower in females; $F$ increased with increase in hemoglobin; $F$ decreased with body weight</td>
<td>(Olsson-Gisleskog et al., 2007)</td>
</tr>
<tr>
<td>Filgrastim</td>
<td>Healthy adults</td>
<td>Parallel zero-order and first-order followed by first order</td>
<td>$F$ Fraction of zero-order Duration of zero-order $k_a$</td>
<td>No</td>
<td>No</td>
<td>(Wiczling et al., 2009)</td>
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<td>Filgrastim</td>
<td>Healthy adults</td>
<td>First-order</td>
<td>$F$ $k_a$</td>
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<td>No</td>
<td>(Krzyzanski et al., 2010)</td>
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<td>Kinetic Model</td>
<td>Kinetics of Elimination</td>
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<td><strong>Growth hormone</strong></td>
<td>Healthy humans and dialysis patients</td>
<td>Michaelis-Menten</td>
<td>$V_{\text{max}}$, $K_m$</td>
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<td>Rats</td>
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<td><strong>Leukaemia inhibitory factor</strong></td>
<td>Healthy postmenopausal women and infertile patients</td>
<td>Zero-order</td>
<td>Duration of zero-order</td>
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<td><strong>Low molecular weight heparin</strong></td>
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<tr>
<td><strong>Pegylated human erythropoietin</strong></td>
<td>Rats, dogs, and monkeys</td>
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<td>$F$</td>
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<td>$k_{a1}/\text{BW}^{0.149} T_{\text{lag}}^{1.810}$</td>
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<td><strong>Peginesatide</strong></td>
<td>Chronic kidney disease patients</td>
<td>First-order</td>
<td>$F$</td>
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<td>$k_{a1}/\text{BMI}, \text{Creatinine}, \text{Ethnicity}, \text{Dialysis status}$</td>
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<td>(Naik et al., 2013)</td>
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</table>

* The estimated parameters values allowed for two processes to occur simultaneously ($D_1=0.725 \text{h}, D_2=37.8\text{h}, T_{\text{lag}}=2.72\text{h}$) (Olsson-Gisleskog et al., 2007)
### Table 3. Selected studies that evaluated SC absorption of monoclonal antibodies and Fc-fusion proteins using population analysis

<table>
<thead>
<tr>
<th>Drug</th>
<th>Population</th>
<th>Population mean F, %</th>
<th>Population mean $k_a$, day⁻¹</th>
<th>Between subject variability for $k_a$, CV%</th>
<th>Covariates for $k_a$</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adalimumab Rheumatoid arthritis</td>
<td>58 value not reported</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(Velagapudi et al., 2005)</td>
</tr>
<tr>
<td>AMG317, fully human IgG2 Healthy humans</td>
<td>24.3</td>
<td>0.185</td>
<td>-</td>
<td>Age (centered at 40y)</td>
<td>-0.72 b</td>
<td>(Kakkar et al., 2011)</td>
</tr>
<tr>
<td>Canakinumab Human healthy and patients</td>
<td>63.3 or 70.0 a</td>
<td>0.299 or 0.269 a</td>
<td>64</td>
<td>Age (centered at 34y)</td>
<td>-0.555 b</td>
<td>(Chakraborty et al., 2012)</td>
</tr>
<tr>
<td>Canakinumab Gouty arthritis</td>
<td>58.8-73.5 b</td>
<td>0.193-0.296 a</td>
<td>50.8</td>
<td>Age (centered at 52y)</td>
<td>-0.319 b</td>
<td>(Chakraborty et al., 2013)</td>
</tr>
<tr>
<td>Denosumab Postmenopausal women with osteopenia or osteoporosis</td>
<td>63.8</td>
<td>0.212</td>
<td>63</td>
<td>Age (centered at 64y)</td>
<td>-0.577 b</td>
<td>(Sutjandra et al., 2011)</td>
</tr>
<tr>
<td>Denosumab Bone metastasis</td>
<td>61.2</td>
<td>0.257</td>
<td>51.5</td>
<td>Age (centered at 54y)</td>
<td>-0.509 b</td>
<td>(Gibiansky et al., 2012)</td>
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<tr>
<td>Efalizumab Psoriasis</td>
<td>56.4</td>
<td>0.242</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(Ng et al., 2005)</td>
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<tr>
<td>Efalizumab Psoriasis</td>
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<td>0.191</td>
<td>0 fixed</td>
<td>-</td>
<td>-</td>
<td>(Sun et al., 2005)</td>
</tr>
<tr>
<td>Etanercept Juvenile rheumatoid arthritis</td>
<td>58 c</td>
<td>1.2</td>
<td>215</td>
<td>-</td>
<td>-</td>
<td>(Yim et al., 2005)</td>
</tr>
<tr>
<td>Etanercept Rheumatoid arthritis</td>
<td>- c</td>
<td>0.797</td>
<td>24.3 (73.1 d)</td>
<td>-</td>
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<td>(Lee et al., 2003)</td>
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<tr>
<td>Etanercept Psoriasis</td>
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<td>0.754</td>
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<td>Fc-osteoprotegerin Healthy postmenopausal women</td>
<td>7.19</td>
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<td>Drug/Protein</td>
<td>Condition</td>
<td>Parameter</td>
<td>F</td>
<td>Vd/F</td>
<td>CL/F</td>
<td>F/Dose</td>
</tr>
<tr>
<td>-------------</td>
<td>----------------------------------</td>
<td>-----------</td>
<td>---</td>
<td>------</td>
<td>------</td>
<td>--------</td>
</tr>
<tr>
<td>Golimumab</td>
<td>Ankylosing spondylitis</td>
<td>- c</td>
<td>1.010</td>
<td>78.6</td>
<td>-</td>
<td>(Xu et al., 2010b)</td>
</tr>
<tr>
<td>IgG subcutaneous</td>
<td>Primary immunodeficiency</td>
<td>66.0</td>
<td>0.439</td>
<td>-</td>
<td>-</td>
<td>(Landersdorfer et al., 2013)</td>
</tr>
<tr>
<td>MTRX1011A, humanized IgG1</td>
<td>Rheumatoid arthritis</td>
<td>52.3 (IIV 18.9CV%)</td>
<td>0.212</td>
<td>68.7</td>
<td>-</td>
<td>(Zheng et al., 2011)</td>
</tr>
<tr>
<td>Omalizumab</td>
<td>Asthma</td>
<td>62 c</td>
<td>0.480</td>
<td>39.9</td>
<td>-</td>
<td>(Hayashi et al., 2007)</td>
</tr>
<tr>
<td>Omalizumab</td>
<td>Asthma</td>
<td>-</td>
<td>0.458</td>
<td>141</td>
<td>-</td>
<td>(Lowe et al., 2009)</td>
</tr>
<tr>
<td>Romiplostim</td>
<td>Healthy subjects</td>
<td>49.9</td>
<td>0.610</td>
<td>-</td>
<td>-</td>
<td>(Wang et al., 2010)</td>
</tr>
<tr>
<td>Sirukumab</td>
<td>Healthy subjects</td>
<td>- c</td>
<td>0.77</td>
<td>60.0</td>
<td>-</td>
<td>(Zhuang et al., 2013)</td>
</tr>
<tr>
<td>TNFR-Fc fusion protein</td>
<td>Ankylosing spondylitis</td>
<td>- c</td>
<td>1.452 (Tlag=1 h)</td>
<td>55.6 (81.8 for Tlag)</td>
<td>-</td>
<td>(Fang et al., 2010)</td>
</tr>
<tr>
<td>Ustekinumab</td>
<td>Psoriasis</td>
<td>- c</td>
<td>0.354</td>
<td>0 fixed</td>
<td>-</td>
<td>(Zhu et al., 2009)</td>
</tr>
<tr>
<td>Ustekinumab</td>
<td>Psoriatic arthritis</td>
<td>- c</td>
<td>0.427</td>
<td>82.4</td>
<td>-</td>
<td>(Zhu et al., 2010)</td>
</tr>
</tbody>
</table>

