*Original Article*

# Physiological Hyperinsulinemia Has No Detectable Effect on Access of Macromolecules to Insulin-Sensitive Tissues in Healthy Humans

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**OBJECTIVE**—Physiologically elevated insulin concentrations promote access of macromolecules to skeletal muscle in dogs. We investigated whether insulin has a stimulating effect on the access of macromolecules to insulin-sensitive tissues in humans as well.

**RESEARCH DESIGN AND METHODS**—In a randomized, controlled trial, euglycemic-hyperinsulinemic clamp (1.2 mU · $kg^{-1}$ · $min^{-1}$ insulin) and saline control experiments were performed in 10 healthy volunteers (aged 27.5 ± 4 years, BMI 22.6 ± 1.6 kg/$m^2$). Distribution and clearance parameters of inulin were determined in a whole-body approach, combining primed intravenous infusion of inulin with compartment modeling. Inulin kinetics were measured in serum using open-flow microperfusion in interstitial fluid of femoral skeletal muscle and subcutaneous adipose tissue.

**RESULTS**—Inulin kinetics in serum were best described using a three-compartment model incorporating a serum and a fast and a slow equilibrating compartment. Inulin kinetics in interstitial fluid of peripheral insulin-sensitive tissues were best represented by the slow equilibrating compartment. Serum and interstitial fluid inulin kinetics were comparable between the insulin and saline groups. Qualitative analysis of inulin kinetics was confirmed by model-derived distribution and clearance parameters of inulin. Physiological hyperinsulinemia (473 ± 6 vs. 18 ± 2 pmol/l for the insulin and saline group, respectively; $P < 0.001$) indicated no effect on distribution volume (98.2 ± 6.2 vs. 102.5 ± 5.7 ml/kg; NS) or exchange parameter (217.6 ± 34.2 vs. 243.1 ± 28.6 ml/min; NS) of inulin to peripheral insulin-sensitive tissues. All other parameters identified by the model were also comparable between the groups.

**CONCLUSIONS**—Our data suggest that in contrast to studies performed in dogs, insulin at physiological concentrations does not augment recruitment of insulin-sensitive tissues in healthy humans. ***Diabetes*** **56:2213–2217, 2007**

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The role of hemodynamic effects of insulin as a regulator of glucose uptake and as a possible factor in the development of insulin resistance has been a matter of controversy during the last decade. Originally, Baron et al. suggested that insulin-mediated vasodilatation may contribute to insulin's overall action on glucose uptake (1) and the development of insulin resistance (2,3). Although these findings were supported by other studies (4,5), several investigators could not reproduce the results (6,7). Refined methods and models have been introduced to investigate vascular actions of insulin closer to the target cells of insulin-sensitive tissues (8–11). While leaving total blood flow unchanged, insulin seems to act via capillary recruitment (10,12) as a regulator to switch flow from the nonnutritive to the nutritive route (13,14). Adequate transport of insulin across the capillary wall and its own availability in interstitial fluid of insulin-sensitive tissues have been identified as crucial factors for insulin action (15,16). In a recent study applying a model in dogs, Ellmerer et al. (9) suggested that insulin could possibly regulate its own distribution to insulin-sensitive tissues. The same investigators also demonstrated that the observed effect of insulin to recruit additional peripheral distribution volume is reduced after diet-induced insulin resistance. The authors speculate that the observed defect may contribute to the pathophysiology of the development of insulin resistance (17).

The objective of the present study was to investigate whether physiological hyperinsulinemia has a stimulatory effect on the recruitment of peripheral insulin-sensitive tissues in humans as well. Similar to the canine model, serum kinetics of inulin was fit to a compartment model as primarily described by Henthorn et al. (18). To gain access to interstitial fluid of insulin-sensitive tissues and to be able to compare compartmental modeling data with actual measurements from interstitial fluid, we applied the open-flow microperfusion technique (19,20) in femoral skeletal muscle and subcutaneous adipose tissue.

