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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIVISIÓN DE NUEVAS CREACIONES

E. S. D.

Re: Expediente N°. 10-160.886

Solicitud de Patente de Invención titulada: "COMPUESTOS DE INSULINA  
MODIFICADA [B28-L-LISINA, B29-L-PROLINA] Y PEGILADA"

Solicitante: ELI LILLY AND COMPANY

Nuestra Ref: P2010/000170

**RESPUESTA AL OFICIO No. 12946, NOTIFICADO POR FIJACIÓN EN LISTA No. 041**

**DE FECHA 14 DE OCTUBRE DE 2014**

**(SEGUNDO EXAMEN DE FONDO)**

CARLOS R. OLARTE, mayor de edad y vecino de Bogotá, identificado tal como aparece al pie de mi firma, en mi carácter de apoderado de la sociedad solicitante, me permito manifestar lo siguiente ante el auto emanado por ese Despacho, dentro del plazo legal de prórroga previsto en el artículo 45 de la Decisión 486, en los siguientes términos:

1. En primer lugar, con el ánimo de brindar mayor claridad a la materia reivindicada, me permito adjuntar un **Capítulo Reivindicatorio Modificado**, que reemplaza en su totalidad al Capítulo Reivindicatorio presentado el 14 de mayo de 2013.

Así entonces, el Capítulo Reivindicatorio Modificado contiene los siguientes cambios:

- se eliminan las Reivindicaciones 2 y 5;
- las antiguas Reivindicaciones 3-4, 6-15 corresponden a las nuevas Reivindicaciones 2-13, respectivamente, y las dependencias fueron modificadas de conformidad;
- se reemplaza la expresión "lispro" por "B28-L lisina, B29 L-prolina" en las Reivindicaciones 1 y 7-10;

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- se introduce la limitación de la antigua Reivindicación 2 en las Reivindicaciones 1 y 9, de tal forma que se restringió el límite superior del intervalo del peso molecular del PEG a 25 kDa;
- en la Reivindicación 9 se introduce la caracterización de A y B tal como se encuentra reclamada en la Reivindicación 1. Adicionalmente, dicha reivindicación se redacta en otro sentido, sin introducir más materia, y simplemente se adapta a un formato más claro.

Valga anotar que las modificaciones efectuadas al Capítulo Reivindicatorio cumplen a cabalidad con lo estipulado en el Artículo 34 de la Decisión 486, ya que éstas de ninguna manera implican una ampliación de la protección correspondiente a la divulgación contenida en la solicitud inicial.

2. a- La Examinadora señala que la Reivindicación 5 reclama materia no patentable.

b- Considero superada la anterior objeción en tanto que la Reivindicación 5 fue eliminada completamente del Capítulo Reivindicatorio Modificado adjunto.

3. a- La Examinadora señala que se modifica el título de la invención, el cual queda de la siguiente manera:

“COMPUESTOS DE INSULINA MODIFICADA [B28-L-LISINA, B29-L-PROLINA] Y  
PEGILADA”

b- Con respecto al punto anterior, estoy completamente de acuerdo con el título propuesto por la Examinadora.

4. a- La Examinadora señala que las reivindicaciones no son claras ni concisas, por las siguientes razones:

– El término *lispro* corresponde a una denominación internacional asignada por la OMS para una insulina modificada y según la guía INN dicho dímero debe citarse siempre indicando las posiciones y los aminoácidos que presentan los cambios, es decir, en la forma [B28-L-lisina, B29-L-prolina];

– las Reivindicaciones 1-3 hacen referencia a un rango o intervalo el cual es impreciso y no permite distinguir la invención del estado de la técnica. Además, no existe sustento para un compuesto que comprenda un PEG de cualquier tamaño comprendido entre dichos intervalos;

- una composición farmacéutica debe estar caracterizada a partir del ingrediente activo y excipientes y las proporciones de los mismos (Reivindicación 6);
- en las Reivindicaciones 7 y 8 se sugiere indicar cuál es el agente de isotonicidad seleccionado para distinguir la invención del estado de la técnica. Además, la expresión “donde la composición tiene una isotonicidad entre 270 mOsm y 330 mOsm” corresponde a un resultado a alcanzar y no a una característica técnica de la composición;
- en la Reivindicación 10 debe indicarse el valor de la cantidad terapéuticamente efectiva de insulina *lispro*;
- la Reivindicación 11 no es clara porque no especifica la condición bajo la cual se cumple que A y B estén “apropiadamente reticulados”. Esta expresión es ambigua y se sugiere reemplazarla por la característica técnica que conlleva a dicha condición. Además, éste proceso no es claro porque caracteriza el proceso por el producto a ser obtenido y no por la serie de pasos secuenciales lógicos necesarios para obtener dicho producto.

b- i) En primer lugar, llamo respetuosamente la atención de la Examinadora sobre las modificaciones realizadas al Capítulo Reivindicatorio Modificado adjunto, tal como se explican en el punto 1 del presente memorial. En este sentido considero superadas todas las objeciones elevadas por la Examinadora en relación con el término *lispro*, el cual fue reemplazado por “B28-L lisina, B29 L-prolina” en las Reivindicaciones 1 y 7-10.

ii) Con respecto a las objeciones en relación con las Reivindicaciones 1 a 3, a diferencia de la opinión de la Examinadora, vale la pena señalar que dicho intervalo si cuenta con el soporte requerido en el Capítulo Descriptivo. En particular, hay soporte para el intervalo reclamado en las Páginas 6, 17, 18, 23, 25, 26, 27, 33, Ejemplos 5-9, Tabla III y Tabla V, por lo tanto no se entiende la razón por la cual la Examinadora levanta este tipo de objeción, aun cuando existe soporte más que suficiente para todo el intervalo reclamado en la presente invención.

Sin embargo, y con el único objetivo de facilitar el trámite, la limitación de la antigua Reivindicación 2 fue introducida en las Reivindicaciones 1 y 9, de tal forma que el límite superior del intervalo del peso molecular del PEG fue **restringido estrictamente a 25 kDa**, lo cual, como se mostró anteriormente, cuenta con todo el soporte requerido en el Capítulo Descriptivo, incluso en los ejemplos, por lo tanto las Reivindicaciones 1 y 2 debe ser aceptadas ya que cualquier persona medianamente versada en la materia estaría en la capacidad de entender con absoluta claridad los límites de la presente invención.

iii) Ahora bien, y respecto a las composiciones farmacéuticas de las Reivindicaciones 4-8 (antiguas Reivindicación 6-10), me permito señalar que el inventor tiene el derecho de reclamar protección para la totalidad de un concepto inventivo desarrollado, sin importar la amplitud de éste, adicionalmente, la patentabilidad de las composiciones generales (tales como las reclamadas en las Reivindicaciones 4-8) recae únicamente en la novedad y nivel inventivo de los compuestos que se encuentran en éstas, más no en las composiciones como tal y mucho menos en los ingredientes específicos que pueda contener. En otros términos, la nueva Reivindicación 4 reclama una composición farmacéutica (cualquier composición) cuya característica técnica principal es que contiene los compuestos de la Reivindicación 1, sin limitación a excipientes, porcentajes o condiciones y sin importar la forma de dosificación en la que se presente. Por lo tanto, considero que no hay necesidad de incluir cual es el agente de isotonicidad, puesto que el alcance de dichas reivindicaciones de composición proporciona protección para cualquier composición en donde esté presente el compuesto de la Reivindicación 1, sin importar si tiene o no agentes de isotonicidad, buffers o cualquier otro tipo de excipiente.

En este sentido, también resulta relevante mencionar que las reivindicaciones no deben incluir elementos no esenciales, sino por el contrario, solamente aquellas características que distinguen la materia reclamada del arte previo. De hecho, incluir elementos específicos no esenciales en las reivindicaciones, tales como ingredientes, excipientes y sus proporciones, limitaría injustamente el derecho del inventor a reclamar el alcance del concepto inventivo desarrollado.

Incluso, la guía de Examen de la SIC establece que si la característica esencial de la composición es el compuesto activo, el Examinador no deberá requerir que se mencionen también otras características estructurales<sup>1</sup>. Adicionalmente, la guía de la SIC también establece que “*Si el objeto de la solicitud es un nuevo compuesto, el solicitante tiene derecho a reivindicar las composiciones farmacéuticas caracterizadas por contener ese nuevo compuesto, pero no está obligado a utilizar ejemplos de cómo diseñar y preparar composiciones concretas que contengan el nuevo compuesto porque “la solución aportada por la invención”, es decir el objeto de la invención, es “el nuevo compuesto” y la persona del oficio normalmente versada en la materia está en capacidad de diseñar y preparar composiciones concretas que contengan el nuevo compuesto, con la información divulgada en la solicitud y con sus conocimientos generales. En tal caso, se considerará que la descripción cumple el requisito de suficiencia.*”<sup>2</sup>. Es por esto, que las composiciones farmacéuticas reclamadas en las Reivindicaciones dependientes 4-8 son completamente aceptables a la luz de las mismas disposiciones y directrices internas de la

<sup>1</sup> Guía para Examen de solicitudes de Patente de Invención y Modelo de Utilidad, 2014, sección 2.6.5.4 Pg 76 (disponible en [http://api.sic.gov.co/Documentos/Guia\\_Examen\\_Patentes.pdf](http://api.sic.gov.co/Documentos/Guia_Examen_Patentes.pdf))

<sup>2</sup> Idem. sección 2.7.3 Pg 94.

Superintendencia de Industria y Comercio, sin necesidad de proporcionar características no esenciales de la invención.

Y aun así, aunque no sea técnicamente correcto, si se consideran resultados a alcanzar los valores de isotonicidad, el solicitante está en todo el derecho de reclamar protección para ello, puesto que la materia contenida en ésta depende de las reivindicaciones que comprenden las características estructurales de la invención, y por consiguiente, los resultados a alcanzar no son indeterminados. En este sentido, me permito resaltar que el Manual Andino de Patentes (MAP) establece que los parámetros, resultados a alcanzar y definiciones funcionales son totalmente aceptables siempre y cuando se incluyan las características estructurales.<sup>3</sup> En este caso, la Reivindicación 1 reclama las características estructurales de la invención y las Reivindicaciones 5-6, siendo dependiente de esta, deberían ser aceptadas.

iv) En lo referente a indicar el valor de la cantidad terapéuticamente efectiva de insulina lispro de la nueva Reivindicación 8 (antigua Reivindicación 10), vale la pena señalar que la presente solicitud no pretende buscar protección sobre un rango particular de dosificación, puesto que en estas cantidades no se evidencia el concepto inventivo de la presente invención. Por el contrario, y como se sustenta a lo largo del Capítulo Descriptivo y en el presente memorial, el concepto inventivo de la materia aquí reclamada recae en la novedad y nivel inventivo de los compuestos compuesto de insulina B28-L lisina, B29 L-prolina PEGilada de la fórmula: P-[(A)-(B)]. Por lo tanto, la presente invención solicita protección para los compuestos como tal, independientemente de la dosificación utilizada en un hipotético producto comercial.

Además, es claro que en la práctica mundial de patentes, y sobre todo en las patentes del sector químico-farmacéutico, se utiliza ésta expresión como una modalidad particular de la invención que *per se* está incluida en el alcance de la Reivindicación 1, la cual proporciona protección para cualquier cantidad, ya sea terapéuticamente efectiva o no, de manera análoga en la que se solicita protección para cualquier composición farmacéutica. Por lo tanto, no hay necesidad técnica ni legal de incluir dicha cantidad en dicha reivindicación, por lo que la Reivindicación 8 debe ser aceptada.

v) En relación con la expresión “apropiadamente reticulada” de antigua Reivindicación 11 (nueva Reivindicación 9), considero superada dicha objeción en tanto que fue sustituida por la expresión “contienen un enlace disulfuro entre la cisteína en la posición 7 de la SEC ID NO: 1 y la cisteína en la posición 7 de la SEC ID NO: 3, un enlace disulfuro entre la cisteína en la posición 20 de la SEC ID NO: 1 y la cisteína en la posición 19 de la SEC ID NO: 3,

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<sup>3</sup> Manual Andino de Patentes, OMPI, EPO, Secretariado de la Comunidad Andina, 2004, secciones 4.6.6 y 4.6.7

y un enlace disulfuro entre la cisteína en la posición 6 de la SEC ID NO: 1 y la cisteína en la posición 11 de la SEC ID NO: 1", lo cual encuentra soporte en la Reivindicación 1.

vi) En relación con el proceso de la Reivindicación 9, es oportuno señalar que dicha reivindicación fue reorganizada y redactada en otro sentido y formato, de tal forma que para cualquier persona medianamente versada en la materia sea más claro y evidente que dicho proceso cuenta con las condiciones y etapas esenciales para caracterizar por completo el objeto de la invención, tal como se muestra a continuación:

9. Un proceso para preparar un compuesto de insulina B28-L lisina, B29 L-prolina PEGilada de la fórmula:

P-[(A)-(B)], o una sal del mismo farmacéuticamente aceptable, caracterizado porque:

A es la cadena A de insulina B28-L lisina, B29 L-prolina (SEC ID NO: 1);

B es la cadena B de insulina B28-L lisina, B29 L-prolina (SEC ID NO: 3); y

P es un PEG que tiene un peso molecular entre 20 kDa y 40 kDa, y en donde

A y B contienen un enlace disulfuro entre la cisteína en la posición 7 de la SEC ID NO: 1 y la cisteína en la posición 7 de la SEC ID NO: 3, un enlace disulfuro entre la cisteína en la posición 20 de la SEC ID NO: 1 y la cisteína en la posición 19 de la SEC ID NO: 3, y un enlace disulfuro entre la cisteína en la posición 6 de la SEC ID NO: 1 y la cisteína en la posición 11 de la SEC ID NO: 1, y

P se une vía un enlace covalente de uretano al grupo épsilon-amino de la lisina en la posición 28 de B,

y donde dicho proceso comprende hacer reaccionar el grupo épsilon-amino de la lisina en la posición 28 de B con monometoxipoli (etilenglicol) p-nitrofenil carbonato (mPEG-NPC); en donde dicho mPEG-NPC tiene un peso molecular promedio ponderado entre 20 kDa y 25 kDa; y donde dicha reacción se lleva a cabo en las siguientes condiciones:

- a. en un solvente acuoso a un pH entre 8,5 y 11,5;
- b. a una temperatura entre 25°C y 30°C;
- c. por un período de tiempo entre 3 y 6 horas.

vii) Finalmente, con base en lo anterior, considero respetuosamente que el Capítulo Reivindicatorio Modificado adjunto cumple con los requisitos de claridad y concisión establecidos en la Decisión 486, y a la luz del Manual Andino de Patentes (MAP) y la Guía de la SIC, permitiéndole a la persona medianamente versada en la materia comprender inequívocamente el alcance de la materia reclamada.

5. a- La Examinadora señala que el documento "Insulin LISPRO"<sup>4</sup> (D1) y las patentes WO2008/015099 (D2), US6890518 (D3) y US2001031726 (D4) afectan el nivel inventivo de la invención.

<sup>4</sup> Holleman, F & Hoekstra, J. 1997. Insulin LISPRO. New England J. Med. 337(3):176-183

i) Con respecto a la Reivindicación 1, la SIC presenta la siguiente tabla comparando las características técnicas esenciales aquí reclamadas y las enseñanzas del arte previo, así:

| Características esenciales                                                                                                                                                                                                                                                                                                                                                                                | D1                                                                                                                                                                                                                                                                                                                                                                                                                           | D3                                                   |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------|
| Compuesto de insulina LISPRO pegilada                                                                                                                                                                                                                                                                                                                                                                     | Análogo de insulina modificado [B28-L-lisina, B29-L-prolina]                                                                                                                                                                                                                                                                                                                                                                 | Insulina PEGilada (Párr. [0010])                     |
| Cadena A SEC ID NO: 1                                                                                                                                                                                                                                                                                                                                                                                     | Cadena A SEC ID NO: 1 (Fig. 2 Pág. 117)                                                                                                                                                                                                                                                                                                                                                                                      |                                                      |
| Cadena B SEC ID NO: 3                                                                                                                                                                                                                                                                                                                                                                                     | Cadena B SEC ID NO: 3 (Fig. 2 Pág. 117)                                                                                                                                                                                                                                                                                                                                                                                      |                                                      |
| donde A y B contienen un enlace disulfuro entre la cisteína en la posición 7 de la SEQ ID NO:1 y la cisteína en la posición 7 de la SEQ ID NO:3, un enlace disulfuro entre la cisteína en la posición 20 de la SEQ ID N:1 y la cisteína en la posición 19 de la SEQ ID NO: 1 y un enlace disulfuro entre la cisteína en la posición 6 de la SEQ ID NO:1 y la cisteína en la posición 11 de la SEQ ID NO:1 | donde A y B contienen un enlace disulfuro entre la cisteína en la posición 7 de la SEQ ID NO:1 y la cisteína en la posición 7 de la SEQ ID NO:3, un enlace disulfuro entre la cisteína en la posición 20 de la SEQ ID N:1 y la cisteína en la posición 19 de la SEQ ID NO: 1 y un enlace disulfuro entre la cisteína en la posición 6 de la SEQ ID NO:1 y la cisteína en la posición 11 de la SEQ ID NO:1 (Fig. 2, Pág. 177) |                                                      |
| PEG de peso molecular de 17.5 kDa hasta 40 kDa unido vía un enlace covalente de uretano, al grupo epsilon-amino de la lisina de la posición 28 de B.                                                                                                                                                                                                                                                      | No menciona                                                                                                                                                                                                                                                                                                                                                                                                                  | PEG de peso molecular de 0.2 a 40kDa (Pcirr. [0084]) |

La Examinadora señala que D1 es considerado el estado de la técnica cercano a la invención definida en la Reivindicación 1 porque divulga un análogo de insulina modificado [B28-L-lisina, B29-L-prolina], que consiste en una cadena A idéntica a la SEQ ID NO: 1 de la presente solicitud y una cadena B idéntica a la SEQ ID NO: 3 de la presente solicitud en la que A y B contienen un enlace disulfuro entre la cisteína en la posición 7 de la cadena A y la cisteína en la posición 7 de la cadena B, entre la cisteína en la posición 20 de la cadena A y la cisteína en la posición 19 de la cadena B y entre la cisteína en la posición 6 de la cadena A y la cisteína en la posición 11 de la cadena A (Pág. 177, Figura 2), donde la característica principal de dicho compuesto es el intercambio de la lisina de la posición 29 con la prolina de la posición 28, de manera que la lisina se encuentra en la posición 28 (Pág. 177, Figura 2).

