Obesity Induced by a High-Fat Diet Is Associated With Reduced Brain Insulin Transport in Dogs

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Insulin transported from plasma into the central nervous system (CNS) is hypothesized to contribute to the negative feedback regulation of body adiposity. Because CNS insulin uptake is likely mediated by insulin receptors, physiological interventions that impair insulin action in the periphery might also reduce the efficiency of CNS insulin uptake and predispose to weight gain. We hypothesized that high-fat feeding, which both reduces insulin sensitivity in peripheral tissues and favors weight gain, reduces the efficiency of insulin uptake from plasma into the CNS. To test this hypothesis, we estimated parameters for cerebrospinal fluid (CSF) insulin uptake and clearance during an intravenous insulin infusion using compartmental modeling in 10 dogs before and after 7 weeks of high-fat feeding. These parameters, together with 24-h plasma insulin levels measured during ad libitum feeding, also permitted estimates of relative CNS insulin concentrations. The percent changes of adiposity, body weight, and food intake after high-fat feeding were each inversely associated with the percent changes of the parameter k_1k_2, which reflects the efficiency of CNS insulin uptake from plasma (r = –0.74, –0.69, –0.63; P = 0.015, 0.03, and 0.05, respectively). These findings were supported by a non–model-based calculation of CNS insulin uptake: the CSF-to-plasma insulin ratio during the insulin infusion. This ratio changed in association with changes of k_1k_2 (r = 0.84, P = 0.002), body weight (r = –0.66, P = 0.04), and relative adiposity (r = –0.72, P = 0.02). By comparison, changes in insulin sensitivity, according to minimal model analysis, were not associated with changes in k_1k_2, suggesting that these parameters are not regulated in parallel. During high-fat feeding, there was a 60% reduction of the estimated CNS insulin level (P = 0.04), and this estimate was inversely associated with percent changes in body weight (r = –0.71, P = 0.03). These results demonstrate that increased food intake and weight gain during high-fat feeding are associated with and may be causally related to reduced insulin delivery into the CNS. *Diabetes* 49:1525–1533, 2000

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AUC, area under the curve; BBB, blood-brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; FM, fat mass; FSIGT, frequently sampled intravenous glucose tolerance test; LBM, lean body mass; S_I, insulin sensitivity index; TBW, total body water.

Insulin secreted by the endocrine pancreas plays a complex role in the regulation of fuel homeostasis. Even as insulin is recognized for its anabolic effects in the periphery, insulin acts in the central nervous system (CNS) as a catabolic agent by inhibiting food intake (1) and stimulating fat oxidation (2). These CNS properties, together with many convergent observations, implicate insulin as a signal that provides negative feedback to the CNS for the long-term regulation of energy balance (3–5). According to this hypothesis, interventions that chronically lower CNS insulin levels will increase the tendency for storing energy in the form of adipose tissue. Hence, interventions that chronically lower the circulating insulin levels, the efficiency of CNS insulin uptake, or both could stimulate energy intake and promote increased adiposity.

In dogs, insulin enters the CNS via a saturable mechanism (6) hypothesized to be mediated by insulin receptors expressed in the endothelium of the blood-brain barrier (BBB) (7–10). Accordingly, CNS insulin uptake, like systemic insulin action, is thought to involve the cellular internalization of hormone-receptor complexes triggered by the binding of insulin to its receptor. Interventions that alter insulin action in peripheral tissues might therefore also alter the efficiency with which insulin enters the CNS. In support of this hypothesis, we previously reported that dexamethasone, a synthetic glucocorticoid that induces insulin resistance and stimulates weight gain, reduces the efficiency of CNS insulin uptake as quantified by compartmental model analysis (11). This pharmacological outcome suggests that physiological interventions that cause insulin resistance might simultaneously reduce the efficiency of CNS insulin uptake and thereby promote weight gain. Reduced efficiency of CNS transport of the adipocyte hormone leptin has also been implicated in the pathogenesis of diet-induced obesity in both rodents (12) and primates (13). High-fat feeding causes insulin resistance (14–17) and promotes weight gain (15,17–23), and some evidence suggests that reduced insulin sensitivity can precede the weight gain (14,15,17).