## RESEARCH DESIGN AND METHODS

The study was performed in healthy volunteers as a monocentric, randomized, controlled, two-period trial. Subjects gave written informed consent after the purpose, nature, and potential risks of the study were explained and before any study-related activities were started. A period of at least 1 week separated the individual experiments. Subjects were urged not to practice strenuous exercise within the last 4 days before each trial day. Furthermore, the intake

TABLE 1
Baseline characteristics ($n$ = 10)

| Variable | Mean ± SD |
|---|---|
| Age (years) | 27.5 ± 4 |
| Weight (kg) | 74 ± 5 |
| BMI (kg/m$^2$) | 22.6 ± 1.6 |
| Systolic blood pressure (mmHg) | 109 ± 7 |
| Diastolic blood pressure (mmHg) | 71 ± 8 |
| Heart rate (bpm) | 64 ± 10 |
| GFR (ml · min$^{-1}$ · 1.7$^{-1}$) | 97 ± 10 |
| Serum creatinine (mg/dl) | 0.99 ± 0.10 |

GFR, glomerular filtration rate.

of caffeine-containing drinks and alcohol was interdicted after midnight the evening before study days. The study was approved by the local ethics committee of the Medical University Graz (Graz, Austria) and conducted in accordance with the Declaration of Helsinki.

Ten lean, healthy, male volunteers (Table 1) participated in this study. One-half of the subjects were smokers, and no subjects took any regular medication during the observation period. All subjects were normotensive and had no evidence of metabolic or cardiovascular disease at the time of the study. Blood samples, withdrawn during the screening visit, were used to quantify fasting glucose (mean ± SD 81.6 ± 7.6 mg/dl) and serum creatinine concentrations (mean 0.99 mg/dl [range 0.86–1.14]). The latter were used to calculate the inulin clearance (118 ± 16 ml/min) and individual inulin infusion using the Cockcroft formula (21).

Subjects arrived after an overnight fasting period at 7:00 A.M. at the clinical research center and prevailed in semisupine position in an air-conditioned room. A conventional 18-gauge catheter was inserted into a forearm vein for insulin (1.2 mU · kg$^{-1}$ · min$^{-1}$ started at −150 and ended at 480 min) (Insulin Actrapid 100 IE/ml; Novo Nordisk, Bagsværd, Denmark) or saline infusion (started at −150 and ended at 480 min) (NaCl 0.9%; Baxter, Wien, Austria). Through the same catheter, a primed infusion of inulin (priming dose 5 g at 0 min and infusion rate 29 ± 4 mg/min; started at 1 and ended and 240 min) (Inutest 25%; Fresenius Kabi Austria, Graz, Austria) was administered.

During the insulin visit, variable rates of glucose (glucose 20%; Fresenius, Kabi, Austria) were infused to clamp the plasma glucose concentration to basal levels throughout the experiment. A second catheter was inserted into a dorsal hand vein of the contralateral arm and placed in a thermoregulated box (at 55°C) to obtain arterialized venous blood samples for the determination of glucose, insulin, and inulin. Samples were collected at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 18, 20, 25, 30, 35, 40, and 45 min and thereafter in 15-min intervals until the end of the inulin-infusion period (0–240 min). Starting from 240 min, the rapid sampling schedule for inulin was repeated until the end of the study (480 min). Serum samples were frozen immediately at −80°C.

**Microperfusion.** The principle of interstitial fluid sampling by open-flow microperfusion has been described in detail previously (22–24) (see supplement: open-flow microperfusion technique [available in an online appendix at http://dx.doi.org/10.2337/db07-0238]). Open-flow microperfusion is based on a macroscopic perforated double-lumen catheter, which permits unrestricted exchange of solutes between the perfusion medium and interstitial fluid. Between −150 and −120 min, four open-flow microperfusion catheters (modified 18-gauge catheters [Angiocath; Becton Dickinson, Sandy, UT]) were inserted after local skin anesthesia using procaine hydrochloride without vasoconstrictor (Novanaest purum 2%; Gebro Pharma, Fieberbrunn, Austria). Two catheters were inserted into rectus femoris muscle and two into the subcutaneous adipose tissue of the periumbilical region. Catheters were placed at least 40 mm apart and immediately perfused with ion-free solution (mannitol in aqueous solution [275 mmol/l, 288 mosmol/l; produced by a local pharmacy]) at a flow rate of 1 μl/min. Continuous flow within the perforated catheter was established using a multichannel peristaltic pump (Minipuls 3; Gilson, Villier le Bel, France). Perfusion flow rate and sample volume were monitored by weighing the vials before and after sampling. After a run-in period of 30 min, interstitial fluid probe effluent was collected in 30-min fractions until the end of the study. The probe effluent samples (PCR Softtubes 0.2 ml; Biozym Scientific, Oldendorf, Germany) were stored on dry ice, covered with parafilm to avoid evaporation, and frozen immediately after sampling at −20°C, to be analyzed for inulin and conductivity measurements. Using open-flow microperfusion mixing between the perfusate and the surrounding interstitial fluid is not complete; i.e., the probe effluent concentration of inulin is smaller than the interstitial fluid concentration. To calculate the interstitial inulin concentration, we applied an internal calibration technique assuming complete equilibration of inulin between interstitial fluid and serum during the last 30 min of continuous inulin infusion. To assure complete equilibration, we performed a separate experiment in six healthy volunteers by applying the no-net-flux calibration technique (25) for inulin (data not shown). Variability of the recovery rate over time was corrected by measuring relative changes of the conductivity in the probe effluent (26).