La diferencia entre el compuesto definido en la Reivindicación 1 y el compuesto divulgado en D1 consiste en que la invención se refiere específicamente a un análogo de insulina modificado [B28-L-lisina, B29-L-prolina] PEGilado mientras que D1 no hace referencia a la conjugación con el polímero PEG. El efecto que logra el incluir la PEGilación de la insulina lispro es que mejora las propiedades farmacocinéticas de la molécula de insulina.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: cómo modificar la insulina lispro ([B28-L-lisina, B29-L-prolina]) conocida en D1 para conseguir un compuesto con propiedades farmacocinéticas mejoradas. Sin embargo, la conjugación de moléculas como la insulina con polímeros PEG para mejorar las propiedades farmacocinéticas, ya está divulgada en D3, el cual se refiere a composiciones de insulina

acoplada covalentemente a una o más moléculas de polietilenglicol (Párr. [0010]), donde PEG está unido a la lisina de la posición 29 de la cadena B, y dicho PEG tiene un peso molecular entre 200 y 40000 daltons (Párr. [0284]), en donde dichos conjugados son útiles para la formulación de composiciones solubles en agua y fisiológicamente activas (Parr. [0010]).

En este sentido, la Examinadora señala que puesto que la característica principal del análogo de insulina modificado lispro = [B28-L-lisina, B29-L-prolina] descrito por la solicitante y conocido en D1 es la ubicación de la lisina en la posición B28, la persona del oficio normalmente versada en la materia se vería motivada a unir el PEG de 20 a 40KDa, tal como se divulga en D3, a la lisina en la posición B28 en el compuesto divulgado en D1, derivando así el objeto de la Reivindicación 1 de la presente solicitud, con la una expectativa razonable de conseguir un efecto prolongado.

ii) Con respecto a las Reivindicaciones 7 y 8, la Examinadora presenta las siguientes tablas comparando las características técnicas esenciales aquí reclamadas y las enseñanzas del arte previo, así:

| Características esenciales                                                | D3                                                                          | D4                                                                   |
|---------------------------------------------------------------------------|-----------------------------------------------------------------------------|----------------------------------------------------------------------|
| Composición farmacéutica que comprende un conjugado insulina LISPRO - PEG | Composición farmacéutica (resumen)<br>Conjugado insulina-PEG (Párr. [0010]) | Composición farmacéutica (resumen)<br>Insulina LISPRO (Párr. [0016]) |
| TRIS a una concentración entre 10 mM a 25mM                               | TRIS (Párr. [0133])                                                         | TRIS a una concentración de 12.3 a 37 mM (Párr. [0011])              |
| Un pH entre 7.0 y 8.0                                                     | pH de 7.8 (Párr. [0191])                                                    | pH entre 7.0 y 8.0 (Párr. [0063])                                    |
| al menos un agente de isotonicidad                                        | No menciona                                                                 | Agente isotónico (resumen)                                           |

| Características esenciales                                                | D3                                                                          | D4                                                                                             |
|---------------------------------------------------------------------------|-----------------------------------------------------------------------------|------------------------------------------------------------------------------------------------|
| Composición farmacéutica que comprende un conjugado insulina LISPRO - PEG | Composición farmacéutica (resumen)<br>Conjugado insulina-PEG (Párr. [0010]) | Composición farmacéutica (resumen)<br>Insulina LISPRO (Párr. [0016])                           |
| Fosfato a concentración entre 5mM y 10 mM a un pH entre 7.0 y 7.5         | Fosfato (Párr. [0133])                                                      | Fosfato con un pH entre 7.3 y 7.5 (Párr. [0064]) y una concentración 1 a 10 mM.(Párr. [0011]). |
| al menos un agente de isotonicidad                                        | No menciona                                                                 | Agente isotónico (resumen)                                                                     |

D3 se considera el estado de la técnica cercano a la invención definida en la Reivindicación 7 y 8 porque divulga polipéptidos tales como insulina acoplados a un polietilenglicol (Párr. [0010]), donde PEG está unido a la lisina de la posición 29 de B, y dicho PEG tiene un peso molecular entre 200 y 40000 daltons (Párr. [0284]). Dichos conjugados son útiles en la formulación de composiciones solubles en agua y fisiológicamente activas (Párr. [0010]), donde los excipientes de dichas composiciones corresponden a agentes amortiguadores como Tris, fosfato o borato

(Párr. [0140]), y agentes quelatantes como EDTA o zinc (Párr. [0142]). La diferencia entre la composición definida en la Reivindicación 7 y 8, y la divulgada en D3 consiste en las concentraciones específicas de TRIS y fosfato así como la adición de un agente de isotonicidad. El efecto que logra el incluir la concentración del agente amortiguador y el agente de isotonicidad es que mejora la estabilidad de la composición. Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: cómo mejorar la estabilidad de la insulina modificada en las posiciones 28 y 29 en una composición.

Sin embargo, la utilización del amortiguador TRIS y fosfato para mejorar la estabilidad del dímero modificado en las posiciones 28 y 29, ya está divulgada en D4 que se refiere a una composición que comprende TRIS/fosfato con un pH entre 7.0 y 8.0/7.3 y 7.5, con una concentración entre 12.3 mM y 37 mM/1 y 10 mM (Párr. [0011]) y que incluye un agente de isotonicidad (Parr. [0010]). Por otra parte, D4 divulga además la posibilidad de incluir otros agentes como zinc a una concentración entre 0.07ug/mL a 0.09ug/mL y m-cresol a una concentración de aproximadamente 2.2 mg/mL. En consecuencia, la persona del oficio normalmente versada en la materia, incluiría las concentraciones de excipientes divulgadas en D4 en la composición divulgada en D3 para llegar así al objeto de la Reivindicación 7 y 8 de la presente solicitud con expectativa razonable de obtener composiciones estables del análogo de insulina modificado [B28-L-lisina, B29-L-prolina].

iii) En relación con la Reivindicación 11, la Examinadora presenta la siguiente tabla comparando las características técnicas esenciales aquí reclamadas y las enseñanzas del arte previo, así:

| Características esenciales                                                                                                                                       | D2                                                                                                                                                                                                                                  | D1                                                                                                     |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|
| Proceso para obtener insulina LISPRO<br>PEGilada de la fbrmula: P-[(A)-(B)]                                                                                      | Proceso para obtener insulina PEGilada (Pdrrafos [0001], [0223])                                                                                                                                                                    | un análogo de insulina modificado [B28-L-lisina, B29-L-prolina] conocido como LISPRO (Fig. 2 Pág. 177) |
| Hacer reaccionar el grupo epsilon-amino de la lisina en la posición 28 de B con mPEG-NPC que tiene un peso molecular entre 20 kDa y 40 kDa en un solvente acuoso | Hacer reaccionar el grupo 6psilon-amino de la lisina de la cadena A o B (Pág. 31 líneas 1 y 2) con mPEGNPC (Pág. 31 línea 36) que tiene un peso molecular de entre 750 Da y 40000 Da (Pág. 32 líneas 14 a 20) en un solvente acuoso |                                                                                                        |
| pH entre 8,5 y 11,5                                                                                                                                              | pH entre 10.2 y 10.4 (Pág. 39 Ej. 2)                                                                                                                                                                                                |                                                                                                        |
| 25°C y 30°C por un periodo de tiempo entre 3 y 6 horas                                                                                                           | Temperatura ambiente durante 160 min (Pág. 39 Ej. 2).                                                                                                                                                                               |                                                                                                        |

La Examinador indica que D2 se considera el estado de la técnica cercano a la invención definida en la Reivindicación 11 porque divulga un procedimiento para obtener insulina PEGilada (Párr. [0001], [0223]) que comprende hacer reaccionar el grupo épsilon-amino de la lisina presente en la cadena A o B de la insulina (Pág. 31 líneas 1 y 2) con mPEG-NPC (Pág. 31 línea 36) que tiene

un peso molecular de entre 750 Da y 40000 Da (Pág. 32 líneas 14 a 20) en un solvente acuoso, donde la reacción se ajusta a un pH entre 10.2 y 10.4 (Pág. 39 Ej. 2) a temperatura ambiente durante 160 min (Pág. 39 Ej. 2).

La diferencia entre la Reivindicación 11 y el procedimiento de D2 consiste en que la insulina PEGilada de la presente solicitud es el análogo de insulina modificado [B28-L-lisina, B29-L-prolina] LISPRO mientras D2 se refiere a la PEGilación de cualquier lisina en la cadena A o B de la insulina nativa. El efecto que dicha diferencia confiere a la invención es que se consigue un procedimiento de PEGilación específico para la insulina LISPRO. Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular así: cómo diseñar un procedimiento específico para PEGilar una insulina LISPRO. Sin embargo, el análogo de insulina modificado lispro equivalente a [B28-L-lisina, B29-L-prolina] ya había sido divulgado previamente en D1, el cual da a conocer una insulina modificada que consiste en una cadena A idéntica a la SEQ ID NO: 1 de la presente solicitud y una cadena B idéntica a la SEQ ID NO: 3 de la presente solicitud en la que A y B contienen un enlace disulfuro entre la cisteína en la posición 7 de la cadena A y la cisteína en la posición 7 de la cadena B, entre la cisteína en la posición 20 de la cadena A y la cisteína en la posición 19 de la cadena B y entre la cisteína en la posición 6 de la cadena A y la cisteína en la posición 11 de la cadena A (Pág. 177, Figura 2), donde la característica principal de dicho compuesto es el intercambio de la lisina de la posición 29 con la prolina de la posición 28, de manera que la lisina se encuentra en la posición 28 (Pág. 177, Figura 2). En consecuencia, la persona del oficio normalmente versada en la materia, seleccionaría la insulina LISPRO conocida en D1 y llevaría a cabo el proceso de PEGilación divulgado en D2 para llegar así al objeto de la Reivindicación 11 de la solicitud. Por lo cual, la solución propuesta se considera obvia y sin nivel inventivo.

b- i) En primer lugar, es preciso resaltar que la presente invención reclama un compuesto de insulina B28-L lisina, B29 L-prolina PEGilada de la fórmula P-[(A)-(B)], o una sal del mismo farmacéuticamente aceptable, caracterizado porque:

A es la cadena A de insulina B28-L lisina, B29 L-prolina (SEC ID NO: 1);

B es la cadena B de insulina B28-L lisina, B29 L-prolina (SEC ID NO: 3); y

P es un PEG que tiene un peso molecular en el intervalo desde 17,5 kDa hasta 25 kDa, y en donde

A y B contienen un enlace disulfuro entre la cisteína en la posición 7 de la SEC ID NO: 1 y la cisteína en la posición 7 de la SEC ID NO: 3, un enlace disulfuro entre la cisteína en la posición 20 de la SEC ID NO: 1 y la cisteína en la posición 19 de la SEC ID NO: 3, y un enlace disulfuro entre la cisteína en la posición 6 de la SEC ID NO: 1 y la cisteína en la posición 11 de

la SEC ID NO: 1, y

P está unido, vía un enlace covalente de uretano, al grupo epsilon-amino de la lisina de la posición 28 de B.

En relación con el arte previo citado, es pertinente señalar que D1 es una publicación académica que realiza una revisión general de datos farmacológicos y clínicos de una clase de insulina lispro y su papel en el tratamiento de pacientes con diabetes mellitus. De hecho, las composiciones mencionadas en D1 comprenden una insulina lispro que **no está PEGilada** y además se administra en regímenes que implican dos inyecciones diarias. D1 no proporciona enseñanza o sugerencia alguna para producir los compuestos de insulina B28-L lisina, B29 L-prolina PEGilada de la fórmula P-[(A)-(B)] con la configuración estructural de la Reivindicación 1.

Ahora bien, contrario a lo argumentado por la Examinadora, D2 alejaría a cualquier persona medianamente versada en la materia de la presente invención, ya que necesariamente se habría motivado a producir compuestos estructuralmente diferentes. D2 enseña diversas insulinas pegiladas que comprenden residuos añadidos a un Carbono o Nitrógeno terminal de las cadenas A o B. Específicamente D2 indica "*... es deseable que no haya **Lys presente en cualquiera de las posiciones 1 a 21 en la cadena A (A1-A21)** y no haya **Lys presente en cualquiera de las posiciones 1 a 30 en la cadena B (B1-B30)***". En contraste, la insulina PEGilada de la presente invención comprende cadenas A y B, las cuales se encuentran unidas a un PEG de alto peso molecular a través de un enlace covalente con el grupo uretano epsilon-amino de la lisina en la posición 28 de la cadena B. Así las cosas, es evidente que la invención aquí reivindicada no habría podido ser derivada evidentemente de los documentos D1 y D2 citados como arte previo por la Examinadora.

En este sentido, la Examinadora podría sostenerse en su argumentación y decir que la única diferencia que existe entre D1 y la invención es la pegilación, puesto que pegar la insulina de D1, con las enseñanzas de D2 era obvio. Sin embargo, a la luz de lo mencionado anteriormente para D1 y D2, es claro que la persona medianamente versada en la material no habría estado motivada a pegar la insulina lispro con el objetivo de aumentar la solubilidad. Incluso, la Examinadora no ha proporcionado evidencia alguna de que la solubilidad sea un problema con la insulina lispro de D1. Ante esto, surge la inquietud de ¿por qué la persona medianamente versada en la material buscaría pegar la insulina lispro de D1 con el objetivo de aumentar la solubilidad, si ni siquiera el arte previo menciona un problema de solubilidad? Evidentemente el análisis de la Examinadora se queda corto en argumentar y demostrar esto, y simplemente evidencia que el análisis de nivel inventivo empleado para D1 y D2 fue realizado en

retrospectiva, ya que, si se hubiera realizado una evaluación objetiva, la Examinadora no solo habría buscado elementos diferenciales entre la invención y D1, sino además habría buscado las sugerencias explícitas en el arte previo que la hubieran llevado a implementar dichas diferencias de manera obvia.

En el presente caso, D1 no enseña ni revela un problema de solubilidad con la insulina lispro allí divulgada. Por el contrario, la finalidad de D1 es mostrarle a la comunidad académica, los resultados terapéuticos y farmacológicos obtenidos con una insulina lispro, que además, **no está pegilada**. En el mismo sentido, D2 enseña insulinas pegiladas, sin embargo, D2 también enseña que la lisina no debe estar presente en las cadenas de insulina, puesto que conllevaría a efectos indeseados. En contraste, y estando totalmente en contra de las enseñanzas de D2, la presente invención reclama lisina dentro de las cadenas de la insulina lispro. En este sentido, vale la pena preguntarse ¿por qué la persona medianamente versada en la materia pegilaría la insulina de D1 con el objetivo de aumentar la solubilidad, si dicha solubilidad **no se encuentra** reportada como un problema a solucionar en D1? ¿Por qué la persona medianamente versada en la materia iría en contra de las enseñanzas de D2 e incluiría una lisina en las cadenas A y B, aun sabiendo que D2 le sugiere específicamente no hacerlo? ¿Si no había un problema de solubilidad por solucionar, por qué la persona medianamente versada en la materia habría pegilado la insulina de D1, y además habría ido en contra de las enseñanzas de D2, incluyendo lisinas indeseables en sus cadenas, aun sabiendo desde el primer momento que no iba a obtener una mejor insulina que las divulgadas en D1 y en D2? Solicito que la Examinadora responda a estos cuestionamientos.