We therefore hypothesized that high-fat feeding reduces the efficiency of CNS insulin uptake, and that this effect is associated with increased food intake and body weight gain. To investigate this hypothesis, we quantified parameters for cerebrospinal fluid (CSF) insulin uptake and clearance in dogs before and after 7 weeks of high-fat feeding by using the compartmental model developed in our laboratory (24) to study the kinetics (6) and pharmacological regulation of CSF insulin transport (11). Changes in body weight, adiposity, and food intake were then analyzed in relation to changes in an index

of CNS insulin levels that comprised CNS insulin transport kinetics and 24-h measurements of circulating insulin levels.

RESEARCH DESIGN AND METHODS

Study animals and protocol. Studies were performed in 10 male mongrel dogs that weighed 27–40 kg and were maintained in accordance with U.S. Department of Agriculture regulations. All studies were approved by the Animal Care Committee at the Puget Sound Veterans Affairs Health Care System. After arterial catheterization, animals were maintained on ad libitum access to standard laboratory diet (Harlan Teklad, Madison, WI) for at least 5 weeks to establish a stable weight baseline, which was recorded at 3-day intervals. During this time, dogs were accustomed to the Pavlov sling used during the frequently sampled intravenous glucose tolerance test (FSIGT) protocol as later described. Animals underwent CNS insulin transport studies, body composition testing, 24-h studies for sampling plasma insulin and glucose levels, and the FSIGT protocol at baseline while on a standard diet and again after a 7-week period on an ad libitum high-fat diet. Results pertaining to the effect of high-fat feeding on glucose tolerance that are not reported here have been published elsewhere (17). Failure of the arterial catheter in 1 dog precluded the performance of the FSIGT and the 24-h plasma-sampling protocol after high-fat feeding.

Arterial catheter insertion. Under general anesthesia, Tygon (Akron, OH) tubing (0.07 in outside diameter × 0.04 in inside diameter) was inserted via the omocervical artery and advanced under guidance of fluoroscopy until the catheter tip resided within the aorta at the level of the diaphragm. The proximal end of the catheter was externalized at the back of the neck via an arterial valve connector (Harvard Apparatus, Holliston, MA) to permit rapid access to arterial blood, and catheter patency was maintained with a heparin solution. Dogs were allowed to recover for at least 5 weeks before being studied.

Diets. The baseline diet consisted of standard laboratory dog food (Harlan Teklad, Madison, WI) that had an energy density of 3.47 kcal/g; 17% of the calories were derived from fat. The high-fat diet provided 80% of the calories as fat and was adapted from a previous work (25) that indicated that a very high-fat diet maximizes the likelihood that some study animals develop obesity, the propensity for which varies widely among dogs of different genetic backgrounds. The high-fat diet regimen entailed 2 feedings per day: morning and afternoon. The afternoon component consisted of a homogeneous mixture of 454 g lard (Armour Foods, Omaha, NE) and 748 g canned dog food. Variety was increased by use of 2 brands of dog food (Blue Mountain Special Menu, Lehigh Valley, PA, and Friskies Alpo Prime Cuts, Glendale, CA) in 3 flavors (chicken, beef, and turkey). Additionally, 71 g of chicken or beef baby food (Heinz, Pittsburgh, PA) was added to each 1,202-g serving of the lard–dog food mixture. This mixture contained 4.13 kcal/g and provided 12.3 g protein/1,000 kcal (the high water content of the dog food substantially dilutes the energy-to-weight ratio). The morning feeding component consisted of 6 food units, each consisting of oil-based tuna fish (19 g) enveloped by lard (57 g) and coated with peanut butter (57 g). Each of these units contained 1,150 kcal and provided 16.2 g protein/1,000 kcal. Sufficient standard and high-fat diet were provided to ensure unlimited access to food at all times. All animals remained healthy throughout the study period.

Estimation of energy intake. Food was weighed before being given to each animal and again upon removal of the food the next day. Estimations of daily caloric intake were based on the weight of food consumed and the energy conversion factors previously described.