F – systemic bioavailability; ka - first-order absorption rate constant

a depending on the drug product;
b Exponent for power model on centered covariate;
c Bioavailability was not modeled as a separate parameter, Vd/F and CL/F were estimated. If the bioavailability value was reported it was taken from previous studies;
d CV% for interoccasion variability.
Figure 1

A

\[ SC \xrightarrow{F, k_a} \text{or} \quad V_{max}, K_M \quad \]

B

\[ SC \xrightarrow{F, k_{a1}} k_{a2} \]

C

Zero-order
\[ F, (1-Frc), t<\tau \]

First-order
\[ F, k_a, Frc, t>T_{lag} \]

\[ \xrightarrow{k_{a2}} \quad k_{a3} \]
Figure 2

Noncannulated

A

\[ \text{SC} \]

\[ k_{\text{blood}} \]

\[ k_{\text{lymph}} \]

\[ k_{\text{loss}} \]

B

\[ \text{SC} \]

\[ k_{\text{blood}} \]

\[ k_{\text{lymph}} \]

\[ k_{\text{input}} \]

\[ k_{\text{loss}} \]

Lymph-cannulated

A

\[ \text{SC} \]

\[ k_{\text{blood}} \]

\[ k_{\text{lymph}} \]

\[ k_{\text{loss}} \]

\[ \boxed{\text{Lymph}} \]

B

\[ \text{SC} \]

\[ k_{\text{blood}} \]

\[ k_{\text{lymph}} \]

\[ k_{\text{loss}} \]

\[ \boxed{\text{Lymph}} \]
Figure 4

Diagram showing the relationships between different types of insulin preparations and their concentrations.

- \( C_{\text{NPH}} \)
- \( NPH \)
- \( C_{\text{len}} \)
- \( X_h \)
- \( X_{\text{dim}} \)
- \( X_{\text{int}} \)
- \( X_{\text{plasma}} \)
- \( C_{\text{ulen}} \)
- \( X_{h,\text{ulen}} \)
- \( X_{h,\text{gla}} \)
- \( P_{\text{gla}} \)

Types of Insulin Preparations:
- Lente
- Monomeric
- Regular
- Ultralente
- Glargine
Figure 7

A

- Consious/during motion: Flow = 7.835BW^{0.795}
- Anesthetized/immobile: Flow = 4.112BW^{0.723}

B

Flow = 3.808BW^{0.729}

Thoracic lymph flow, mL/h

Body weight, kg

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