De esta manera, queda claro que, a diferencia del análisis en retrospectiva que realiza la SIC, la persona medianamente versada en la materia, siguiendo las enseñanzas de D1 y D2 habría estado motivada necesariamente a producir insulinas lispro sin pegilar (puesto que no existe un problema de solubilidad), y que además no contiene lisina dentro de las cadenas A y B (puesto que generarían un efecto indeseado), lo cual resulta absolutamente opuesto y diferente de las características estructurales de la insulina reclamada en la presente invención.

ii) Con respecto a D3, dicho documento se refiere a derivados hidrofílicos polímero-modificados de insulina que son útiles para entregar dicha insulina en los pulmones con propiedades farmacocinéticas y/o farmacodinámicas mejoradas comparadas con insulinas nativas. Particularmente, los derivados pegilados de insulina de D3 muestran ejemplos de PEG que tienen pesos moleculares de 750, 2000 o 5000 Da, mientras que la presente invención reclama compuestos PEG con rangos desde 17,5 kDa hasta 25 kDa, lo cual resulta completamente alejado y diferente de lo sugerido en D3. Incluso, aunque astutamente la Examinadora hace caso omiso a

este aspecto de pesos molecular, es importante también señalar que D3 se encuentra en el campo técnico de **pegilar insulinas nativas** y los efectos relacionados con ello, mas no en el campo técnico de pegilar insulinas lispro de la presente invención. Además, documentos relacionados con formulaciones pulmonares, como lo es D3, no son relevantes para evaluar el nivel inventivo de la presente invención ya que la presente invención hace referencia a formulaciones inyectables. Es decir, la presente invención reclama insulina lispro pegilada, la cual, como la Examinadora debe saberlo, presenta configuraciones estructurales completamente diferentes de la insulina nativa, por lo que no es posible extrapolar y comparar directamente los hipotéticos resultados que tendría pegilar una insulina nativa y una insulina lispro. Vale la pena recordarle a la Examinadora que las invenciones en el campo químico/biotecnológico corresponden a un arte impredecible, y por mucha información técnica que esté disponible, jamás podrán predecirse los efectos y resultados de hacer cambios estructurales en las moléculas, porque de ser así, los laboratorios de investigación no existirían, y los avances de la ciencia simplemente se harían con papel y lápiz.

En relación con D4, este documento enseña composiciones farmacéuticas de polipéptidos (y sus derivados, preferiblemente insulina monomérica) que comprende un buffer TRIS-fosfato, zinc, un preservante fenólico y un agente de isotonicidad como glicerol. Por lo tanto, aunque D4 mencione insulina lispro, dicho documento no enseña ni mucho menos sugiere insulinas lispro pegiladas o derivados del mismo, y mucho menos los pesos moleculares de PEG reclamados en la presente invención.

En este orden de ideas, es evidente que la única razón que podría llevar a considerar que la presente invención no es inventiva, es que se esté realizando un análisis retrospectivo, donde, a partir de los conocimientos adquiridos al leer la presente solicitud, intenta encontrar en el estado de la técnica fragmentos que demuestren que **teóricamente se podría haber llegado a la invención que aquí se reivindica.**

Sea esta la oportunidad para recordarle a la Examinadora que la Guía para el Examen de Solicitudes de Patentes de Invención y Modelos de Utilidad de la Superintendencia de Industria y Comercio señala: *"Es importante tener en cuenta que una invención reivindicada que, **a primera vista, parece evidente, puede ser inventiva.** Una vez formulada una nueva idea, **a menudo se puede demostrar teóricamente el modo en el que se podría llegar a ella,** a partir de algo conocido, a través de una serie de etapas aparentemente fáciles. **El examinador de patentes debe evitar el uso de un análisis ex post facto de este tipo.***

*‘Es decir, el estado de la técnica se debe examinar sin el beneficio del conocimiento ofrecido retrospectivamente por la invención reivindicada. La indicación o la sugerencia que permita llegar a la invención reivindicada deben proceder del estado de la técnica o de los conocimientos generales de la persona del oficio normalmente versada en la materia, y no basarse en la divulgación del solicitante.*

*Uno de los factores que deben tomarse en consideración para establecer si la persona del oficio normalmente versada en la materia hubiera estado motivada a combinar los documentos del estado de la técnica es la probabilidad razonable de éxito como resultado de tal combinación de sugerencias del estado de la técnica, consideradas colectivamente.*

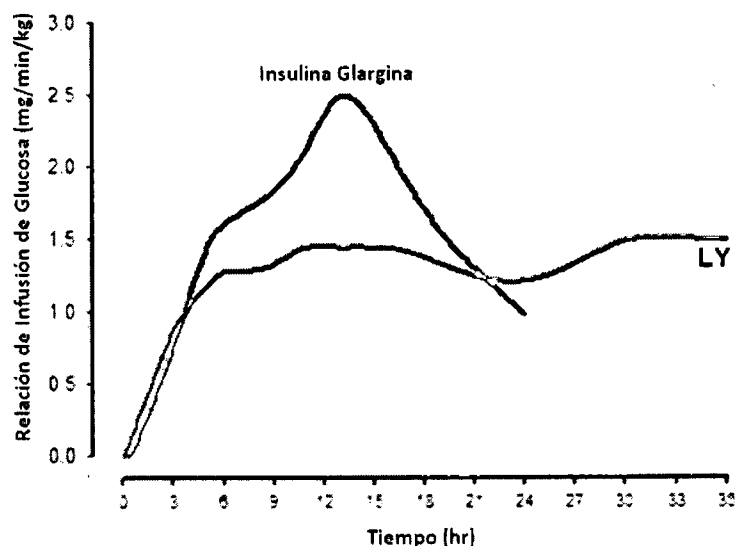
*En cualquier caso, el Examinador de patentes deberá esforzarse por hacer una evaluación práctica conforme a la realidad. Deberá tener en cuenta todo lo que se sabe en cuanto a los antecedentes de la invención reivindicada y dar el valor justo a los argumentos o pruebas pertinentes presentados por el solicitante.”<sup>5</sup> (Mi énfasis).*

Para el presente caso, nada en D1, D2, D3 o D4, solos ni en combinación, llevaría a establecer que existía la probabilidad razonable de éxito, como resultado de tal combinación de sugerencias del estado de la técnica, de obtener una insulina lispro pegilada tal y como la reclama la presente invención. Nada en el arte previo citado proporciona enseñanza o sugerencia alguna que le permita a la persona medianamente versada en la materia considerar una configuración estructural tal y como se reclama en la presente invención, ni mucho menos sugiere en absoluto, que ir en contra de las enseñanzas de dichos documentos tendría un aumento beneficioso en la solubilidad y el efecto prolongado, aun más si dicho arte previo no menciona estos dos efectos como un problema a solucionar. Por estas razones, la invención reivindicada no puede ser derivada del estado de la técnica y por lo tanto, la invención reivindicada no es obvia y reviste nivel inventivo.

iii) Adicionalmente, con el ánimo de proporcionar mayor evidencia del nivel inventivo de la presente invención, me permito llamar la atención sobre la Figura 2 del Capítulo Descriptivo, la cual ilustra una gráfica de relaciones de infusión de glucosa (GIR) en humanos después de una dosis subcutánea, ya sea insulina lispro de PEG (LY; 0.225 mg/kg) o insulina glargina (0,5 U/kg), que demuestra las ventajas de la presente invención:

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<sup>5</sup> Guía para Examen de Solicitudes de Patentes de Invención y Modelo de utilidad. Superintendencia de Industria y Comercio. 2012. Sección 2.13.6.3 Página 76. Relativo al *Análisis ex post facto o retrospectivo o hindsight* (disponible electrónicamente en [http://api.sic.gov.co/Documentos/Guia\\_Examen\\_Patentes.pdf](http://api.sic.gov.co/Documentos/Guia_Examen_Patentes.pdf))



De otra parte, llamo la atención de la Examinadora sobre el Capítulo Descriptivo (página 101, línea 11) que señala que en el Ejemplo 12 que la insulina lispro PEGilada (LY) de la presente invención proporciona una larga duración de acción, una vida media aparente que va de 24 a 44 horas y características basales, es decir, una proporción de pico de menos de 2 (Figura 2). De hecho, la duración de acción para LY es mayor que la de insulina glargina (Figura 2). Además, la variabilidad entre sujetos en los glucodinámicos es menos del 30% (datos no mostrados) lo cual es similar a, o mejor que la glargina. Finalmente, los datos glucodinámicos de la Parte C del estudio (0.5 mg/kg) resultan en un perfil de velocidad de infusión de glucosa (GIR, por sus siglas en inglés) para LY que sin picos, donde la glucodinámica se mantiene por más de 36 horas, y excede el pico de respuesta GIR para glargina (0.5 U/kg; datos no mostrados). Evidentemente, los anteriores resultados no eran previsibles a partir de las enseñanzas de D1-D4, puesto que ni siquiera mencionaban un problema a resolver.

De otra parte, llamo también la atención sobre los siguientes los documentos adjuntos al presente memorial, los cuales demuestran los efectos sorprendentes e inesperados de la materia reclamada en el Capítulo Reivindicatorio Modificado adjunto, así:

El primer documento adjunto “*Steady-State pharmacokinetics and glucodynamics of the novel, long-acting basal insulin LY2605541 dosed once-daily in patients with type 2 diabetes mellitus*”<sup>6</sup> (ANEXO 1) confirma los beneficios del LY2605541, es decir, de la insulina lispro PEGilada tal y como se reclama en la presente invención. En especial, estos datos en humanos obtenidos en estudios clínicos demuestran los beneficios de dicha molécula. Incluso, al comienzo de la página

<sup>6</sup> Sinha, V. P., et al. Steady-State pharmacokinetics and glucodynamics of the novel, long-acting basal insulin LY2605541 dosed once-daily in patients with type 2 diabetes mellitus. Diabetes, Obesity and Metabolism 16: 344-350.2014.

349, hay una mención de la actividad hepato-preferencial de este compuesto, lo que, claramente no fue sugerido ni motivado por el arte previo citado.

Es por esto, que me permito llamar la atención sobre el segundo documento “*Novel PEGylated Basal Insulin LY2605541 Has a Preferential Hepatic Effect on Glucose Metabolism*”<sup>7</sup> (ANEXO 2), el cual describe en detalle la actividad hepatopreferencial evidencia por este compuesto. En especial, en la sección de Discusión de este artículo, se puede apreciar claramente que la actividad hepatopreferencial usual de la insulina natural es bastante cercana comparada con la del compuesto reclamado. Es por esto que resulta importante señalar que el hecho que el compuesto reclamado imite de manera más cercana y parecida la distribución de la insulina nativa o natural (es decir, que sea hepatopreferencial) corresponde claramente a un efecto sorprendente e inesperado, el cual no hubiera podido ser derivado a partir de las escasas y lejanas enseñanzas del arte previo citado, los cuales ni siquiera mencionan esta actividad.

En conclusión, es evidente que una persona medianamente versada en la materia, siguiendo las enseñanzas del arte previo citado, solos o en combinación, no habría encontrado enseñanza o motivación alguna que lo llevara a derivar de manera evidente compuestos de insulina lispro PEGilada como los aquí reivindicados, y mucho menos lo llevaría a esperar, con alguna expectativa de éxito, que dichos compuestos presentarían las propiedades inesperadas y sorprendentes mencionadas en la Descripción (Ejemplo 12, Figura 2) y en los documentos anexos. Adicionalmente, lo anterior también se encuentra en concordancia con los lineamientos del MAP, que establece que resultados y mejoras sorprendentes constituyen indicios de no obviedad, de tal manera que propiedades ventajosas constituyen indicios aceptables de nivel inventivo<sup>8</sup>, por lo que indudablemente, la presente invención reviste nivel inventivo.

Además, en este punto vale la pena señalar que el Manual Andino de Patentes establece que para que pueda negarse la existencia de nivel inventivo, es necesario no solamente que la combinación de las enseñanzas pueda hacerse, sino también que exista una sugerencia o razón tal, que lleve al técnico con conocimientos medios a combinar las enseñanzas de los documentos<sup>9</sup>. Además, el Manual Andino de Patentes también establece que las invenciones en el campo de la química tienen nivel inventivo cuando los compuestos tienen una **estructura inesperada** (no derivable por la persona medianamente versada en la materia) o cuando presentan un **efecto técnico**

<sup>7</sup> Moore, M. C., et al. Novel PEGylated Basal Insulin LY2605541 Has a Preferential Hepatic Effect on Glucose Metabolism. Diabetes. Vol. 63. February 2014.

<sup>8</sup> Manual Andino de Patentes. OMPI, EPO, Secretaría de la Comunidad Andina. 2004. Sección 4.6.1.

<sup>9</sup> Manual Andino de Patentes. (2004). Sección 10.2.1 Ejemplo 1 (disponible en <http://www.comunidadandina.org/public/patentes.pdf>).

# OLARTEMOURE

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inesperado o mejorado impredecible del arte previo<sup>10</sup>. En conclusión, y de acuerdo con los lineamientos citados anteriormente, nada en el arte previo (D1, D2, D3 y D4) habría guiado específica e inequívocamente a la persona medianamente versada en la materia a desarrollar una insulina lispro pegilada tal y como se reclama en la presente invención, y mucho menos con alguna expectativa de éxito que tal configuración estructural incrementaría la solubilidad, incrementara el perfil de acción y además presentara un comportamiento hepatopreferencial tal como el mostrado por la insulina nativa, por lo que respetuosamente considero que el nivel inventivo debe ser reconocido.

6. Por todo lo anterior, considero que las objeciones presentadas por la Examinadora en su segundo examen de fondo, han sido resueltas y por consiguiente solicito respetuosamente que el Despacho tenga por cumplidos los requisitos de patentabilidad y proceda con la concesión de la patente.

Atentamente,



CARLOS R. OLARTE

C.C. No. 79.782.747 de Bogotá

T.P. 74.295

Anexos:

- Capítulo Reivindicatorio Modificado.
- ANEXO 1: Artículo Científico: Steady-State pharmacokinetics and glucodynamics of the novel, long-acting basal insulin LY2605541 dosed once-daily in patients with type 2 diabetes mellitus.
- ANEXO 2: Artículo Científico: Novel PEGylated Basal Insulin LY2605541 Has a Preferential Hepatic Effect on Glucose Metabolism.

<sup>10</sup> Manual Andino de Patentes. (2004). Sección 10.8.1 (disponible en <http://www.comunidadandina.org/public/patentes.pdf>).

## CAPITULO REIVINDICATORIO MODIFICADO

### (Segundo Examen de Fondo)

1. Un compuesto de insulina B28-L lisina, B29 L-prolina PEGilada de la fórmula:  
P-[(A)-(B)], o una sal del mismo farmacéuticamente aceptable, caracterizado porque:  
A es la cadena A de insulina B28-L lisina, B29 L-prolina (SEC ID NO: 1);  
B es la cadena B de insulina B28-L lisina, B29 L-prolina (SEC ID NO: 3); y  
P es un PEG que tiene un peso molecular en el intervalo desde 17,5 kDa hasta 25 kDa, y en donde  
A y B contienen un enlace disulfuro entre la cisteína en la posición 7 de la SEC ID NO: 1 y la cisteína en la posición 7 de la SEC ID NO: 3, un enlace disulfuro entre la cisteína en la posición 20 de la SEC ID NO: 1 y la cisteína en la posición 19 de la SEC ID NO: 3, y un enlace disulfuro entre la cisteína en la posición 6 de la SEC ID NO: 1 y la cisteína en la posición 11 de la SEC ID NO: 1, y  
P está unido, vía un enlace covalente de uretano, al grupo epsilon-amino de la lisina de la posición 28 de B.
2. El compuesto de la Reivindicación 1, caracterizado porque el PEG tiene un peso molecular en el intervalo de 20 kDa a 25 kDa.
3. El compuesto de conformidad cualquiera de las Reivindicaciones 1 a 2, caracterizado porque el PEG tiene un peso molecular de 20 kDa.
4. Una composición farmacéutica, caracterizada porque comprende un compuesto de conformidad con cualquiera de las Reivindicaciones 1 a 3, y uno o más excipientes, diluyentes o portadores farmacéuticamente aceptables.
5. La composición farmacéutica de conformidad con la Reivindicación 4, caracterizada porque adicionalmente comprende un amortiguador TRIS a una concentración en el intervalo de 10 mM a 25 mM de TRIS, en donde la composición farmacéutica tiene un pH entre 7,0 y 8,0 y al menos un agente de isotonicidad, en donde la composición tiene una isotonicidad entre 270 mOsm y 330 mOsm.
6. La composición farmacéutica de conformidad con la Reivindicación 4, caracterizada porque adicionalmente comprende un amortiguador de fosfato a una concentración entre 5 mM y 10 mM y en donde la composición farmacéutica tiene un pH entre 7,0 y 7,5 y al menos un agente de isotonicidad, en donde la composición tiene

una isotonicidad entre 270 mOsm y 330 mOsm.

7. La composición farmacéutica de conformidad con cualquiera de las Reivindicaciones 4 a 6, caracterizada porque comprende el compuesto de insulina B28-L lisina, B29 L-prolina PEGilada a una concentración desde 15 mg/mL hasta 40 mg/mL, zinc a una relación de zinc a compuesto de insulina B28-L lisina, B29 L-prolina PEGilada desde 0,33 a 0,83, y m-cresol a una concentración desde 10 mM hasta 40 mM a un pH entre 7,0 y 8,0.