Quantification of body composition. Body composition was estimated with the isotope dilution technique (26). As a bolus, 24 ml of a stock solution of sterile isotonic saline containing approximately 2 μCi/ml of ^3H_2O was administered intravenously. The syringe was weighed before and after injection for precise determination of the volume administered. Blood samples were collected before and 3 h after the injection for determination of plasma radioactivity (disintegrations per minute). Plasma radioactivity was converted to body water radioactivity on the assumption that plasma is 94% water (27). For each body composition determination, a single assay using a liquid-scintillation counter (Packwood Tri-Carb 1600 TR; Meriden, CT) quantified disintegrations per minute in each of three 0.3-ml samples of baseline and equilibrium plasma and in three 0.3-ml samples of scintillation fluid containing 1 μl of radioactive stock. The difference in the mean dpm/ml between pre- and postinjection plasma samples was taken as the equilibrium concentration of the ^3H_2O, which provides a measure of total body water (TBW) from which lean body mass (LBM) is estimated on the assumption that TBW represents a constant fraction of LBM (0.74 in the dog) (26). Fat mass (FM) was determined as the difference between LBM and total body mass. The percent body fat was calculated as 100 · FM/total body mass.

Quantification of changes of relative adiposity. To characterize changes in relative adiposity, we determined the change in the ratio of FM to LBM calculated as (FM/LBM after high-fat feeding – FM/LBM at baseline) × 100. Each term of this equation represents FM normalized to a relatively stable index of body size (LBM). Thus, because the change in relative FM represents the change in FM scaled according to body size, comparisons between animals that differ with respect to LBM are made more meaningful. Additionally, unlike changes in the percent body fat, the change in relative FM tends to be linearly related to changes in both FM and body weight, which facilitates the interpretation of associations with other variables, such as parameters for CNS insulin transport.

Measurement of 24-h insulin levels. Serial sampling of arterial plasma was performed in the animal's home cage over a 24-h period during ad libitum provision of food and water. For each dog, baseline 24-h studies occurred within 2 weeks of beginning high-fat feeding and were repeated during the 7th week of high-fat feeding. Dogs were not fasted before these studies, which began at 9:30 A.M. Arterial blood samples were drawn every 30 min for the first 15 h, every 1 h for the next 4 h, and at 30-min intervals for the final 5 h (a total of 44 samples). Circulating insulin levels were quantified using the trapezoidal rule to compute the area under the 24-h insulin curve. Plasma glucose was quantified as the mean of the 44 values obtained.

FSIGT procedure. Tolbutamide-modified FSIGT studies were conducted at 10:30 A.M. after an overnight fast. At $t = 0$ min, a glucose bolus (0.3g/kg of the baseline body weight) was infused over a 40-s period into a forelimb vein. At $t = 20$ min, tolbutamide was administered (3 mg/kg i.v.) to improve identifiability of parameters derived from minimal model analysis (28). Arterial blood was collected for determination of insulin and glucose levels at $t = -20, -10, -1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160$, and 180 min.

Quantification of insulin sensitivity. The minimal model method (29–31), which was developed originally in dogs, was used to analyze FSIGTs for quantification of the insulin sensitivity index (S_I). S_I measures the ability of a unit increment of insulin to enhance glucose disappearance. Data regarding the effect of the high-fat diet on S_I in animals studied here have been reported in a separate article (17).