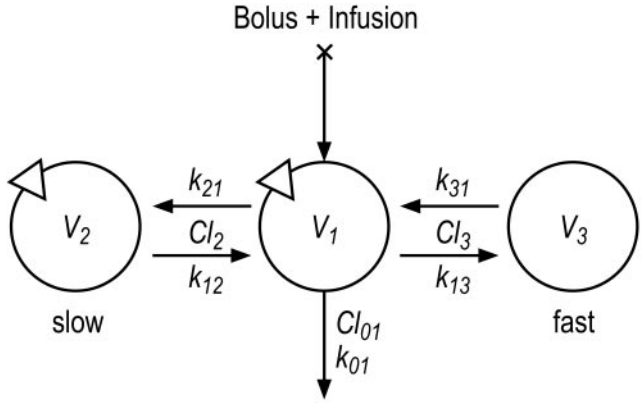


**FIG. 1. Three-compartment model for the distribution and clearance of inulin as originally proposed by Henthorn et al. (ref. 18).**

**Assays.** On-line plasma glucose was assayed using a glucose oxidase method (Beckman Glucose Analyzer 2; Beckman Instruments, Fullerton, CA) (intra-assay coefficient of variation [CV] 2%). Insulin was measured using a solid-phase two-site enzyme-linked immunosorbent assay (Ultra-Sensitive Insulin ELISA; Mercodia, Uppsala, Sweden) (intra- and interrun assay CV ~15%). Inulin concentrations in serum (1 ml) and microperfusion effluent samples (15 μl) were measured photometrically with an adapted enzymatic kit (Glucoquant; Roche Diagnostic, Mannheim, Germany) using a standard laboratory analyzer (Cobas Mira; Hoffmann-La Roche, Basel, Switzerland) (intra- and interrun assay CV ~10%). Relative conductivity of the microperfusion probe effluent was detected using a TraceDec conductivity measurement (S.I.T., Strasshof, Austria).

**Pharmacokinetic data analysis: inulin distribution model.** Inulin, an inert polysaccharide of similar molecular size and weight as insulin (5 vs. 5.9 kDa for inulin vs. insulin, respectively), is a commonly used marker to characterize extracellular space in humans (27). The principles underlying our investigational approach are that inulin is not metabolized and traces the kinetic events of extracellular distribution space (28). Serum and interstitial fluid profiles of inulin covering the inulin study period from 0 to 480 min were best described using a three-compartment model (Fig. 1). The model as originally applied in animal studies by Henthorn et al. (9) and modified by Ellmerer et al. (17) is characterized by a central compartment representing the serum volume ($V_1$) and a slow ($V_2$) and a rapid ($V_3$) equilibrating compartment representing interstitial fluid spaces of insulin-sensitive tissues. The slow equilibrating compartment ($V_2$) is assumed to be supplied by capillaries with a continuous basement membrane, as described for skeletal muscle and subcutaneous adipose tissue (i.e., it accounts for peripheral insulin-sensitive tissues), whereas the fast equilibrating compartment ($V_3$), provided by discontinuous and fenestrated capillaries is suggested to represent mainly the splanchnic bed and liver. Inulin was assumed to be irreversibly lost from the serum compartment by renal clearance ($Cl_{01}$). Transport parameters between serum and slow ($k_{21}$, $k_{12}$) and serum and fast ($k_{31}$, $k_{13}$) equilibrating compartments were introduced by assuming passive diffusion as a transport mechanism across the capillary endothelium (29,30). Distribution volumes were expressed as milliliters per kilogram body weight, and clearance and tissue-specific transport parameters were expressed as milliliters per minute ($Cl_{01} = k_{01} \times V_1$, $Cl_2 = k_{21} \times V_1$, and $Cl_3 = k_{31} \times V_1$). Model parameters were identified using serum inulin only.