8. La composición farmacéutica de conformidad con cualquiera de las Reivindicaciones 4 a 7, caracterizada porque adicionalmente comprende una cantidad terapéuticamente efectiva de insulina B28-L lisina, B29 L-prolina.

9. Un proceso para preparar un compuesto de insulina B28-L lisina, B29 L-prolina PEGilada de la fórmula:

P-[(A)-(B)], o una sal del mismo farmacéuticamente aceptable, caracterizado porque:

A es la cadena A de insulina B28-L lisina, B29 L-prolina (SEC ID NO: 1);

B es la cadena B de insulina B28-L lisina, B29 L-prolina (SEC ID NO: 3); y

P es un PEG que tiene un peso molecular entre 20 kDa y 40 kDa, y en donde

A y B contienen un enlace disulfuro entre la cisteína en la posición 7 de la SEC ID NO: 1 y la cisteína en la posición 7 de la SEC ID NO: 3, un enlace disulfuro entre la cisteína en la posición 20 de la SEC ID NO: 1 y la cisteína en la posición 19 de la SEC ID NO: 3, y un enlace disulfuro entre la cisteína en la posición 6 de la SEC ID NO: 1 y la cisteína en la posición 11 de la SEC ID NO: 1, y

P se une vía un enlace covalente de uretano al grupo epsilon-amino de la lisina en la posición 28 de B,

y donde dicho proceso comprende hacer reaccionar el grupo epsilon-amino de la lisina en la posición 28 de B con monometoxipoli (etilenglicol) p-nitrofenil carbonato (mPEG-NPC); en donde dicho mPEG-NPC tiene un peso molecular promedio ponderado entre 20 kDa y 25 kDa; y donde dicha reacción se lleva a cabo en las siguientes condiciones:

- a. en un solvente acuoso a un pH entre 8,5 y 11,5;
- b. a una temperatura entre 25°C y 30°C;
- c. por un período de tiempo entre 3 y 6 horas.

10. El proceso de conformidad con la Reivindicación 9, caracterizado porque tiene una relación molar de PEG:insulina B28-L lisina, B29 L-prolina entre 2,5 y 5,0.

11. El proceso de conformidad con la Reivindicación 9 o 10, caracterizado porque el peso molecular promedio ponderado del mPEG-NPC es 20 kDa.
12. El proceso de conformidad con cualquiera de las Reivindicaciones 9 a 11, caracterizado porque el pH de la reacción se mantiene entre 10,5 y 11,5.
13. El proceso de conformidad con cualquiera de las Reivindicaciones 9 a 12, caracterizado porque la reacción se lleva a cabo entre 25°C y 30°C por 3 horas.

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## Steady-state pharmacokinetics and glucodynamics of the novel, long-acting basal insulin LY2605541 dosed once-daily in patients with type 2 diabetes mellitus

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**Aims:** To assess the pharmacokinetics (PK) and glucodynamics (GD) of LY2605541 in patients with type 2 diabetes mellitus.

**Methods:** This parallel-group, open-label, dose-escalation study examined the PK and GD of basal insulin LY2605541 after single and multiple-dose administration. Fixed doses of LY2605541 (0.33–1.00 U/kg) were given once-daily (OD) for 14 days to insulin-treated patients with type 2 diabetes. A 24-h euglycaemic glucose clamp was conducted on days 1 and 14.

**Results:** PK steady state was achieved within 7–10 days and the peak-to-trough fluctuation was <2, translating to a nearly 'peakless' glucose infusion rate at steady state and with a duration of action of at least 24 h. Across dose levels  $t_{1/2}$  ranged from 44.7 to 75.5 h (~2–3 days). As steady state was achieved, there were dose-dependent reductions in the prandial insulin dose and in fasting blood glucose, which decreased to 60–100 mg/dl across dose levels. Within-patient variability was <14 and <26% for the area under the concentration versus time curve (AUC) of the 8-point blood glucose profile and fasting blood glucose, respectively. The nocturnal glucose control between 03:00 and 09:00 hours was relatively unchanged. Mild hypoglycaemia was the most common adverse event.

**Conclusions:** In this Phase I study of fixed LY2605541 doses without titration, LY2605541 was well-tolerated and demonstrated a flat PK and GD profile accompanied by glucose normalization, prandial insulin dose reduction and no severe hypoglycaemia.

**Keywords:** insulin analogues, pharmacodynamics, pharmacokinetics, type 2 diabetes

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### Introduction

Basal insulin, in combination with oral antidiabetes medications, has been a successful initial therapy option for patients with type 2 diabetes mellitus [1–4]. Its success can be measured by the ability to provide several desirable characteristics, including a solution-based formulation that can readily be integrated into various delivery devices; a prolonged activity profile that supports a once-daily dosing regimen; no pronounced peaks in activity that can induce hypoglycaemia; reproducible absorption from the subcutaneous tissues with minimal variation in blood glucose-lowering activity; and a weight-gain neutral or weight-loss profile. While improvements have been made in the pharmacokinetic/pharmacodynamic (PK/PD) properties of basal insulin over the last decade, currently available basal insulins still do not meet all desired characteristics. In particular, neutral protamine Hagedorn (NPH) insulin exhibits a marked peak in its PK profile [5–7], with the basal insulin analogues, insulin glargine and insulin detemir exhibiting a smaller peak [8]. Additionally, NPH, insulin glargine and insulin detemir do not always cover 24-h insulin needs of patients with once-daily

administration [7,9,10]. PD variation is observed in particular with either NPH or glargine [11].

The novel basal insulin analogue, LY2605541, is a long-acting insulin composed of insulin lispro covalently bound, via a urethane bond, to a 20-kDa polyethylene glycol (PEG) chain. LY2605541 has a large functional size and a prolonged duration of action. This extended activity is believed to be mediated through slower absorption and reduced renal clearance [12]. Additionally, the tissue distribution of LY2605541 is believed to be similar to endogenous insulin, with restricted peripheral activity and retained hepatic activity [13].

This study assessed the PK and glucodynamic (GD) profile of LY2605541 after single dose and multiple daily doses in patients with type 2 diabetes previously treated with insulin. Secondary objectives included assessing the safety, tolerability and intra-patient variability of LY2605541 PK and GD parameters.

### Patients and Methods

#### Patients and Study design

This Phase I, open-label, parallel, four-arm, multiple-ascending dose study was completed at a single center in Germany between April 2009 and July 2009. The sample size of eight patients per cohort was expected to provide adequate power

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for parameter estimation and is consistent with other Phase I studies evaluating safety, PK and/or GD parameters. The study was approved by an ethical review board. All study procedures were carried out in compliance with the Declaration of Helsinki and Good Clinical Practices. All subjects provided written informed consent.

Eligible patients were men or women, 18–65 years of age (inclusive), with type 2 diabetes mellitus diagnosed at least 1 year prior to enrollment, and who were treated with basal and bolus insulin for at least 3 months prior to screening. Patients were required to have a glycosylated haemoglobin (HbA1c)  $\leq 10\%$  and a fasting C-peptide level of  $\leq 1.0$  nmol/l at screening to prevent stimulation of endogenous insulin through glucose infusion during the clamp experiments. Patients were excluded if they used a total daily dose of basal insulin that exceeded 0.8 (1)U/kg/day had taken any glucose-lowering medications other than insulin for at least 3 months before the screening visit, had  $>1$  episode of severe hypoglycaemia, or had received chronic systemic glucocorticoid therapy (excluding topical, intra-articular and inhaled preparations) in the past year, or any glucocorticoid therapy within 30 days prior to the screening visit.

Patients were assigned to 1 of 4 treatment cohorts of ascending LY2605541 dose. LY2605541 was administered as mg/kg (nmols/kg) with dose conversion to units (U) as follows: 0.025 mg  $\approx$  1 nmol and 9 nmols = 1 U of LY2605541. Given this conversion from mg/kg to U/kg, dose levels were 0.33 U/kg (Cohort 1), 0.67 U/kg (Cohort 2), 1.00 U/kg (Cohort 3) and 0.50 U/kg (Cohort 4). The planned dose in Cohort 4 was intended to be 1.33 U/kg; however, this dose was reduced due to the number of events of mild hypoglycaemia in Cohort 3.

### Study Procedures

Patients were admitted to the Clinical Research Unit on day 2 and remained resident throughout the dosing period (days 1 through 14), until discharge on day 16. The last dose of basal insulin glargine or insulin detemir on day 2 was required to be administered prior to bedtime (23:00 hours at the latest) in order to safely avoid any carryover effects of long-acting insulin preparations. Patients were allowed to subcutaneously administer short-acting insulin (Humulin<sup>®</sup>, Eli Lilly and Company, Indianapolis, IN, USA) for mealtime coverage on day 1. LY2605541 was administered subcutaneously, once daily in the morning on days 1–14. Upon discharge, patients were followed on an outpatient basis until at least 30 days after the

last dose of LY2605541. Immunogenicity testing was conducted approximately 30 days after the last dose of study drug.

Euglycaemic glucose clamps (24-h duration) were performed on days 1 and 14. Patients were fasted (except for water) from approximately 8 h prior to dosing until completion of the 24-h clamp period. Intravenous (i.v.) insulin infusion of regular human insulin (Humulin<sup>®</sup>) was started at least 4 h before administration of LY2605541 to achieve a target blood glucose level of 90 mg/dl (5.0 mmol/l). The goal was to achieve this target by at least 2 h before dosing so that the Humulin<sup>®</sup> infusion rate could be lowered to a level where the blood glucose remained stable and at the same time no glucose infusion was required. The i.v. insulin infusion was stopped completely 15 min before trial drug administration. After the administration of LY2605541, a 20% glucose solution was infused at a variable rate (via Biostat<sup>™</sup>, Miles Laboratories, Elkhart, IN, USA) in order to maintain blood glucose at the clamp level of 90 mg/dl. The rate of glucose delivery was adjusted by the Biostat<sup>™</sup> in response to changes in blood glucose at 1-min interval until 24-h post dosing.

Fixed meals were administered to patients on all inpatient days, except days 1 and 14. The daily caloric intake was 2200 kcal/day, which was increased to 2600 kcal/day in Cohort 3 to ensure stabilization of blood glucose values. An adjustable dose of short-acting bolus insulin was administered on an individual basis to achieve pre-prandial glucose values between 70 and 180 mg/dl. In the event that blood glucose concentrations fell below 60 mg/dl (confirmed by a repeat measurement), or in the event that a patient experienced symptoms consistent with hypoglycaemia, an adequate portion (at least 10 g) of carbohydrates were administered orally and blood glucose testing was performed for every 20 min until the blood glucose level rose above 70 mg/dl.

Serum samples for PK analysis were obtained on day 1 (pre-dose, 0.5, 2, 4, 6, 12 and 24 hours), day 2 (pre-dose), day 7 (pre-dose, 4, 8 and 12 h), days 10, 13 and 14 (pre-dose, 2, 4, 6, 8 and 12 h), days 15, 17, 19, 21, 23 and 25. Samples were analyzed using a validated LY2605541-specific, enzyme-linked immunosorbent assay (ELISA) method in which the lower limit of quantification was 75 pM and the upper limit of quantification was 900 pM. Samples above the limit of quantification were diluted and reanalyzed to yield results within the calibrated range. Serum samples for GD measures were additionally obtained during the clamp procedure and 8-point glucose profiles were performed daily from day 2 through day 16.

Table 1. Demographic and baseline characteristics.

|                          | 0.33 U/kg (n = 8) | 0.50 U/kg (n = 8) | 0.67 U/kg (n = 8)  | 1.00 U/kg (n = 8) |
|--------------------------|-------------------|-------------------|--------------------|-------------------|
| Age (years)              | 55.0 (42–65)      | 56.5 (50–64)      | 58.9 (47–65)       | 55.3 (49–62)      |
| Gender, male, n (%)      | 8 (100)           | 6 (75)            | 8 (100)            | 8 (100)           |
| BMI (kg/m <sup>2</sup> ) | 31.4 (29.3–36.9)  | 30.4 (27.5–33.3)  | 32.0 (28.9–36.2)   | 30.2 (27.0–33.7)  |
| Body weight (kg)         | 97.7 (81.5–123.6) | 89.9 (69.5–105.1) | 103.6 (89.5–114.5) | 96.8 (86.9–106.6) |
| Duration of T2DM (years) | 13.7 (7.1–21.8)   | 17.9 (7.3–28.3)   | 13.4 (9.0–21.3)    | 19.8 (12.9–39.3)  |
| HbA1c (%)                | 7.3 (6.3–8.5)     | 8.2 (6.6–9.6)     | 8.1 (6.2–9.9)      | 7.4 (6.1–8.6)     |

Data are mean (range), with the exception of gender which is presented as number (n) and percentage (%) of male patients. BMI, body mass index; T2DM, type 2 diabetes mellitus; HbA1c, glycosylated haemoglobin.

**Table 2.** Summary of LY2605541 PK parameters following multiple once-daily doses in patients with type 2 diabetes.

| PK parameter                    | 0.33 U/kg n = 8 |                | 0.50 U/kg n = 8 |                  | 0.67 U/kg n = 8 |                  | 1.00 U/kg n = 8 |                 |
|---------------------------------|-----------------|----------------|-----------------|------------------|-----------------|------------------|-----------------|-----------------|
|                                 | Day 7           | Day 14         | Day 7           | Day 14           | Day 7           | Day 14           | Day 7           | Day 14          |
| $C_{max,ss}$ (pmol/l)           | 4690 (32)       | 4000 (19)      | 7250 (25)       | 5460 (22)        | 9210 (26)       | 8180 (11)        | 16000 (28)      | 12800 (33)      |
| $C_{av,ss}$ (pmol/l)            | 3520 (31)       | 3210 (28)      | 6400 (25)       | 4420 (20)        | 7450† (27)      | 7110 (10)        | 13400 (25)      | 10800 (33)      |
| $AUC_{(0-12),ss}$ (pmol·h/l)    | 42200 (31)      | 38600 (28)     | 76800 (25)      | 53000 (20)       | 89400† (27)     | 85300 (10)       | 161000 (25)     | 130000 (33)     |
| $AUC_{(0-23.97),ss}$ (pmol·h/l) | —               | 75700 (24)     | —               | 101000 (22)      | —               | 169000 (13)      | —               | 242000 (30)     |
| $AUC_{(0-23.97),ss}$ (pmol·h/l) | —               | 75800 (24)     | —               | 101000 (22)      | —               | 168000 (13)      | —               | 242000 (30)     |
| $t_{max,ss}$ (h)                | —               | 6.0 (2.0–24.0) | —               | 0.00§ (0.0–12.0) | —               | 7.0 (2.0–12.0)   | —               | 2.0 (0.0–4.0)   |
| $t_{1/2,ss}$ (h)                | —               | 67 (52.7–91.8) | —               | 64.5 (42.4–91.5) | —               | 44.7 (35.4–62.5) | —               | 75.5 (58.8–111) |
| PTF                             | —               | 1.56 (22)      | —               | 1.48 (10)        | —               | 1.42 (13)        | —               | 1.49 (7)        |
| $CL_{ss}/F$ (l/h)               | —               | 3.73 (27)      | —               | 3.86 (16)        | —               | 3.54 (20)        | —               | 3.52 (32)       |
| Ra                              | —               | 7.48 (46)      | —               | 7.24 (57)        | —               | 7.52 (52)        | —               | 12.2 (44)       |

$AUC_{(0-12),ss}$ , area under the concentration versus time curve from time zero to time 12 h at steady state;  $AUC_{(0-23.97),ss}$ , area under the concentration versus time curve from zero to 23.97 h during a dosing interval at steady state;  $C_{av,ss}$ , average drug concentration under steady state conditions based on the equation:  $AUC_{(0-12),ss}/12$ ;  $CL_{ss}/F$ , apparent total body clearance of drug at steady state after extra-vascular administration;  $C_{max,ss}$ , maximum observed drug concentration at steady state; CV%, coefficient of variation; n, number of subjects; PK, pharmacokinetic; PTF, the peak-to-trough fluctuation ratio calculated based on the equation:  $C_{max,ss}/C_{min,ss}$ , where  $C_{max}$  and  $C_{min}$  are the maximum and minimum drug concentrations in the dosing interval, respectively; Ra, accumulation ratio calculated from  $AUC_{(0-23.97),ss}/t_{1/2,ss}$ ;  $t_{1/2,ss}$ , half-life associated with the terminal rate constant ( $\lambda_z$ ) in non-compartmental analysis at steady state;  $t_{max,ss}$ , time of maximum observed drug concentration at steady state.

\*The geometric mean (CV%)  $C_{max}$  values on day 1 for the 0.33, 0.5, 0.67, and 1.0 U/kg dose groups were 628 (33), 949 (49), 1530 (43) and 1310 (62) pmol/l, respectively.

†n = 7.

‡Median (range).

§In Cohort 1 (the 0.50 U/kg dose level), all patients had a  $t_{max,ss}$  of 00:00 hours, except one who had a  $t_{max,ss}$  of 12 h; hence, the median  $t_{max,ss}$  was 00:00 hours.

||Geometric mean (range).