CNS insulin transport studies. The protocol for quantifying CNS insulin transport and the compartmental model of CNS insulin transport has been described in detail elsewhere (6,11,24). After an overnight fast, dogs were anesthetized with thiamylal (Surital; Parke-Davis, Morris Plains, NJ) 20 mg/kg i.v. and placed on mechanical ventilation with 1–2% halothane and 40% O_2. Arterial blood gas analysis was performed at frequent intervals throughout the study, and the ventilatory rate was adjusted as necessary to maintain arterial pH between 7.35 and 7.45 and P_{CO_2} between 35 and 45 mmHg. An intravenous catheter was placed in a hindlimb vein for insulin and glucose infusion, and blood samples were obtained via an indwelling arterial catheter. Samples of CSF were obtained from a 22-gauge spinal needle inserted into the cisternum magnum using the sterile technique. Each study entailed a 90-min primed intravenous insulin infusion period with frequent sampling of plasma and CSF for a total of 490 min. Insulin was administered intravenously as a 3-min priming infusion (8.5 mU/kg of baseline body wt/min) starting at $t = 0$ min, followed by a continuous infusion for 87 min at 1.5 mU/kg of baseline body wt/min. Thus, the total amount of insulin infused into each dog was constant across pre– and post–high-fat diet studies. Blood samples (1.8 ml) were drawn at $t = -10, -5, 1, 2, 3, 4, 6, 8, 10, 13, 16, 20, 25, 30, 35, 40, 65$, and 90 min, then at 5-min intervals for $90 \leq t \leq 150$ min and at 20-min intervals for $150 \leq t \leq 490$ min. CSF samples (0.4 ml) were drawn at $t = -10, -5, 20, 40, 65, 90, 95, 100, 110, 120, 135$, and 150 min and every 20 min thereafter until 490 min. Euglycemia was maintained by variable rate infusion of 50% dextrose with real-time monitoring of blood glucose levels via a hand-held glucose meter (Glucoscan, Lifescan; American Medical Systems, Cincinnati, OH). For each study, we determined the ratio of the incremental area under the CSF insulin curve (AUC) measured over the entire 490 min study period to the incremental plasma insulin AUC measured during the 90-min infusion period (CSF-to–plasma insulin ratio). AUCs were quantified based on actual data values using KaleidaGraph (Synergy Software, Reading, PA).

To determine the contribution of changes in the efficiency of CSF insulin uptake and clearance to differences in the CSF-to–plasma insulin ratio, we used a compartmental model developed in our laboratory (24). Kinetic modeling of parameters for CNS insulin transport relies on a 3-compartment model (Fig. 1) according to which insulin derived from plasma appears in CSF after passing through an intermediate compartment hypothesized to be brain interstitial fluid. Insulin from plasma enters the intermediate compartment with a rate constant k_1. From the intermediate compartment, insulin can either enter the CSF compartment with a rate constant k_2 or be cleared by a route independent of CSF with a rate constant k_3. Insulin in CSF is cleared with a rate constant k_4. Because CSF represents the only CNS compartment that is sampled for insulin, the parameter k_1 cannot be uniquely estimated. However, the product $k_1 \cdot k_2$ can be estimated, along with the sum $(k_2 + k_3)$, and k_4. k_1k_2 represents

the efficiency with which insulin from plasma enters the intermediate compartment and subsequently enters CSF. The mathematical modeling program SAAM (32) was used to estimate the rate constants k_1k_2 (min^{-2}), $k_2 + k_3$ (min^{-1}), and k_4 (min^{-1}).

Index of 24-h brain insulin levels. According to our compartmental model for CNS insulin transport (Fig. 1) (11,24), at any given time, t_i, the rate at which insulin enters a unit volume of the intermediate compartment in pmol · l^{-1} min^{-1}, equals

$$k_1 \cdot [INS]_p$$

where $[INS]_p$ is the plasma insulin concentration in pmol/l. In a 24-h (1,440 min) time period, therefore, total insulin delivery into a unit-volume of the intermediate compartment equals

$$(k_1) \cdot \int_0^{1440} [INS]_p dt = (k_1) \cdot (AUC_{[INS]p})$$

where $(AUC_{[INS]p})$ is the area under the 1,440-min plasma insulin curve.

Assuming that brain interstitial fluid (ISF_b), is represented by the intermediate compartment, the rate of removal at t_i of insulin from ISF_b in pmol · l^{-1} · min^{-1} equals

$$(k_2 + k_3) \cdot [INS]_{ISFb}$$

where $[INS]_{ISFb}$ is the insulin concentration in brain interstitial fluid.

Thus, in a 1,440-min time period, total insulin clearance from a unit volume of ISF_b is

$$(k_2 + k_3) \cdot (AUC_{[INS]ISFb})$$

where $(AUC_{[INS]ISFb})$ is the area under the 1,440-min ISF_b insulin curve.

If we assume that in 1,440 min the total insulin delivery into ISF_b approximates total insulin clearance from this compartment, then

$$k_1 \cdot (AUC_{[INS]p}) \approx ([k_2 + k_3] \cdot [AUC_{(INS)ISFb}])$$

This can be rearranged as

$$AUC_{[INS]ISFb} \approx k_1 \cdot (k_2 + k_3)^{-1} (AUC_{[INS]p})$$

Therefore, using k_1k_2 as an index of k_1, an index of the average 24-h CNS insulin level was computed as

$$k_1k_2 \cdot (k_2 + k_3)^{-1} \cdot AUC_{[INS]p}$$

We termed this parameter the 24-h CNS insulin index.