**Numerical methods and data analysis.** Model parameters were identified using the Windows version of the SAAM (Simulation, Analysis, and Modeling) program (National Institutes of Health, Bethesda, MD), implemented on a personal computer. Numerical values of unknown parameters were obtained by nonlinear least squares using a modified Gauss-Newton algorithm with inverse variance weights. Identified parameters were used to predict the kinetic response of the peripheral distribution compartments. This predicted response was compared with the measured profiles of inulin in serum and interstitial fluid on a qualitative basis. Paired Student's $t$ test was applied to calculate statistical significances within and between subsets of data using noncompartmental analysis. Statistical analysis was performed using SPSS software. Data are shown as means ± SE unless otherwise noted. $P < 0.05$ was considered statistically significant.

## RESULTS

**Basal metabolic parameters.** Plasma glucose concentrations were comparable between the two experiments and were constant during the inulin study period (91 ± 2 vs. 88 ± 2 mg/dl for the insulin vs. saline group, respectively; NS). Steady-state glucose infusion rates during the euglycemic-hyperinsulinemic clamp were within an expected range for healthy humans as known from previous studies and indicated low intersubject variability (10.2 ± 0.3 mg · $kg^{-1}$ · $min^{-1}$). Per definition, serum insulin was physiologically elevated in the insulin vs. saline group (473 ± 6 vs. 18 ± 2 pmol/l; $P < 0.001$) and remained constant during the inulin study period (0–480 min).

**Inulin kinetics.** The kinetic profile of serum inulin was not altered by physiological hyperinsulinemia. A tendency for a parallel shift of the profile in the insulin group was observed (Fig. 2*A*). Modeling and simulation analysis could attribute this shift to the additional infusion volume caused by variable exogenous glucose infusion as required for the hyperinsulinemic clamp experiment. Overall, steady-state inulin concentrations were not different between groups (237 ± 7 vs. 250 ± 8 mg/l for the insulin vs. saline group, respectively; NS). The interstitial fluid response to the primed intravenous inulin infusion was delayed in comparison with the serum response (Fig. 2*B* and *C*). Interstitial fluid concentrations of inulin in skeletal muscle and adipose tissue were almost superimposable, suggesting that inulin transport across the capillary wall is comparable in these tissues. Similar to the effect on serum inulin kinetics, hyperinsulinemia indicated no effect on inulin kinetics in skeletal muscle and adipose tissue.

**Three-compartment model analysis.** Model evaluation and analysis was performed blinded and in randomized order. Model parameters of serum inulin kinetics were best identifiable using a three-compartment modeling approach. The precision of individual parameter estimates was evaluated as fractional SD achieving ≤18% for individual parameters (Table 2). To further evaluate the model fit over the time course of the data, the residual plot was used as a measure to indicate relative deviation of model prediction from the measured data (Fig. 2*A*, *inlay*). The kinetic response of inulin measured in skeletal muscle and adipose tissue was well described by model predictions from serum data for the slow equilibrating compartment ($V_2$) as indicated in Fig. 2*B* and *C*, respectively. All model parameter estimates were comparable between insulin and saline experiments (Table 2).

**Open-flow microperfusion recovery rate.** Mean recovery rates of interstitial fluid in open-flow microperfusion probe effluent were comparable between skeletal muscle and subcutaneous adipose tissue for both insulin (muscle 23 ± 2 vs. adipose 25 ± 4%; NS) and saline (muscle 26 ± 1 vs. adipose 22 ± 3%; NS) experiments.