Safety was assessed via monitoring of adverse events (AEs), clinical laboratory values, physical examinations and electrocardiograms (ECGs). Additional blood samples were obtained for the determination of fasting blood glucose concentration and C-peptide concentration.

### Pharmacokinetic and Glucodynamic Analysis

PK parameters were analyzed by conventional non-compartmental analysis using WinNonlin Enterprise Edition® software (version 5.01, Pharsight Corp., St. Louis, MO, USA). Serum insulin concentrations were used to calculate PK parameters, including maximum observed drug concentration ( $C_{max}$ ), time of maximum observed drug concentration ( $t_{max}$ ) and area under the concentration versus time curve (AUC). PK parameters were individually calculated for each patient. Log-transformed  $C_{max}$  and AUC were evaluated using a model to estimate dose-normalized geometric means for day 1, day 14 and ratio of day 14 over day 1 (with corresponding 90% confidence intervals). PK parameter estimates were evaluated to delineate dose proportionality at day 14 [14]. The serum concentration of LY2605541 was compared between days 1, 7 and 14 to assess the accumulation and wash-out effect. Within- and between-patient coefficient variation (CV) in PK parameters was assessed based on steady-state data.

For GD parameters, data were determined from the euglycaemic glucose clamp procedure, where the glucose infusion rate (GIR) over time was used as a measure of insulin effect. A locally weighted scatterplot smoothing (LOESS) function (smoothing parameter of 0.2) was applied to all individual GIR versus time profiles in each treatment group and/or period using S Plus® software (version 7.0.6, Insightful Corp., Seattle, WA, USA). The mean LOESS fits

from all patients in each treatment group was used for the generation of mean GIR plots over time whereas unsmoothed data was used to calculate GD parameters. The primary GD parameters, maximum GIR ( $R_{max}$ ) and total glucose infusion over the clamp duration ( $G_{tot}$ ) were log-transformed and compared between day 1 and day 14. Fasting and postprandial glucose concentrations were summarized over time to assess the accumulation and wash-out effect of LY2605541 on glucose.

## Results

### Patient Disposition and Demographics

The demographics and baseline (day 1) characteristics of the study population are summarized in Table 1. A total of 32 patients (30 male and 2 female) with type 2 diabetes were enrolled in the study with 8 patients enrolled into each of four dose cohorts. All patients received study treatment and completed the study. Age and body mass index (BMI) were comparable across all dose cohorts. All patients were Caucasian. The average duration of type 2 diabetes was approximately 16 years (range of 7–39 years).

### Pharmacokinetic Evaluations

LY2605541 PK parameters across all dose levels are summarized in Table 2. The concentration-time profile suggests a relatively flat profile in steady state with a peak-to-trough fluctuation at day 14 of <2 across all dose levels. Concentrations increased with steady state being achieved 7–10 days after dosing (figures 1, 2). Trough concentrations at day 7 were higher than those on day 14; however, the average concentrations were in the range of those observed on day 14 indicating that

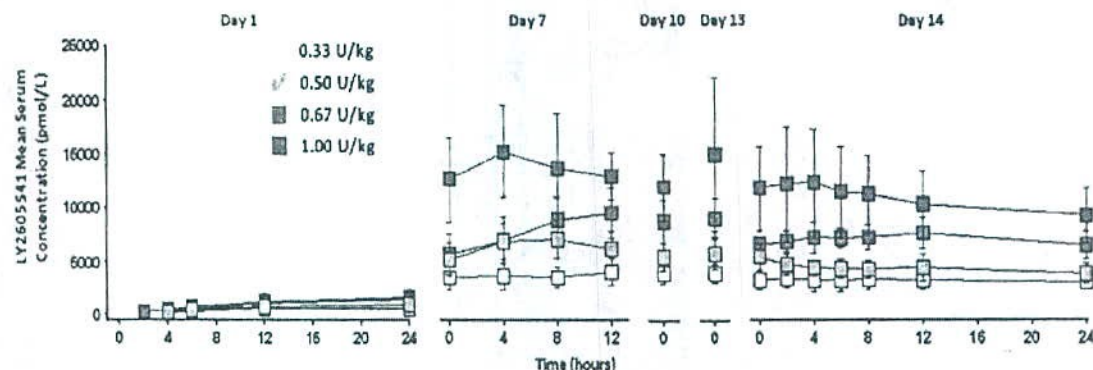


Figure 1. LY2605541 serum concentration-time profiles following single doses and multiple once-daily doses in patients with type 2 diabetes.

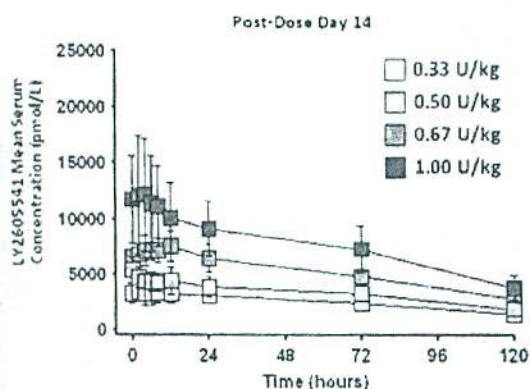


Figure 2. LY2605541 serum concentration-time profile post-dose day 14.

steady state had been reached by day 7. Monitoring of PK samples for 2 weeks postdosing showed an apparent half-life of 2–3 days (44.7–75.5 h).

A slightly more than proportional increase in PK parameter values was observed across the dose range of 0.33–1.00 U/kg as demonstrated by the ratio of dose-normalized means ( $R_{\text{dnm}}$ ) for AUC [1.12 (CI 0.93, 1.36)] and  $C_{\text{max}}$  [1.09 (CI 0.91, 1.31)].

For all PK parameters, the within-patient variability was <35% [ $\text{AUC}_{(0-24,97)}$  of 34.32% and  $C_{\text{max}}$  of 29.35%] and the between-patient variability was <25% [ $\text{AUC}_{(0-24,97)}$  of 24.06% and  $C_{\text{max}}$  of 21.22%].

#### Glucodynamic Evaluations

LY2605541 GD parameters are shown in Table 3. Consistent with PK observations, administration of multiple once-daily dosing of LY2605541 resulted in an essentially peakless GIR profile at steady state. The glucose-lowering effect of LY2605541 was evenly distributed over the first and second 12 h after injection with  $\text{AUC-GIR}_{0-12\text{h}}/\text{AUC-GIR}_{12-24\text{h}}$  being 53.5% (8.97). There was a dose-dependent glucose effect on the GIR (figure 3). The similarity in  $G_{\text{IR}}$  between the 0.50 and 0.67 U/kg dose likely reflects the between-subject variability in drug concentrations between the two dose groups.

Dose response was demonstrated with regard to glucose infusion rate, fasting blood glucose and bolus insulin requirements. The fasting glucose declined over time with an average change from baseline (day 1) to day 14 of approximately 39 mg/dl (2.2 mmol/l) and was decreased to 60–100 mg/dl (3.3–5.6 mmol/l) across the dose range (figure 4). Further decrease in fasting glucose was prevented by oral carbohydrate intake. Nocturnal glucose control between 03:00 and 09:00 hours was relatively unchanged. The bolus insulin dose declined from study start, with no bolus administrations after day 7 for the 1.00 U/kg cohort.

Within-patient variability was <14 and <26% for the AUC of the 8-point blood glucose profile and fasting blood glucose, respectively.

#### Safety

Mild hypoglycaemia was the most common treatment-emergent adverse event (TEAE) [44 events (67.7%)] in 38% of patients. All hypoglycaemia events were of mild severity. No local injection site reactions were observed. No serious adverse events (SAEs) were reported. No patients discontinued the study due to an adverse event.

Clinical laboratory tests, vital signs, ECGs and antibodies to LY2605541 did not reveal clinically significant results or changes from baseline. During the course of the study, three patients had elevated alanine aminotransferase (ALT) levels; however, all observed elevations in ALT were below three times the upper limit of normal and all bilirubin levels were normal for these patients.

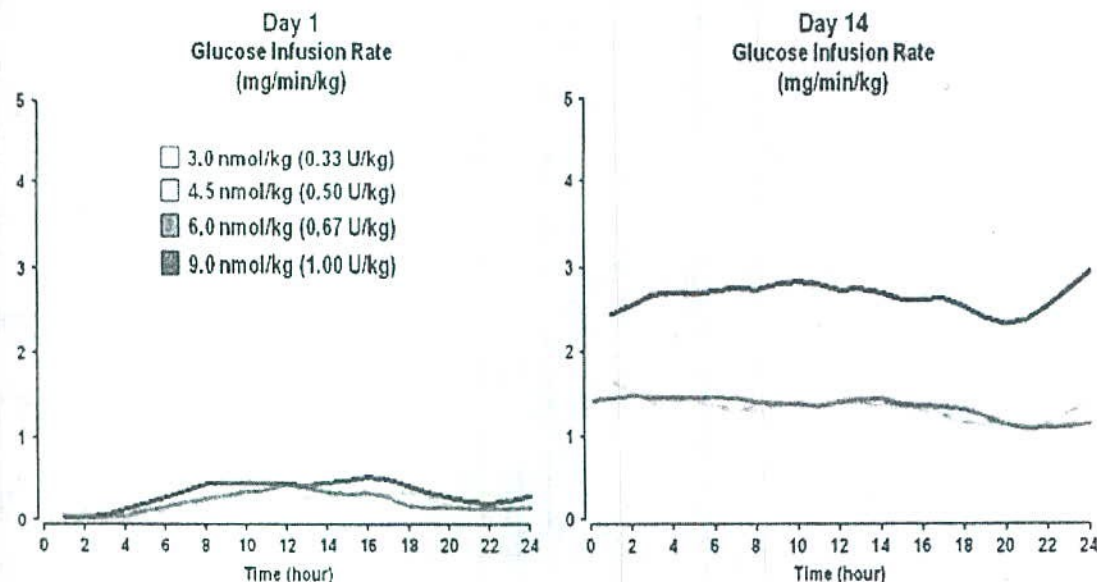
#### Discussion

In this Phase I study, LY2605541 was well-tolerated and demonstrated a flat PK and GD profile with no evidence of severe hypoglycaemia. Atypical of insulin dosing, this study was conducted as a 'fixed' dose over 14 days; patients were not titrated, nor were doses decreased within a cohort. This allowed investigating the PK and GD properties of LY2605541 without the need to account for dose adjustments or a potential delay in drug response. LY2605541 has a duration of action of > 24 h,

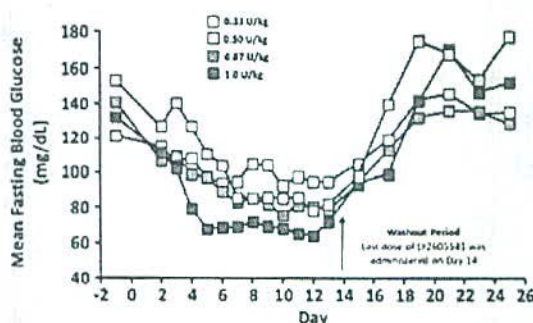
**Table 3.** Summary of LY2605541 GD parameters following single doses and multiple once-daily doses in patients with type 2 diabetes.

| GD parameters         | 0.33 U/kg   |           | 0.50 U/kg    |            | 0.67 U/kg    |           | 1.00 U/kg   |           |
|-----------------------|-------------|-----------|--------------|------------|--------------|-----------|-------------|-----------|
|                       | Day 1       | Day 14    | Day 1        | Day 14     | Day 1        | Day 14    | Day 1       | Day 14    |
| N                     | 7           | 8         | 7            | 8          | 8            | 8         | 8           | 8         |
| $R_{max}$ (mg/min/kg) | 0.259 (947) | 1.20 (25) | 0.143 (1455) | 1.72 (82)  | 0.261 (1134) | 1.60 (55) | 0.299 (674) | 2.98 (54) |
| $G_{tot}$ (mg/kg)     | 122 (911)   | 920 (37)  | 54.2 (4540)  | 1520 (109) | 107 (992)    | 1720 (60) | 147 (1442)  | 3320 (60) |

CV%, coefficient of variation; GD, glucodynamic;  $G_{tot}$ , total glucose infused over the clamp duration;  $R_{max}$ , maximum glucose infusion rate.



**Figure 3.** Mean locally weighted scatterplot smoothing (LOESS)-fitted glucose infusion rate profiles following single doses and multiple once-daily doses in patients with type 2 diabetes.



**Figure 4.** Mean fasting blood glucose following multiple once-daily doses in patients with type 2 diabetes.

a half-life of approximately 2–3 days, and minimal variability in patients with type 2 diabetes.

This study illustrates the favourable characteristics of LY2605541 as a long-acting basal insulin. The apparent half-life of LY2605541 (2–3 days) is considerably longer than that reported for insulin glargine (12.5 h) [15], insulin detemir (6–7 h) [16,17] and insulin degludec (25.4 h) [15]. The long

half-life relative to the dosing interval (QD) of LY2605541 explains the low fluctuation in PK levels (i.e. the low peak-to-trough fluctuation <2) in steady state. The long half-life of LY2605541 translates into a prolonged duration of action and long and flat GD profile. While all clamps were stopped after 24 h in this study (and therefore duration of action could not be fully assessed), the mean GD profiles on day 14 were very flat and showed considerably less peak-to-trough fluctuation than observed with insulin glargine or insulin detemir [6,7,18].

Trough concentrations on day 7 were higher than those on day 14. While the reason for this is not clearly understood, a dilution effect is a possibility, due to the large volume of fluid infused on day 14 during the euglycaemic clamp procedure. Further, day 14 PK levels were sampled under clamp conditions with the subjects being supine in contrast to day 7 where subjects were usually sitting for blood draws. Trough levels on days 10 and 13 were also equivalent to those on day 7, indicating that steady state was achieved on day 7.

Administration of LY2605541 produced a long, flat GD profile with small peak-to-trough fluctuations. The GIR profile at steady state mirrored the flat PK profile (concentration vs. time profiles) at steady state. On day 14, the GIR and  $G_{tot}$  are indicative of a dose-dependent response with a

GIR ~ 1–2 mg/min/kg in the 0.50–0.67 U/kg dose range of LY2605541. In accordance with the hypothesized mechanism of action, LY2605541 may have restricted peripheral activity, but retains hepatic activity to mimic endogenous insulin distribution [13]. As such, the GIR may not reflect the full effect of LY2605541. Normalization of fasting blood glucose was in a time and dose-dependent manner.

The nocturnal glucose control between 03:00 and 09:00 hours was relatively unchanged, thus potentially providing patients with type 2 diabetes with a lower risk of nocturnal hypoglycaemia. These results are consistent with the GD properties observed in the randomized, controlled study of once-daily LY2605541 versus insulin glargine in patients with type 2 diabetes in which the rate of nocturnal hypoglycaemia was statistically significantly lower with LY2605541 [19].

The GD response for LY2605541 demonstrated low intra-subject variability which is an important consideration for insulin dosing with respect to both reliability and limiting the risk of hypoglycaemia. The intra-subject variability results in this study are consistent with results from the Phase II trial conducted by Bergenstal et al., in which the intra-day blood glucose variability was statistically significantly lower with LY2605541 versus insulin glargine [19]. In terms of PK response, the intra-subject variability (CV%) for  $C_{max}$  (21.22%) observed in this trial is lower than both NPH insulin (24%) and insulin glargine (34%), and slightly higher than that of insulin detemir (18%) reported in a randomized, double-blind study comparing the intra-subject variability of the three insulins [11].

LY2605541 was generally well tolerated in patients with type 2 diabetes when administered subcutaneously as multiple doses up to 1.00 U/kg. All events of hypoglycaemia were mild, and no events were prolonged or difficult to manage. Hypoglycaemia appeared to be dose-dependent with 30 events occurring in Cohort 3 (1.00 U/kg). A *post hoc* analysis of the distribution of hypoglycaemia by time of day did not show any trends in terms of time-related occurrence. The daily caloric intake was increased from 2200 to 2600 kcal/day in Cohort 3 to ensure stabilization of blood glucose values. No bolus insulin doses were administered to patients in Cohort 3 after day 7. This is a clear indication of the strong pharmacological effect of LY2605541 at steady state in high doses which may require a reduction in pre-meal insulin requirements at least in some patients. A similar phenomenon was observed in a clinical study in patients with type 1 diabetes who also had to reduce their average short-acting insulin dose [20].

In conclusion, the use of LY2605541 exhibits desirable characteristics of a long-acting basal insulin, including a prolonged activity profile that supports once-daily dosing, no pronounced peaks in activity and minimal variability. Taken together, these data support the clinical investigation of LY2605541 in a once-daily dosing regimen. Clinical studies assessing the glucose-lowering effects and safety and tolerability of LY2605541 in patients with type 1 and type 2 diabetes are ongoing and LY2605541 is currently in Phase III development.