Statistical analysis. Paired Student's *t* tests were performed to assess the significance of changes in CNS insulin transport parameters and the CNS insulin index. Because paired differences in k_1k_2 parameter estimates were not normally distributed, k_1k_2 values were log transformed before statistical analysis of paired differences. Least squares linear regression analysis was performed to assess the significance of bivariate associations. Correlational analyses involving k_1k_2 did not involve log transformation of this variable. Data are reported as means ± SE, and a 2-sided α level of 0.05 was accepted as statistically significant.

RESULTS

Changes in body composition, S_I, and 24-h circulating insulin levels. The effects of high-fat feeding on body weight and composition, 24-h insulin levels, and S_I were measured in 9 of the 10 dogs and were reported elsewhere (17); they are also summarized in Table 1. S_I decreased in all 9 animals (range –19.6 to –85.0%, mean –56.6%, P = 0.003), and the mean 24-h insulin profile measured during ad libitum feeding was reduced in 8 of the 9 dogs, with the mean value being significantly reduced by 44.0% (range 11 to –70.9%, P = 0.004). For the entire set of 10 dogs, the mean body weight increased from 35.0 ± 1.5 to 40.9 ± 2.4 kg (P = 0.01), FM increased from 5.6 ± 0.8 to 11.6 ± 2.1 kg (P = 0.009), and relative adiposity (measured as 100 · ΔFM/LBM) increased 2-fold from 20.7 ± 4.4 to 42.0 ± 8.7% (P = 0.008). After high-fat feeding, however, estimates of FM for 3 of the dogs were slightly below (mean –0.40 kg) those at baseline, and total body weight in each of these animals differed by <1.0 kg from baseline (mean –0.3 kg). Thus, high-fat feeding induced insulin resistance in all of the dogs, and it reduced 24-h insulin levels in 8 of 9 dogs; however, it had more variable effects on body fat content, with an increase detected in 7 of the 10 dogs (Table 1).

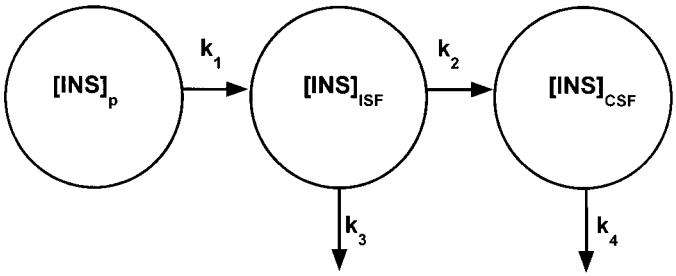

FIG. 1. A schematic illustration of the 3-compartment model used for kinetic analysis of insulin uptake from plasma into CSF. The rate constant k_1 represents the efficiency of insulin uptake, which is hypothesized to occur across the BBB, from plasma into an intermediate compartment. k_2 represents the efficiency of insulin transport from the intermediate compartment, which is hypothesized to be brain interstitial fluid, into CSF. Insulin also exits the intermediate compartment independently of CSF with an efficiency k_3. Insulin in CSF is cleared with a rate constant k_4. Mathematical modeling using plasma and CSF insulin data (Fig. 2*B*) allows identification of the k_1k_2 product, $(k_2 + k_3)$, and k_4.

Relationship between changes in body weight and food intake. Estimated caloric intake averaged over the last 2-week period of standard diet feeding was 3,078 ± 136 kcal/day. In contrast, estimated energy intake averaged over the 7 weeks of high-fat feeding was 4,284 ± 332 kcal/day, a 39% increase above that at baseline (P < 0.01). Regression analysis revealed significant positive associations between the change in caloric intake and the change in body weight (r = 0.68, P = 0.03), the change in FM (r = 0.78, P = 0.007), and the change in relative adiposity (r = 0.76, P = 0.01).