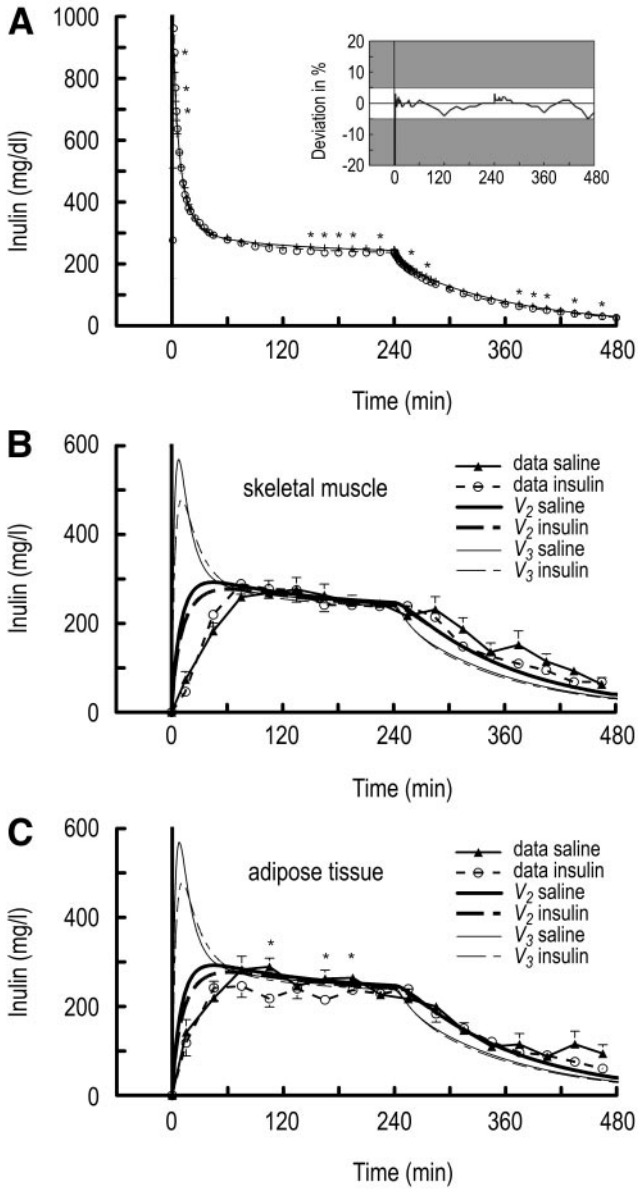


**FIG. 2. *A*: Serum inulin kinetics. Inulin concentrations are indicated for the insulin (○) and saline (+) group and compared with model predictions of serum inulin kinetics, as suggested by compartmental modeling (dashed and solid lines represent the insulin and saline group, respectively). The *inlay* indicates the overall mean residuals for the deviation between model predictions and measured data expressed as percentage of deviation from measured data. *B* and *C*: Interstitial inulin kinetics. Comparison of model predictions for slow ($V_2$) and fast ($V_3$) equilibrating compartments and measured interstitial fluid inulin kinetics for skeletal muscle (*B*) and adipose tissue (*C*). Measured inulin concentrations are indicated for insulin and saline. *$P < 0.05$.**

## DISCUSSION

The present results indicate that physiological hyperinsulinemia has no detectable effect on distribution volumes or transport parameters of the extracellular marker inulin in healthy male volunteers. Applying compartmental modeling of inulin kinetics during insulin versus saline control experiments, we were able to demonstrate that all model parameters describing serum inulin kinetics were comparable between groups. Our results suggest that, in contrast to the findings of recent studies performed in a canine model, physiological hyperinsulinemia does not augment access of macromolecules to insulin-sensitive tissues in healthy humans.

Inulin, commonly used to estimate glomerular filtration rate (30–32), has originally been applied by Atkinson and colleagues (18,33) for the characterization of extracellular fluid compartments using a mathematical modeling approach. In the present study, the extracellular tracer inulin was used to investigate possible hemodynamic effects of insulin on transport and distribution parameters of macromolecules in peripheral insulin-sensitive tissues. In comparison to a two- or a four- compartmental modeling approach (data not shown), serum inulin kinetics could be best characterized by a three-compartment model. Model