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## Conflict of Interest

The institution of T. H. received research grants from the following pharmaceutical companies: Astellas, Bayer, Becton Dickinson, Biocon, Boehringer Ingelheim, Evolva, Glaxo Smith Kline, Hoffmann LaRoche, Johnson&Johnson, Eli Lilly, Novo Nordisk, Noxxon, Prosidion, Sanofi and Skye Pharma in the past 12 months. In addition, T. H. received travel grants, consulting fees and speaker honoraria from Novo Nordisk and Boehringer Ingelheim. V. P. S. and D. C. H. are former employees, and current stockholders of Eli Lilly and Company. S. L. C. and K. F. M. are employees and stockholders of Eli Lilly and Company. V. P. S., D. C. H., K. F. M. and T. H. designed this study. V. P. S., D. C. H. and K. F. M. conducted this study. V. P. S. performed statistical analysis and data analysis. V. P. S., D. C. H., S. L. C., K. F. M. and T. H. helped in preparation and writing of manuscript and approval of manuscript. S. L. C. performed PK/GD analysis. T. H. carried out data acquisition and interpretation of data.

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## Novel PEGylated Basal Insulin LY2605541 Has a Preferential Hepatic Effect on Glucose Metabolism



The impact of the novel basal insulin LY2605541 (LY) on hepatic and nonhepatic glucose uptake (non-HGU) was evaluated. Conscious dogs underwent euglycemic clamps with tracer and hepatic balance measurements. Clamp period infusions were peripheral venous regular insulin ( $0.1 \text{ nmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  [control],  $n = 6$ ) or LY (bolus [ $\text{nmol/kg}$ ], continuous [ $\text{nmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ ]:  $0.5, 0.5$  [ $n = 6$ ];  $0.375, 0.375$  [ $n = 5$ ];  $0.25, 0.25$  [ $n = 4$ ]), somatostatin, and glucose, as well as intraportal glucagon (basal). During the clamp, the dogs switched from net hepatic glucose output to uptake (rates reached  $2.1 \pm 1.2, 0.9 \pm 2.1, 8.6 \pm 2.3$ , and  $6.0 \pm 1.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  within 5 h in control, LY<sub>0.25</sub>, LY<sub>0.375</sub>, and LY<sub>0.5</sub>, respectively). Non-HGU in LY increased less than in control; the ratio of change from basal in non-HGU to change in net hepatic glucose balance, calculated when glucose infusion rates (GIRs) were  $\sim 20 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in all groups, was higher in control ( $1.17 \pm 0.38$ ) versus LY<sub>0.25</sub> ( $0.39 \pm 0.33$ ), LY<sub>0.375</sub> ( $-0.01 \pm 0.13$ ), and LY<sub>0.5</sub> ( $-0.09 \pm 0.07$ ). Likewise, the change from baseline in glucose  $R_d$ -to- $R_a$  ratio was greatest in control ( $1.4 \pm 0.3$  vs.  $0.6 \pm 0.4, 0.5 \pm 0.2$ , and  $0.6 \pm 0.2$  in LY<sub>0.25</sub>, LY<sub>0.375</sub>, and LY<sub>0.5</sub>, respectively). In contrast to exogenously administered human insulin, LY demonstrated preferential hepatic effects, similar

to endogenously secreted insulin. Therefore, the analog might reduce complications associated with current insulin therapy.

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Marked improvements have been made in insulin analogs for basal-bolus therapy over the last few years. Insulins detemir and glargine reduce the risk of hypoglycemia compared with the older NPH insulin (1) and come closer to the ideal of creating a peakless basal insulin (2). However, neither detemir nor glargine maintains 24-h basal insulinemia for all individuals on all days (3), and thus improved analogs are needed. Insulin degludec, approved for use in Europe, Japan, and Mexico, is the first of the next-generation analogs to reach the market. The  $T_{1/2}$  of insulin degludec is approximately two times that of glargine (4), with the prolonged activity being achieved by the formation of soluble multihexamers upon subcutaneous injection, followed by gradual release of insulin monomers from the aggregate (5). Compared with glargine in treat-to-target trials, insulin degludec has brought about similar reduction in HbA<sub>1c</sub> at lower dosages and with lower rates of nocturnal hypoglycemia (6,7). Another new basal insulin, LY2605541 (LY), is currently in phase 3 trials.

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V.P.S. is currently affiliated with the U.S. Food and Drug Administration, Silver Spring, MD.

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See accompanying commentary, p. 390.

LY is insulin lispro with a 20-kDa polyethylene glycol moiety covalently attached to lysine B28 via a urethane bond (8,9). Its large hydrodynamic (i.e., functional) size (10) may contribute to slowing absorption and reducing clearance, resulting in prolonged duration of action. In phase I trials, the half-life of LY was between 1 and 3 days and reached steady-state concentrations within 7–10 days with ~8- to 13-fold increase in exposure at steady-state. After a single dose, the requirement for glucose infusion was at least 36 h, indicating prolonged pharmacodynamic action. At steady-state, nearly peakless glucose infusion profiles were observed due to low peak-to-trough pharmacokinetic fluctuation (11,12). In two phase II trials in patients with type 1 and type 2 diabetes, LY was associated with a lower rate of nocturnal hypoglycemia and glucose variability compared with insulin glargine. At the same time, it resulted in similar glycemic control in the type 2 diabetic patients but better glycemic control in the type 1 diabetic patients. In the type 1 diabetic patients, there was a slightly higher rate of total hypoglycemia but a lower prandial insulin requirement (13,14). In addition, these patients, who were previously treated with other basal insulins, showed modest weight loss when switched to LY compared with modest weight gain with insulin glargine. They also exhibited modestly higher triglycerides compared with those on insulin glargine (13,14). These effects prompted an examination of the effect of LY on specific tissues, including the liver, kidney, and skeletal muscle. The chronically catheterized conscious dog was chosen as the model because it permits cannulation of the vessels required for assessing glucose balance across these organs/tissues and because of the similarity in insulin's regulation of glucose metabolism in the human and the dog.

## RESEARCH DESIGN AND METHODS

### Animals and Surgical Procedures

The protocol was approved by the Vanderbilt University Institutional Animal Care and Use Committee, and the animals were housed and cared for according to Association for Assessment and Accreditation of Laboratory Animal Care International guidelines. Approximately 16 days prior to study, each dog underwent surgical preparation under general anesthesia. In the initial studies (part A;  $n = 12$ ), sampling catheters were placed in a femoral artery, the hepatic portal vein, the left common hepatic vein, a renal vein, and an iliac vein; infusion catheters were inserted in a jejunal and a splenic vein; and ultrasonic flow probes (Transonic Systems, Ithaca, NY) were placed around the hepatic artery, the portal vein, a renal artery, and an external iliac artery as previously described (15–18). In follow-up experiments (part B), the renal and iliac cannulas and flow probes were not placed, but all other aspects of the preparation were as described above. Criteria for use in a study were as previously described (17).

### Experimental Protocol

On the morning of study, the proximal ends of the flow probes and catheters were exteriorized under local anesthesia. A primed, continuous infusion of [ $^3\text{H}$ ]glucose was initiated via a peripheral vein, and the dog was allowed to rest quietly in a Pavlov harness throughout the period of tracer equilibration (90 min). This was followed by 30 min of baseline sampling (90–120 min) and a euglycemic clamp period lasting 5 h (from 120 to 420 min) in part A. At 120 min, infusions of somatostatin (Bachem, Torrance, CA) and insulin were begun via a peripheral vein, while glucagon was administered intraportally at a basal rate ( $0.5 \text{ ng} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). The dogs in part A were randomly assigned to receive LY initiated with a  $0.5 \text{ nmol/kg}$  priming dose followed by continuous infusion at  $0.5 \text{ nmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  (LY<sub>0.5</sub> group,  $n = 6$ ) or regular human insulin (control group,  $n = 6$ ) at  $0.11 \text{ nmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  with no priming dose. The analog is designed for subcutaneous injection, but it was infused via peripheral vein in these experiments in order to come to near-steady-state in a reasonable period of time and to mimic the route by which subcutaneously injected insulin arrives at the liver and other insulin-sensitive tissues. There is no known modification of the molecule during the absorption from the subcutaneous tissues, and after absorption it circulates in the same manner as endogenous insulin.

The insulin infusion rates used (determined in pilot studies; data not shown) in the two groups required equivalent glucose infusion rates (GIRs) to maintain euglycemia over the time course of the experiments. Glucose (50%; Hospira, Lake Forest, IL) was infused via peripheral vein as needed to maintain euglycemia. All sampling catheters functioned with the exception of a renal catheter in one LY<sub>0.5</sub> animal and a hindlimb catheter in one control dog. At the end of each experiment, the dog was anesthetized, and hepatic biopsies were freeze clamped *in situ* as previously described (19). Subsequently, the dog was killed and the hypothalamus was removed and freeze clamped (20). Tissues were stored at  $-80^\circ\text{C}$  to await further analysis.

Part B studies were identical to those in part A, except that the euglycemic clamp lasted 7 h (from 120 to 540 min), and the dogs were randomized to either the LY<sub>0.375</sub> ( $n = 5$ ;  $0.375 \text{ nmol/kg}$  prime,  $0.375 \text{ nmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  infusion) or LY<sub>0.25</sub> ( $n = 4$ ;  $0.25 \text{ nmol/kg}$  prime,  $0.25 \text{ nmol} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  infusion) groups. The purpose of part B was to determine whether the hepato-preferential effects of LY, observed in part A, would be evident at different dosages of the analog. Additionally, the longer clamp period was instituted to determine whether lower LY infusion rates sustained the hepato-preferential effects.

### Sample Analyses

Plasma glucose, [ $^3\text{H}$ ]glucose, glucagon, canine and human insulin, and nonesterified fatty acid (NEFA) levels and blood lactate and glycerol concentrations were measured

using standard methods as previously described (19,21). Plasma triglycerides were assayed spectrophotometrically (TRO100; Sigma-Aldrich, St. Louis, MO).

Serum concentrations of LY were assayed using a dual-antibody ELISA specific for LY (Charles River Laboratories, Senneville, QC, Canada). The quantitation range was 20–500 pmol/L, with the standard curve range 15–1,000 pmol/L. Samples with concentrations higher than 500 pmol/L were diluted so that the resulting concentration was within the quantitation range. Standard curve and quality control samples were analyzed with each set of study samples and passed acceptance criteria (within  $\pm 20\%$  except at lower limit of quantitation: within  $\pm 25\%$ ).

Western blotting was carried out on hepatic and hypothalamic tissue from control and LY<sub>0.5</sub> dogs; all analytic procedures have previously been described (22). Antibodies against total and phosphorylated Akt (Ser<sup>473</sup>), signal transducer and activator of transcription 3 (STAT3) (Tyr<sup>705</sup>), and glycogen synthase kinase-3 $\beta$  (GSK3 $\beta$ ) (Ser<sup>9</sup>) were purchased from Cell Signaling (Danvers, MA). Protein bands were quantified using ImageJ software (<http://rsb.info.nih.gov/ij/>).

### Calculations

Organ balance calculations and estimation of hepatic sinusoidal hormone concentrations and net hepatic carbon retention were carried out as previously described in detail (15–18). Tracer-determined glucose turnover was calculated with the circulatory model of Mari et al. (23). Unidirectional hepatic glucose uptake was calculated endogenous glucose  $R_a$  minus net hepatic glucose balance.

Two-way repeated-measures ANOVA (group  $\times$  time) was used for statistical analysis of time course data, with post hoc analysis by Tukey test (Sigmaplot 11; Systat Software, San Jose, CA). Since part A was 2 h shorter than part B, statistical comparisons do not include the data from the last 2 h of part B. Unpaired *t* tests were used for comparison of tissue analytes. A *P* value  $<0.05$  was considered statistically significant.

## RESULTS

### Hormone Concentrations and Hepatic Blood Flow

Portal vein blood flow declined 15–25% in response to somatostatin infusion ( $P < 0.05$  for  $\Delta$  from baseline in all groups), while there was a slight (albeit nonsignificant) 0–10% rise in hepatic artery blood flow (Table 1).

Arterial plasma C-peptide concentrations declined to near the limits of detection in all groups during the clamp period, demonstrating that insulin secretion was fully suppressed (data not shown). Arterial and hepatic sinusoidal glucagon concentrations remained near basal throughout the clamp period and did not differ significantly among groups ( $P = 0.22$ ) (Table 1).

Plasma insulin concentrations during the baseline period were similar in all groups (data shown for control group only [Fig. 1A]). In the control group, the arterial

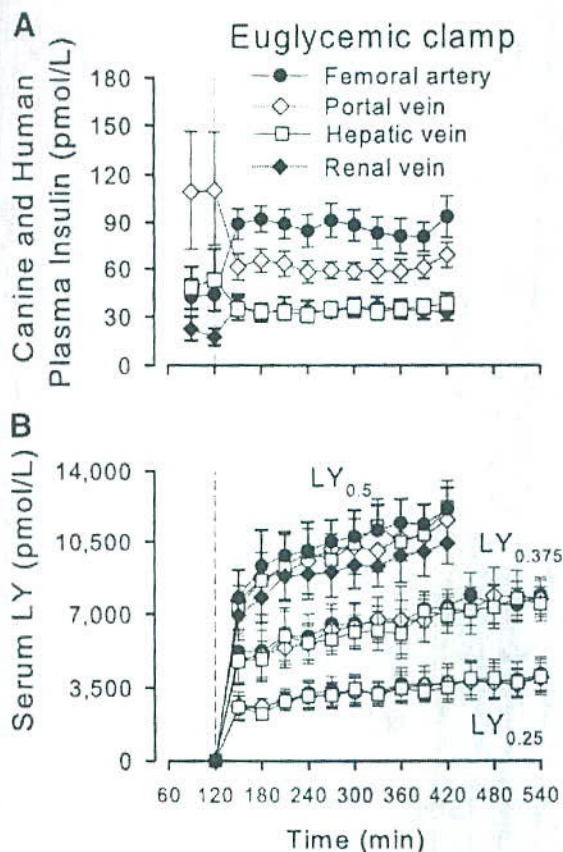
**TABLE 1—Hepatic blood flow and plasma glucagon concentrations**

|                                                                                              | Baseline period | Clamp period   |
|----------------------------------------------------------------------------------------------|-----------------|----------------|
| <b>Hepatic artery blood flow</b><br>(mL $\cdot$ kg <sup>-1</sup> $\cdot$ min <sup>-1</sup> ) |                 |                |
| Control                                                                                      | 6.9 $\pm$ 1.2   | 7.3 $\pm$ 1.1  |
| LY <sub>0.5</sub>                                                                            | 5.7 $\pm$ 0.7   | 6.3 $\pm$ 0.7  |
| LY <sub>0.375</sub>                                                                          | 6.8 $\pm$ 0.5   | 7.4 $\pm$ 0.7  |
| LY <sub>0.25</sub>                                                                           | 5.3 $\pm$ 0.7   | 5.2 $\pm$ 0.9  |
| <b>Portal vein blood flow</b><br>(mL $\cdot$ kg <sup>-1</sup> $\cdot$ min <sup>-1</sup> )    |                 |                |
| Control                                                                                      | 25.8 $\pm$ 1.5  | 19.5 $\pm$ 1.5 |
| LY <sub>0.5</sub>                                                                            | 28.0 $\pm$ 2.3  | 21.7 $\pm$ 1.5 |
| LY <sub>0.375</sub>                                                                          | 25.0 $\pm$ 1.8  | 21.2 $\pm$ 1.7 |
| LY <sub>0.25</sub>                                                                           | 22.2 $\pm$ 2.2  | 17.3 $\pm$ 1.3 |
| <b>Arterial plasma glucagon</b><br>(ng/L)                                                    |                 |                |
| Control                                                                                      | 50 $\pm$ 3      | 43 $\pm$ 2     |
| LY <sub>0.5</sub>                                                                            | 50 $\pm$ 6      | 45 $\pm$ 4     |
| LY <sub>0.375</sub>                                                                          | 50 $\pm$ 3      | 45 $\pm$ 5     |
| LY <sub>0.25</sub>                                                                           | 62 $\pm$ 8      | 55 $\pm$ 8     |
| <b>Hepatic sinusoidal plasma glucagon</b><br>(ng/L)                                          |                 |                |
| Control                                                                                      | 58 $\pm$ 3      | 52 $\pm$ 2     |
| LY <sub>0.5</sub>                                                                            | 54 $\pm$ 6      | 57 $\pm$ 7     |
| LY <sub>0.375</sub>                                                                          | 56 $\pm$ 6      | 55 $\pm$ 5     |
| LY <sub>0.25</sub>                                                                           | 74 $\pm$ 4      | 67 $\pm$ 6     |

Data are mean  $\pm$  SEM. *n* = 6/group for control and LY<sub>0.5</sub>; *n* = 5 for LY<sub>0.375</sub>, and *n* = 4 for LY<sub>0.25</sub>. Basal period values are the mean of two sampling times (90 and 120 min); clamp period values are the mean of the sampling points between 120 and 420 min (for control and LY<sub>0.5</sub>) and between 120 and 540 min (for LY<sub>0.375</sub> and LY<sub>0.25</sub>). There were no significant differences among groups.

concentrations during the clamp period were twofold basal levels, but peripheral venous insulin delivery resulted in a fall in the portal vein concentrations to ~50% of basal. Consequently, hepatic sinusoidal insulin concentrations in the control group tended to decline (from 96  $\pm$  30 pmol/L [baseline] to 68  $\pm$  7 pmol/L [clamp period];  $P = 0.14$ ), while renal vein plasma insulin concentrations increased in parallel with the rise in arterial insulin. The mean arterial LY concentrations during the final hour (near-steady-state) were 11,651  $\pm$  946, 7,589  $\pm$  843, and 3,906  $\pm$  858 pmol/L in LY<sub>0.5</sub>, LY<sub>0.375</sub>, and LY<sub>0.25</sub>, respectively (Fig. 1B).