Relationship of initial adiposity to subsequent weight gain. Body weight before high-fat feeding and weight gain during this diet were not associated (r = 0.03, P = 0.93). Similarly, there was no association between baseline relative adiposity and subsequent weight gain on the high-fat diet (r = 0.005, P = 0.99). The only variable measured at baseline that was correlated with subsequent weight gain was the mean plasma insulin level during the 90-min insulin infusion period during the baseline CSF insulin transport studies (r = –0.68, P = 0.03).

Plasma insulin levels during CNS insulin uptake studies. Despite maintaining the insulin dose constant for each dog across pre– versus post–high-fat diet studies, the average plasma insulin level during the 90-min insulin infusion period was 50% higher in the study during high-fat feeding (1,182 ± 58 pmol/l vs. 786 ± 77 pmol/l, P = 0.001) (Fig. 2*A*). There was a significant positive association between the percent change in plasma insulin during the infusion and the percent change in body weight (r = 0.78, P = 0.008).

Changes in the CSF-to–plasma insulin ratio. High-fat feeding resulted in a significant decrease in the CSF-to–plasma insulin ratio (0.59 ± 0.05 after vs. 0.89 ± 0.01% before high fat feeding; P = 0.02) (Table 1). Among the 7 dogs that did gain

TABLE 1
Effects of 7 weeks of high-fat feeding on insulin levels, insulin sensitivity, body composition, and CNS insulin transport parameters

	Δ Fasting insulin (pmol/l)	Δ 24-h Insulin (pmol/l)	Δ S_1 × 10^{-5}/min/pmol/l	Δ Body weight (kg)	Δ Food intake (kcal)	Δ Fat mass (kg)	Δ k_1k_2 × 10^{-6} min^{-2}	Δ $(k_2 + k_3)$ × 10^{-2}/min	Δ k_4 × 10^{-2}/min	ΔCSF: plasma insulin ratio (% units)
Dog										
1	–6 (–7.7)	–130 (–57)	–5.5 (–56)	2.0 (5)	717 (26)	1.0 (30)	–0.47 (–12)	–0.47 (–41)	1.56 (55)	–0.36 (–50)
2	30 (83)	–203 (–71)	–2.1 (–20)	0.8 (2)	–288 (–10)	–0.1 (–1)	1.65 (157)	0.23 (44)	3.05 (169)	0.28 (99)
3	48 (100)	–93 (–44)	–3.4 (–20)	5.0 (16)	1,229 (50)	6.2 (56)	–5.5 (–44)	–0.27 (–15)	–0.88 (–23)	–0.26 (–29)
4	30 (56)	–145 (–54)	–4.7 (–47)	–1.5 (–4)	1,494 (45)	–0.2 (–4)	1.48 (58)	–0.21 (–17)	1.73 (134)	–0.21 (–24)
5	24 (57)	–249 (–62)	–17 (–75)	7.7 (24)	1,199 (38)	8.1 (630)	–1.08 (–37)	–0.17 (–17)	0.89 (59)	–0.42 (–51)
6	12 (13)	–114 (–30)	–2.9 (–52)	7.7 (19)	1,787 (49)	10.4 (229)	–10.78 (–69)	–1.92 (–70)	–2.53 (–88)	–0.79 (–49)
7	78 (144)	–48 (–85)	–11.9 (–79)	8.7 (22)	1,991 (63)	9.8 (123)	3.60 (77)	–0.01 (–1)	1.07 (53)	–0.33 (–32)
8	24 (57)	–2 (–5)	–6.6 (–50)	17.0 (49)	2,024 (66)	15.7 (320)	–3.10 (–58)	0.99 (277)	–4.84 (–60)	–0.52 (–59)
9	12 (22)	10 (10)	–15.1 (–85)	11.7 (37)	1,535 (40)	10.4 (153)	–7.11 (–83)	0.47 (53)	–3.41 (–70)	–0.50 (–47)
10	NM	NM	NM	–0.2 (–1)	374 (14)	–0.9 (–20)	6.17 (144)	1.47 (82)	1.60 (79)	0.16 (27)
Mean Δ ± SE	25.5 ± 7.6	–113 ± 28	–7.7 ± 1.9	5.9 ± 1.8	1,206 ± 235	6.0 ± 1.8	–1.51 ± 1.63	0.01 ± 0.29	–0.18 ± 0.82	–0.30 ± 0.10
% of Control	50 ± 14	–45 ± 11	–57 ± 14	17 ± 5	39 ± 8	108 ± 33	–25 ± 27	1 ± 22	–6 ± 27	–21 ± 15

Data are the magnitude of change (% change from baseline) and means ± SE. NM, not measured.