TABLE 2
Basal metabolic and model parameters

| | Saline | Insulin | *P* |
|---|---|---|---|
| Glucose (mg/d) | 88 ± 2 | 91 ± 2 | NS |
| Insulin (pmol/l) | 18 ± 2 | 473 ± 6 | <0.001 |
| $V_1$ (ml/kg) | 49.6 ± 6.9 (0.09) | 44.4 ± 4.1 (0.08) | 0.36 |
| $V_2$ (ml/kg) | 102.5 ± 5.7(0.08) | 98.2 ± 6.2 (0.06) | 0.49 |
| $V_3$ (ml/kg) | 25.4 ± 4.4 (0.12) | 31.8 ± 4.5 (0.18) | 0.20 |
| $Cl_{01}$(ml/min) | 124 ± 4 (0.01) | 127 ± 3 (0.01) | 0.21 |
| $Cl_2$ (ml/min) | 243 ± 29 (0.15) | 218 ± 34 (0.13 | 0.34 |
| $Cl_3$ (ml/min) | 452 ± 76 (0.16) | 398 ± 53 (0.17) | 0.48 |

Data are means ± SE ($n$ = 10). Data in parentheses indicate precision of parameter estimates expressed as fractional SDs. Results from three-compartment model analysis for the saline and insulin groups. NS, not significant.

predictions for the serum compartment ($V_1$: 3.5 ± 0.3 l), the total inulin distribution volume ($V_{inulin}$: 13.0 ± 0.6 l), and the renal clearance were in accordance with previous findings (33–35). In particular, the model prediction of the renal clearance was in very good agreement with values as determined using the Cockcroft formula (21). Also, all model parameters were identifiable a priori, suggesting that the three-compartment model is adequate to describe inulin kinetics in humans.

Interstitial kinetics of inulin indicated an almost superimposable response of inulin in skeletal muscle and adipose tissue, suggesting that the capillary endothelium is structurally comparable between the two tissues. Furthermore, the appearance of inulin was delayed in interstitial fluid in comparison with that in serum, indicating that the capillary wall of skeletal muscle and subcutaneous adipose tissue constitutes a barrier for the exchange of inulin in humans. Both appearance and clearance kinetics of inulin in interstitial fluid were well described using the simulated response for the slow equilibrating compartment ($V_2$), which was independently obtained using serum inulin kinetics. Therefore, results from interstitial fluid inulin kinetics support the concept that peripheral insulin-sensitive tissues are part of the slow equilibrating compartment for the distribution of inulin in humans as well (18,33).

Over past years, very different methodologies and models have been invented to determine insulin's hemodynamic effects. Investigators focused their work mainly on three different vascular levels: systemic blood flow (macrovascular level), capillary recruitment (microvascular level), and tissue recruitment (interstitial level). At the microvascular level, findings from different investigators have consistently shown that insulin can stimulate the recruitment of capillaries independently of bulk blood flow and that glucose uptake is influenced by capillary recruitment, both in animals (36) and in humans (13). Investigation of the extravasation of macromolecules in skeletal muscle in obese versus lean Zucker rats (37) suggests that there might be a dissociation between the regulation of microcirculation and capillary permeability. The authors observed increased vasopermeability in obese versus lean Zucker rats, which may compensate for the loss of nitric oxide–dependent vasodilatation and capillary recruitment. The question remains open whether the above-mentioned effects can also be observed at the level of interstitial fluid. Bonadonna et al. (8) combined measurements of L-glucose with a multicompartmental model applied across the human forearm. Distribution of L-glucose and tissue-specific flow was not affected by physiological hyperinsulinemia. Although the results of the present study are in support of the findings by Bonadonna et al., they stand in contrast to the results of a canine model applied by Ellmerer et al. (9), who observed a distinct effect of physiological hyperinsulinemia on tissue recruitment that was diminished in a model of diet-induced insulin resistance (17). In the present study, we used a methodological approach very similar to that applied by Ellmerer et al. in the canine model but in healthy humans. In the present experiments, all three distribution volumes (expressed as percentage of body weight) and also the transport rates from serum to the slow ($k_{21}$) and the fast ($k_{31}$) equilibrating compartments were very comparable with the results from the canine model, but no effect of physiological hyperinsulinemia on tissue recruitment was observed. Overall, a combination of the different methodologies as they are presently used may shed more light on the mechanism and physiological relevance of vascular effects of insulin at the microvascular and interstitial levels and its importance in the development of insulin resistance.

In conclusion, our data suggest that in contrast to similar studies performed in dogs, insulin at physiologically elevated concentrations does not augment recruitment of insulin-sensitive tissues in healthy male volunteers.

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