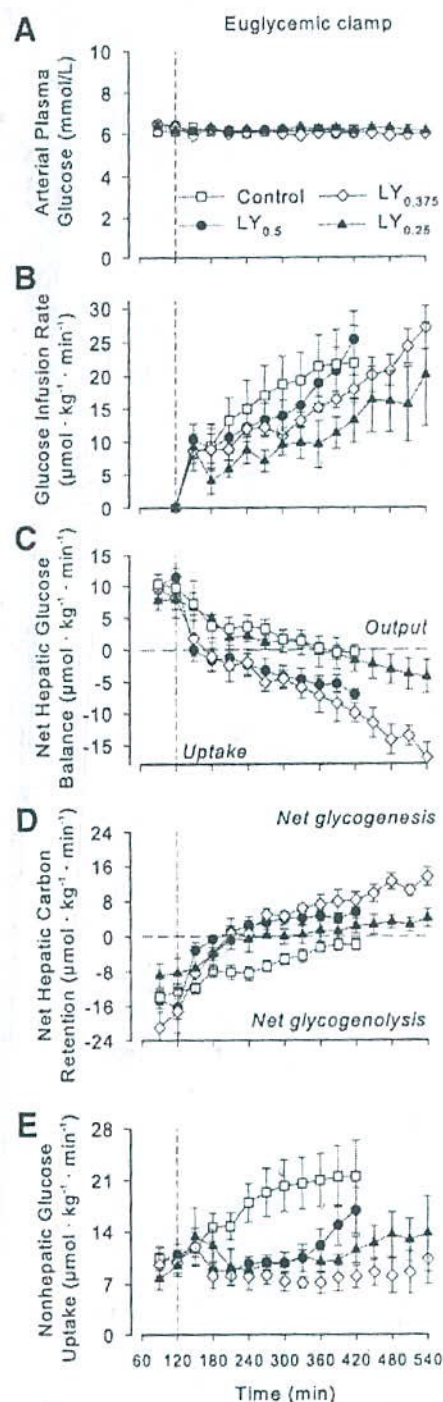
Fractional hepatic, intestinal, and renal extractions of regular insulin during the clamp were 50  $\pm$  3%, 29  $\pm$  2%, and 61  $\pm$  3%, respectively. The extraction of LY by the liver could not be precisely quantified because of the high analog concentrations and relatively low tissue clearance. The extraction by the intestinal tract averaged 5  $\pm$  3% at the highest dose, but at the lower LY infusion rates, the analog concentrations were too similar in the artery and portal vein to detect intestinal extraction. In LY<sub>0.5</sub>, renal extraction of the LY was 13  $\pm$  4% (not evaluated in LY<sub>0.375</sub> and LY<sub>0.25</sub>).



**Figure 1**—Plasma concentrations of endogenous insulin (basal period) and regular human insulin (clamp period) in control dogs (A) and serum concentrations of LY (B). Arterial, portal vein, hepatic vein, and renal vein (available for control and LY<sub>0.5</sub> groups only) concentrations of insulin or analog. Data are mean  $\pm$  SEM;  $n = 6$ , 6, 5, and 4 for control, LY<sub>0.5</sub>, LY<sub>0.375</sub>, and LY<sub>0.25</sub>, respectively.

### Glucose Metabolism

Arterial plasma glucose during the clamp period remained at basal concentrations [ $\sim 6.1$  mmol/L in all groups (Fig. 2A)]. The GIRs were not significantly different between control and LY<sub>0.5</sub> or LY<sub>0.375</sub> (Fig. 2B) but were lower ( $P < 0.05$ ) in LY<sub>0.25</sub> than in control. All groups exhibited net hepatic glucose output (NHGO) ( $8\text{--}11 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) during the baseline period. In the control group, NHGO gradually declined from baseline, reaching approximately zero by 420 min ( $\Delta 10.3 \pm 1.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) (Fig. 2C). In LY<sub>0.5</sub>, on the other hand, there was a rapid and marked impact at the liver, such that there was a switch from NHGO to net hepatic glucose uptake (NHGU) within 30 min of beginning the LY<sub>0.5</sub> infusion, and the change from baseline in net hepatic glucose balance reached  $16.8 \pm 2.2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  by 420 min ( $P < 0.05$  between groups). The change from baseline in LY<sub>0.375</sub> and LY<sub>0.25</sub> averaged  $18.8 \pm 2.6$  ( $P < 0.05$  vs. control) and  $9.5 \pm 3.1$  (NS vs. control)



**Figure 2**—Arterial plasma glucose concentrations (A), GIRs (B), net hepatic glucose balance (NHGB) (C), net hepatic carbon retention (NHCR) (D), and non-HGU (E). Control ( $n = 6$ ), LY<sub>0.5</sub> ( $n = 6$ ), LY<sub>0.375</sub> ( $n = 5$ ), and LY<sub>0.25</sub> ( $n = 4$ ). Significant differences ( $P < 0.05$ ): LY<sub>0.5</sub> and LY<sub>0.375</sub> vs. control for NHGB, NHCR, and non-HGU; LY<sub>0.25</sub> vs. control for GIR, NHCR, and non-HGU; LY<sub>0.375</sub> vs. LY<sub>0.5</sub> for non-HGU; LY<sub>0.25</sub> vs. LY<sub>0.5</sub> for NHGB, GIR, and NHCR; LY<sub>0.25</sub> vs. LY<sub>0.375</sub> for NHGB and NHCR.

$\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  by 420 min. All LY dosages were associated with significantly greater net hepatic carbon retention than exhibited by the control group (Fig. 2D). The effect of each treatment on endogenous glucose  $R_a$  was very similar to its effect on net hepatic glucose balance (Fig. 3A). In the control group, there was a gradual decline in glucose  $R_a$ , with the fall from basal totaling  $8.9 \pm 1.9 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  by the final hour. When the change in endogenous glucose  $R_a$  between the basal period and 360–420 min (the final hour during part A) was calculated, it was clear that the change was significantly greater in LY<sub>0.5</sub> and LY<sub>0.375</sub> than in control (Fig. 3C).

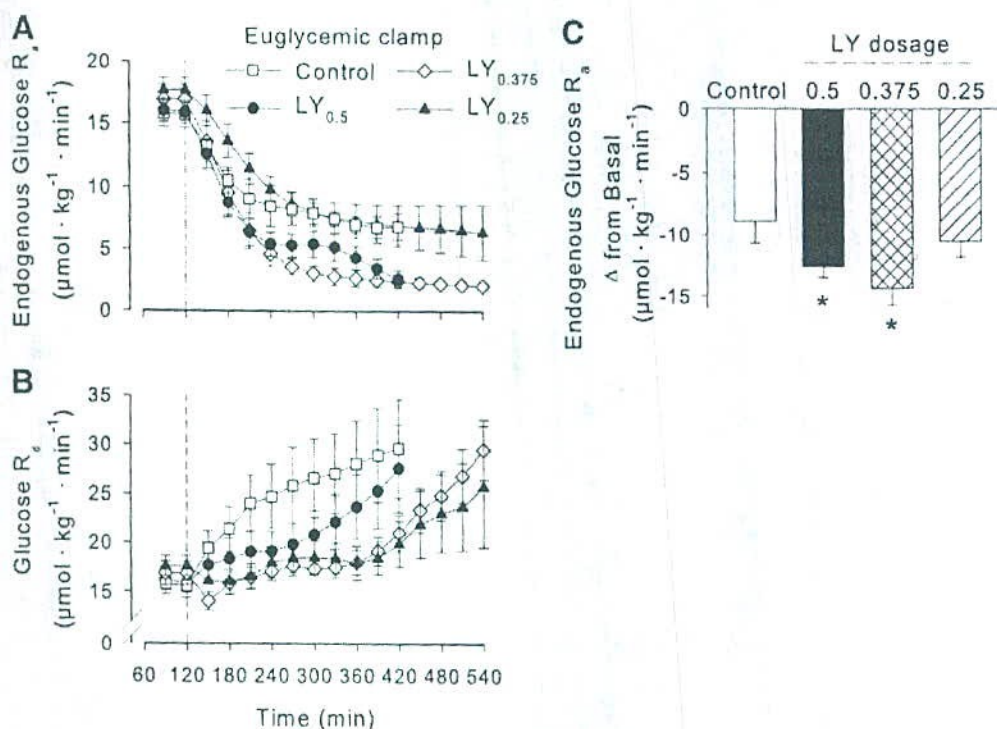
Peripheral insulin infusion in the control group had an immediate impact on nonhepatic glucose uptake (non-HGU), and by the final hour non-HGU had increased  $11.1 \pm 4.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  over the basal rate (Fig. 2E). In the LY groups, non-HGU rates initially fell below basal. In the LY<sub>0.5</sub> group, it began to increase gradually after 330 min, with the increase from basal in the final hour being  $4.1 \pm 1.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  ( $P = 0.14$  versus control). The LY<sub>0.25</sub> group also exhibited a small increase in non-HGU during the late part of the clamp, while the rate did not rise above basal at any time in the LY<sub>0.375</sub> group. In an effort to compare the control and the different LY groups under equivalent conditions, we

calculated the ratio of the change from basal in non-HGU to the change in NHGU at a time when GIRs were equivalent in all groups ( $\sim 20 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ). The lower the ratio in Table 2, the more hepato-preferential the action. The ratio was much higher in control than in LY<sub>0.5</sub> and LY<sub>0.375</sub> ( $P < 0.01$ ), and it tended to be higher than in LY<sub>0.25</sub>. No significant differences were seen in either net renal or hindlimb glucose balance between the control and LY<sub>0.5</sub> groups (data not shown).

LY<sub>0.375</sub> and LY<sub>0.25</sub>, but not LY<sub>0.5</sub>, stimulated glucose  $R_d$  significantly less than regular insulin through 420 min (Fig. 3B). When the ratio of change from baseline in glucose  $R_d$  to change in glucose  $R_a$  was calculated at the time when GIR was  $\sim 20 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in each group, the control group exhibited a ratio  $>$ twofold that in the LY groups (Table 2).

### Lactate Metabolism

All groups exhibited net hepatic lactate output (NHLO) during the baseline period, with the rates in LY<sub>0.375</sub> and LY<sub>0.25</sub> being significantly higher and lower, respectively, than in the control group (Table 3). During the clamp period, the control group exhibited a slight increase in NHLO followed by a return to the baseline rate. In all of the LY groups, NHLO during the clamp period declined to a nadir no different from  $0 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in



**Figure 3**—Tracer-determined rate of endogenous glucose  $R_a$  (A) and  $R_d$  (B). C: Magnitude of the decline in endogenous glucose  $R_a$  between the baseline period and 360–420 min (the last hour of study for the control and LY<sub>0.5</sub> groups). Control ( $n = 6$ ), LY<sub>0.5</sub> ( $n = 6$ ), LY<sub>0.375</sub> ( $n = 5$ ), and LY<sub>0.25</sub> ( $n = 4$ ).  $P < 0.05$  for LY<sub>0.375</sub> vs. control for both  $R_a$  and  $R_d$ .  $P < 0.05$  for LY<sub>0.25</sub> vs. control for  $R_d$ . \* $P < 0.05$  vs. control.

**TABLE 2—Ratios of change from baseline in non-HGU to change in NHGU, as well as change from basal in tracer-determined glucose  $R_d$  to the change in endogenous glucose  $R_a$**

| Group               | $\Delta \text{non-HGU}:\Delta \text{NHGU}$ | $\Delta R_d:\Delta R_a$ |
|---------------------|--------------------------------------------|-------------------------|
| Control             | $1.17 \pm 0.38$                            | $1.37 \pm 0.32$         |
| LY <sub>0.5</sub>   | $0.09 \pm 0.07^*$                          | $0.60 \pm 0.24\ddagger$ |
| LY <sub>0.375</sub> | $-0.01 \pm 0.13^*$                         | $0.51 \pm 0.22\ddagger$ |
| LY <sub>0.25</sub>  | $0.39 \pm 0.33\ddagger$                    | $0.61 \pm 0.43\ddagger$ |

Data are mean  $\pm$  SEM.  $n = 6/\text{group}$  for control and LY<sub>0.5</sub>;  $n = 5$  for LY<sub>0.375</sub>, and  $n = 4$  for LY<sub>0.25</sub>. Ratios were calculated at the point at which GIR in each group was  $\sim 20 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (330, 360, 450, and 540 min for control, LY<sub>0.5</sub>, LY<sub>0.375</sub>, and LY<sub>0.25</sub>, respectively). There were no significant differences among the LY groups.  $^*P < 0.01$  vs. control,  $\ddagger P = 0.07$  vs. control,  $\pm P < 0.05$  vs. control.

LY<sub>0.5</sub>. The LY<sub>0.375</sub> and LY<sub>0.25</sub> groups actually switched to net hepatic lactate uptake. NHLO resumed during the latter part of the clamp but did not reach baseline rates by the end of the experiment in any of the LY groups. As a result of these changes, blood lactate concentrations tended to fall initially and then return toward baseline levels in the LY groups.

The control group exhibited net hindlimb lactate uptake throughout the clamp, with uptake increasing significantly as NHLO peaked and then declining to near-baseline rates. Conversely, net hindlimb lactate uptake decreased in LY<sub>0.5</sub> as NHLO declined, with a subsequent small increase in net limb lactate uptake corresponding to the increase in NHLO by the end of the clamp. Net renal lactate uptake did not change significantly from

baseline in either control or LY<sub>0.5</sub>, and the two groups did not differ at any time point (data not shown).

#### Glycerol, Fatty Acid, and Triglyceride Metabolism

Arterial glycerol and NEFA concentrations in the control group declined promptly under clamp conditions in response to the peripheral hyperinsulinemia. In contrast, the glycerol and NEFA concentrations initially increased in the LY groups and then gradually declined (Fig. 4A and C). Net hepatic and renal (renal not shown) uptakes of glycerol and NEFA decreased during the clamp in the control group and initially increased in the LY groups, corresponding to the changes in the substrate concentrations (Fig. 4B and D). Net hindlimb glycerol output did not differ between the control and LY<sub>0.5</sub> groups at any time (data not shown). During the clamp, arterial plasma triglyceride concentrations declined significantly in all groups (Fig. 4E). By 420 min, triglyceride concentrations had fallen  $\sim 40\%$  below baseline in the control and LY<sub>0.5</sub> and LY<sub>0.375</sub> groups and 15% in LY<sub>0.25</sub>. The concentrations were not significantly different among groups at 420 min.

#### Tissue Analyses

There was no significant difference between regular insulin and LY<sub>0.5</sub> in phosphorylation of Akt in the hypothalamus or STAT3 protein in the liver (Fig. 5A and B; Supplementary Fig. 1). On the other hand, phosphorylation of Akt and GSK3 $\beta$  in the liver was significantly enhanced with LY<sub>0.5</sub> versus regular insulin (Fig. 5C and D).