FM, this ratio decreased by 45% after high-fat feeding (mean –0.46 ± 0.07%), whereas 2 of the 3 dogs that did not gain FM exhibited increases (mean increase 0.07 ± 0.15%). The group difference was significant ($P = 0.005$). The change in the CSF-to–plasma insulin ratio was inversely associated with both the change in body weight ($r = 0.66$, $P = 0.04$) and the change in relative adiposity ($r = 0.72$, $P = 0.02$).

Changes in CNS insulin uptake and clearance parameters. Representative examples of plasma and CSF insulin values and optimal curve fits to CSF insulin data obtained before and during high-fat feeding are shown in Fig. 2. Among dogs that did not gain FM, mean CSF insulin values increased more rapidly in response to the plasma insulin infusion during high-fat feeding compared with values obtained before high-fat feeding (Fig. 2). In contrast, in dogs that did gain FM, the mean CSF insulin values during high-fat feeding were similar to those at baseline, despite the increase in plasma insulin levels (Fig. 2).

Overall, the mean log-transformed value of the parameter indicating the efficiency of insulin uptake from plasma into the intermediate compartment and subsequently into CSF (k_1k_2) was not significantly different from baseline when measured during high-fat feeding ($P = 0.49$). However, in dogs that did not experience an increase in adiposity (Figs. 2*B* and 3*B*), k_1k_2 values were higher during high-fat feeding (the ratio of k_1k_2 during high-fat feeding to baseline k_1k_2 was 2.15) (Fig. 3*A*), and, despite the small sample size, this change was significant ($P = 0.04$ for log-transformed values). In contrast, log-transformed values of k_1k_2 tended to decrease in the subset of 7 animals that gained FM ($P = 0.07$) (Fig. 3*C*), so that the mean value of k_1k_2 during high-fat feeding was 53% of the baseline value (Figs. 2*B* and 3C).

A strong correlation was found between the percent change in k_1k_2 and the percent change in the CSF-to–plasma insulin ratio ($r = 0.84$, $P = 0.002$) (Fig. 4*A*), and this ratio was also inversely associated with the change in relative adiposity in the 10 animals ($r = 0.63$, $P = 0.05$). Similar inverse associations were found for the percent change in k_1k_2 vs. the percent change in body weight ($r = –0.69$, $P = 0.03$) and for the percent change in k_1k_2 vs. the change in relative adiposity ($r = –0.74$, $P = 0.015$) (Fig. 4*B*). There was also a significant inverse association between the percent change in k_1k_2 and the percent change in mean caloric intake based on caloric intake averaged over the 7 weeks of high-fat feeding relative to energy intake averaged over the final 2 weeks of chow feeding ($r = –0.63$, $P = 0.05$) (Fig. 4*C*).

In contrast, high-fat feeding was not associated with a change from baseline in the mean value of $(k_2 + k_3)$, the parameter associated with clearance of insulin from the intermediate compartment (baseline 1.31 ± 0.22 vs. 1.32 ± 0.24 × 10^{-2}/min at follow-up, $P = 0.97$), and the percent change in $(k_2 + k_3)$ was not significantly associated with the change in relative FM ($r = 0.32$, $P = 0.36$). Similarly, the mean value of the parameter for the clearance of insulin from CSF, k_4, was also unchanged during high-fat feeding (baseline 3.11 ± 0.66 vs. 2.93 ± 0.42 × 10^{-2}/min at follow-up, $P = 0.84$), despite a significant inverse correlation of the percent change in k_4 with the change in relative FM ($r = –0.82$, $P = 0.003$) and the percent change in body weight ($r = 0.75$, $P = 0.01$). However, of the 7 dogs that increased in adiposity, values of k_4 increased in 3 and decreased in the other 4 animals during high-fat feeding. Thus, unlike k_1k_2, the sign of changes of k_4 did not correspond to