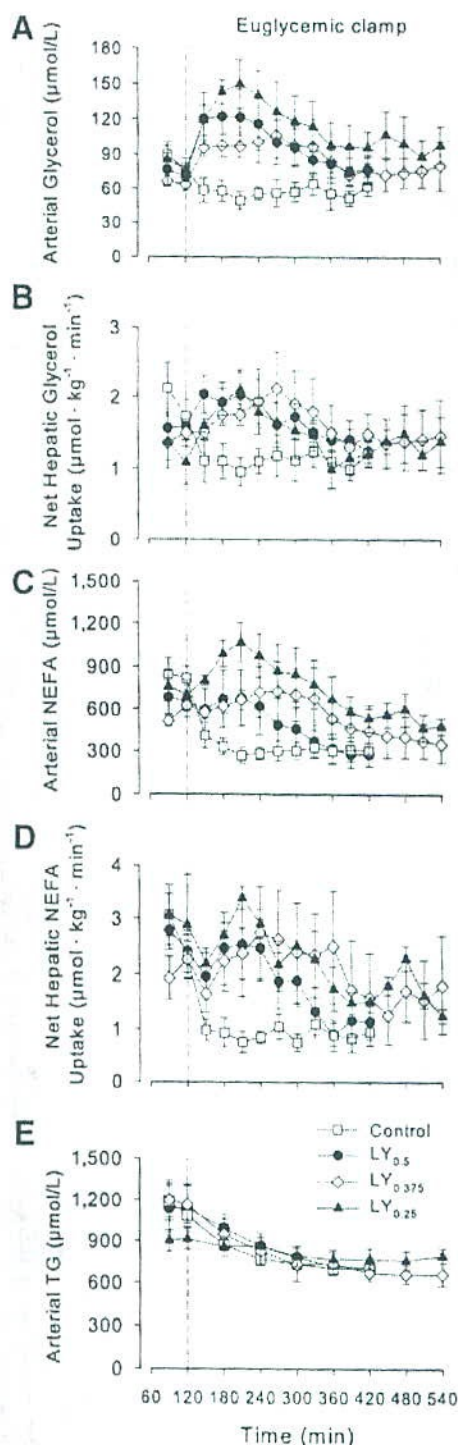
#### DISCUSSION

Insulin secretion into the portal vein results in  $\sim$ three-fold greater exposure of the liver, versus other tissues, to insulin. The increased exposure of the liver to the

**TABLE 3—Lactate data**

|                                                                                  | Baseline<br>period | Clamp period time (min) |             |             |             |             |            |           |
|----------------------------------------------------------------------------------|--------------------|-------------------------|-------------|-------------|-------------|-------------|------------|-----------|
|                                                                                  |                    | 180                     | 240         | 300         | 360         | 420         | 480        | 540       |
| Arterial lactate (μmol/L)                                                        |                    |                         |             |             |             |             |            |           |
| Control                                                                          | 701 ± 69           | 624 ± 72                | 623 ± 105   | 623 ± 81    | 648 ± 73    | 650 ± 106   | —          | —         |
| LY <sub>0.5</sub>                                                                | 771 ± 122          | 580 ± 83                | 483 ± 63    | 503 ± 77    | 548 ± 93    | 548 ± 117   | —          | —         |
| LY <sub>0.375</sub>                                                              | 952 ± 150*         | 802 ± 176               | 552 ± 107   | 471 ± 41    | 534 ± 71    | 602 ± 62    | 743 ± 68   | 761 ± 71  |
| LY <sub>0.25</sub>                                                               | 684 ± 42           | 551 ± 96                | 539 ± 73    | 482 ± 113   | 498 ± 125   | 542 ± 84    | 628 ± 125  | 887 ± 344 |
| Net hepatic<br>lactate balance<br>(μmol · kg <sup>-1</sup> · min <sup>-1</sup> ) |                    |                         |             |             |             |             |            |           |
| Control                                                                          | 6.4 ± 1.9          | 8.4 ± 0.9               | 9.8 ± 0.9   | 7.2 ± 1.0   | 4.7 ± 1.2   | 4.6 ± 1.4   | —          | —         |
| LY <sub>0.5</sub>                                                                | 9.7 ± 2.9          | 5.6 ± 1.9               | 0.6 ± 2.1*  | 1.4 ± 1.4*  | 3.0 ± 1.2*  | 3.5 ± 0.5   | —          | —         |
| LY <sub>0.375</sub>                                                              | 20.6 ± 6.7*        | 9.9 ± 4.1               | 3.0 ± 4.2   | -0.1 ± 1.7* | 0.0 ± 1.5*  | 3.6 ± 1.7   | 3.4 ± 1.7  | 7.0 ± 0.9 |
| LY <sub>0.25</sub>                                                               | 1.7 ± 2.4*         | -1.8 ± 2.2*             | -2.9 ± 2.2* | -2.6 ± 1.9* | -2.0 ± 1.7* | -1.3 ± 0.7* | -0.4 ± 2.0 | 0.2 ± 1.3 |

Data are mean  $\pm$  SEM.  $n = 6, 6, 5$ , and  $4$  for control, LY<sub>0.5</sub>, LY<sub>0.375</sub>, and LY<sub>0.25</sub>, respectively. Basal period values are the mean of two sampling times; the clamp period took place between 120 and 420 min (for control and LY<sub>0.5</sub>) and between 120 and 540 min (for LY<sub>0.375</sub> and LY<sub>0.25</sub>). Positive values for balance indicate net hepatic output. There were no significant differences among the LY groups;  $P = 0.1$  for basal period net balance in LY<sub>0.375</sub> compared with the rates in LY<sub>0.5</sub> and LY<sub>0.25</sub>.  $^*P < 0.05$  vs. control.

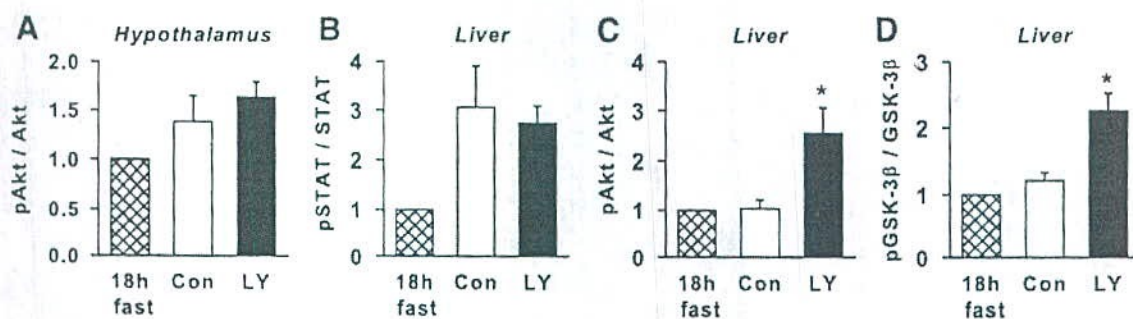


**Figure 4**—Arterial blood glycerol (A), plasma NEFA (C), and plasma triglyceride (TG) (E) concentrations and net hepatic uptakes of glycerol (B) and NEFA (D). Control ( $n = 6$ ), LY<sub>0.5</sub> ( $n = 6$ ), LY<sub>0.375</sub> ( $n = 5$ ), and LY<sub>0.25</sub> ( $n = 4$ ).  $P < 0.05$  for all LY groups vs. control for arterial glycerol and NEFA and for net hepatic uptake of glycerol and NEFA. There were no significant differences among groups in TG concentrations. There were no significant differences among LY groups for any parameter.

hormone results from its secretion into a limited vascular pool and the fact that the liver extracts 50–60% of the insulin to which it is exposed (24). In contrast, when insulin is administered subcutaneously, the normal portal-peripheral insulin gradient is lost so that the peripheral tissues are comparatively hyperinsulinemic while the liver is comparatively hypoinsulinemic (25,26). The portal vein, hepatic vein, and hepatic sinusoidal insulin concentrations in the control group during the clamp period were ~30–40% below basal, while the arterial concentrations were approximately two times basal. Although the arterial concentrations did not increase as much as would be anticipated after a mixed meal (six-fold basal or more), they were inappropriately high in relation to the other blood vessels sampled, which also exhibit sixfold or more increases in insulin after meal ingestion (27). Partly as a result of the relative overinsulinization of peripheral tissues, even individuals with well-controlled insulin-treated diabetes are apt to experience glycemic fluctuations, hypoglycemia, and dyslipidemia, which have been suggested to contribute to microvascular and macrovascular complications (28). Theoretically, insulin analogs with hepato-preferential actions could reduce the risk of these complications (29,30).

While it may seem counterintuitive that a hepato-preferential insulin could reduce hypoglycemia, since the liver's response is key to restoring euglycemia, relative overinsulinization of peripheral tissues contributes to hypoglycemia and impaired counterregulation. Lipolysis is an important contributor to gluconeogenesis under hypoglycemic conditions, and the relative overinsulinization of the peripheral lipid depots resulting from current peripherally administered insulin therapies would be expected to impede gluconeogenesis (31–33). Moreover, even though skeletal muscle glucose uptake decreases during hypoglycemia associated with conventional insulin therapy, it does not cease. Due to the relatively large mass of skeletal muscle, this results in significant ongoing glucose disposal, a process that would be curtailed by a hepato-preferential insulin. More importantly, a hepato-preferential insulin puts the primary burden of hypoglycemia on the organ best suited to cope because hepatic insulin signaling is markedly reduced in the presence of hypoglycemia; i.e., the insulin receptor is essentially disengaged (34). Even when overinsulinized and deprived of increases in counterregulatory hormones and central neural stimuli, the liver is capable of responding directly to hypoglycemia by increasing its glucose output (35–37).

At the doses examined, LY exhibited a clear hepato-preferential effect compared with human insulin when both products were delivered via peripheral vein. This was demonstrated by two independent measures of glucose metabolism: organ balance and tracer-determined turnover. It should be noted that a single control group was used as a comparator for all three LY dosages. In



**Figure 5**—Molecular markers of insulin signaling in the control and LY<sub>0.5</sub> groups. Hypothalamic phosphorylated Akt (pAkt), expressed relative to total Akt (A), as well as hepatic pSTAT3/STAT3 (B), pAkt/Akt (C), and pGSK3β/GSK3β (D), in control (con) (white bars) and LY<sub>0.5</sub> (black bars) ( $n = 6$ /group). The hatched bars are mean data from 2 additional dogs, not included in the experiments, that were fasted for 18 h and then killed to provide reference (non-insulin stimulated) tissue. See Supplementary Fig. 1 for representative blots. \* $P < 0.05$  vs. control.

order for groups to be compared, in terms of glucose disposition, they must be matched for at least one parameter of interest. In that regard, the LY<sub>0.5</sub> and LY<sub>0.375</sub> groups were not significantly different in GIR from the control group ( $P = 0.72$  and  $0.35$ , respectively). Both LY<sub>0.5</sub> and LY<sub>0.375</sub> exhibited a faster and greater effect on net hepatic glucose balance than regular insulin, quickly shifting the liver from NHGO to NHGU. Conversely, their effect on non-HGU was delayed and minimal compared with regular insulin. At the lowest LY dose, the analog's effects on net hepatic glucose balance were comparable to those in the control group throughout 420 min ( $P = 0.974$ ), making possible a comparison of nonhepatic effects in the presence of similar rates of NHGO. The impact of LY<sub>0.25</sub> on non-HGU was markedly lower than that of regular insulin. Of the three LY dosages examined, LY<sub>0.375</sub> appeared to have the greatest effect at the liver concomitant with the least effect on nonhepatic tissues.

Tracer methodology yielded results consistent with hepatic glucose balance data. The two higher doses of LY caused a significantly greater fall in endogenous glucose  $R_a$  than regular insulin (even though significant differences from control in the rates were not evident as early as they were with organ balance data), while the effect of LY<sub>0.25</sub> was very similar to that of regular insulin ( $P = 0.34$ ). On the other hand, both LY<sub>0.375</sub> and LY<sub>0.25</sub> stimulated glucose  $R_d$  significantly less than regular insulin. Moreover, all doses of LY, compared with regular insulin, were associated with a significantly smaller change in glucose  $R_d$  relative to the impact on endogenous  $R_a$ .

In contrast to its rapid effects on the liver, as noted above, LY's effect on nonhepatic tissues was more gradual than that of regular insulin. In particular, LY's effect on fat metabolism appeared to be slow. Lipolysis is extremely sensitive to an elevation of regular insulin, and thus it was quickly suppressed in the control group, with glycerol concentrations reaching a stable nadir within 30 min of the start of the clamp. In none of the LY groups did the glycerol concentrations fall below the

baseline values at any time, suggesting a lack of lipolytic inhibition by the analog at these doses. NEFA levels, on the other hand, fell below baseline levels during the clamp in all LY groups, consistent with an increase in reesterification. Nevertheless, regular insulin lowered NEFA much more promptly than LY. The nadir of NEFA concentrations ( $271 \pm 57 \mu\text{mol/L}$ ) occurred within 90 min after the start of the clamp period in the control group, while the nadir in LY<sub>0.5</sub> ( $278 \pm 92 \mu\text{mol/L}$ ) was not reached until 270 min after the start of the clamp, and LY<sub>0.375</sub> and LY<sub>0.25</sub> did not reach their lowest concentrations ( $353 \pm 130$  and  $467 \pm 96 \mu\text{mol/L}$ , respectively) until the last hour. An insulin-induced fall in circulating NEFA provides an indirect means for reducing hepatic glucose production (38,39), and thus the sluggish response of glycerol and NEFA concentrations to LY administration suggests that it was the direct effect of the analog that inhibited hepatic glucose production.

A decrease in NEFA levels and hepatic NEFA uptake is associated with an increase in hepatic glycolysis (39). The exact mechanism for this is unclear, but it may well result from a fall in intrahepatic citrate, one of the major inhibitors of phosphofructokinase, the first rate-determining enzyme in the glycolytic pathway. Alternatively, the fall in NEFA could alter the intrahepatic redox state, increasing the NADH-to-NAD ratio and leading to an activation of lactate dehydrogenase. (For further discussion, see Sindelar et al. [39]) Thus, when net hepatic NEFA uptake decreased in the control group, there was an increase in the release of glycolytic carbon (lactate) from the liver. Conversely, there appeared to be a prompt suppression of glycolysis (hepatic lactate release) in response to LY, likely related to the increase in lipolysis early in the experimental period. The two lower rates of LY infusion not only reduced NHLO but also actually prompted the liver to shift to net uptake of lactate, consistent with increased storage of carbon as glycogen in the liver.

The liver is a key organ in the extraction of regular insulin, but in contrast, hepatic extraction of LY

appeared to be very low. As with other slowly cleared insulin analogs, such as detemir (40), the plasma LY concentrations were high relative to those of regular insulin. However, it is evident that we achieved three distinct LY levels and near-steady-state concentrations, particularly with the longer studies in Part B. With such high circulating concentrations and low clearance, it is difficult to quantify tissue LY extraction precisely. Even though the kidney was an important site of extraction for LY, renal LY extraction was proportionally much less than with human insulin, as designed (9). When LY was tested in subjects with renal impairment (ranging from mild to end-stage renal disease), the apparent clearance and half-life of the drug did not differ from that in subjects with normal renal function (41). Thus, the pharmacokinetic properties of LY do not appear to be affected by impairment in renal function.

Because of the large hydrodynamic size of LY, it might not be expected to cross the blood-brain barrier (42). Nevertheless, hypothalamic Akt phosphorylation at the end of the experiment was not different in the control and LY-treated animals. The affinity of LY for the insulin receptor, although low (<6% of that of insulin lispro [43]), might permit some passage into the central nervous system (CNS) via saturable mechanisms. Additionally, there is a potential for LY to reach the CNS via extracellular pathways such as are used by some very large molecules, e.g., albumin and certain antibodies (44–46). The current findings are not unique or completely unexpected, since the insulin analog detemir (which is tightly bound to serum albumin [47]) has been reported not to cross the mouse blood-brain barrier (48), and yet it has a more rapid and pronounced effect on hypothalamic insulin receptor phosphorylation than human insulin when both are injected intravenously in mice (49). The similarity of phosphorylated STAT3/STAT3 in the livers of the control and LY<sub>0.5</sub> groups is consistent with an effect of LY in the CNS, since hepatic STAT3 phosphorylation has been suggested to be a marker of brain insulin signaling (50,51) that can be blunted with infusion of an ATP-sensitive K<sup>+</sup>-channel inhibitor or a phosphatidylinositol-3 kinase inhibitor into the 3rd cerebroventricle of the dog (51). Nevertheless, hepatic STAT3 phosphorylation in response to brain insulin signaling occurs slowly also (50–52), affecting gene transcription slowly, and thus could not have been involved in bringing about the rapid response effect on liver glucose metabolism observed with LY infusion.

The significant stimulation of hepatic Akt and GSK3 $\beta$  phosphorylation in the LY-treated versus control animals is in keeping with the hepato-preferential metabolic actions of the analog. Conversely, the lack of apparent stimulation of phosphorylation of these proteins by regular insulin (in view of the data from the 18-h-fasted, non-insulin-treated animals) is in keeping with the lack of increase in hepatic insulin concentrations during the clamp; indeed, the hepatic sinusoidal insulin

concentrations were below basal in the control group. GSK3 $\beta$  is inactivated by phosphorylation, thus allowing glycogen synthesis to proceed. Consistent with this, net hepatic carbon retention, a good surrogate measure for hepatic glycogen synthesis (53), was enhanced in the LY-treated groups.

The physiologic basis of the hepato-preferential effect of LY is not fully understood but is likely related to its large hydrodynamic size. While this may impede passage from the capillaries into the interstitium of peripheral target tissues, i.e., muscle and adipose, the hepatic sinusoidal capillaries are fenestrated, with openings of 100–200 Å in diameter, allowing entry of larger molecules into the organ (e.g., lipoproteins), with many factors affecting hepatic clearance (54,55).

In conclusion, LY at all dosages examined exhibited a more hepato-preferential effect than regular human insulin when both products were delivered by peripheral vein. The effect on the liver was evident throughout the duration of the clamp period, with the use of both tracer and organ balance techniques. If these effects can be sustained during long-term dosing, peripherally administered LY appears to have the potential to reproduce the hepato-preferential effects of endogenously secreted insulin—an effect that might reduce complications associated with current insulin therapy.

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**Author Contributions.** M.C.M. carried out the studies, interpreted data, and drafted the manuscript. M.S.S. carried out the studies and analyses. V.P.S., S.J.J., and A.D.C. participated in the design of the studies. Interpreted data, and reviewed and edited the manuscript. J.M.B. interpreted data and reviewed and edited the manuscript. M.D.M. participated in the design of the studies and reviewed and edited the manuscript. A.D.C. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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