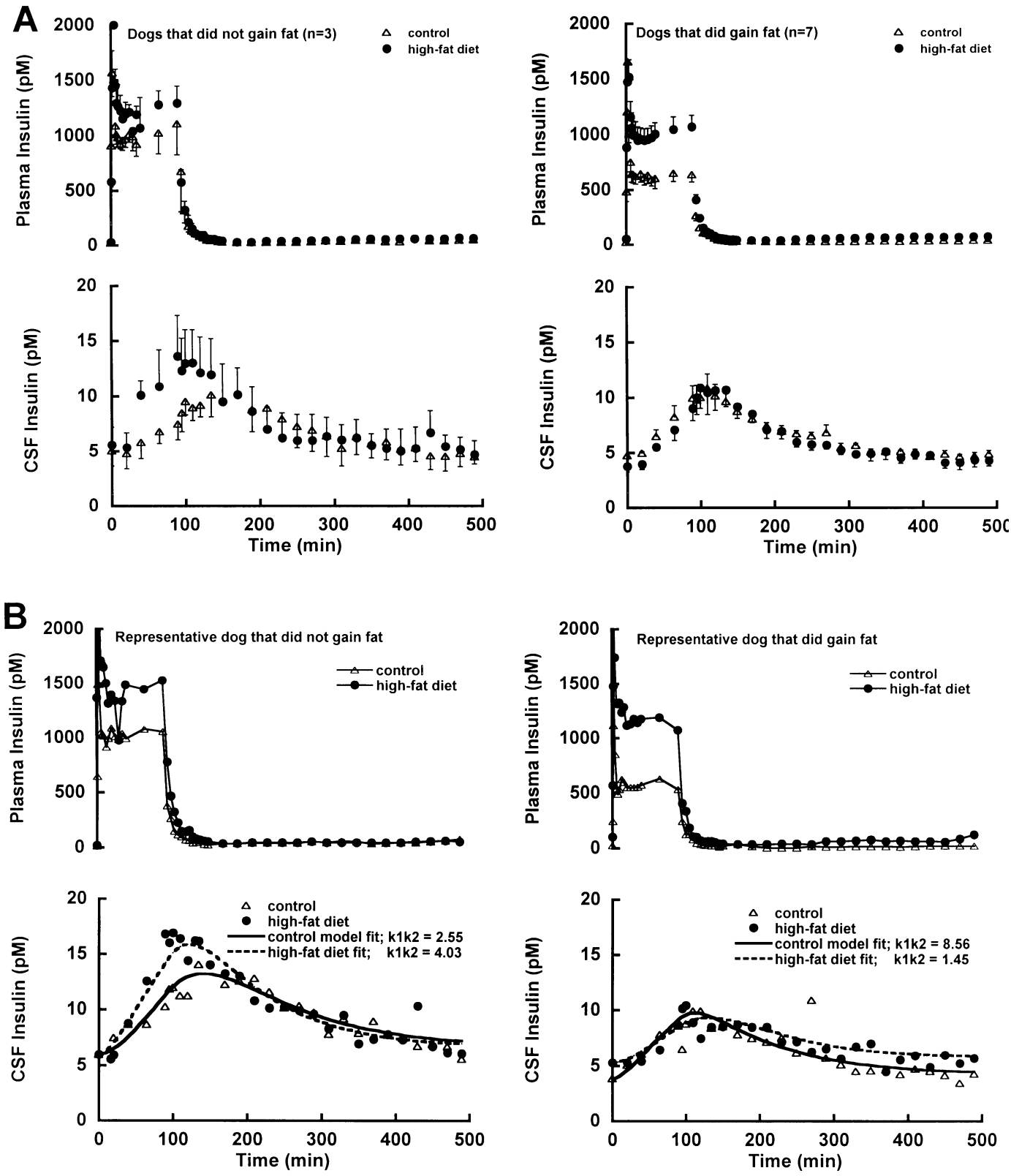

FIG. 2. ***A*****: Mean values of plasma and CSF insulin during CSF insulin uptake studies for dogs that did (right panels) and did not (left panels) gain fat.** ***B*****: Representative plasma and CSF insulin profiles for 1 dog that did (right panels) and 1 that did not (left panels) gain fat. Three-compartment model–fits corresponding to sets of parameter estimates, as described in Fig. 1, are shown as smooth curves through CSF insulin data. Units of k_1k_2 are 10^{-6} min^{-2}.**

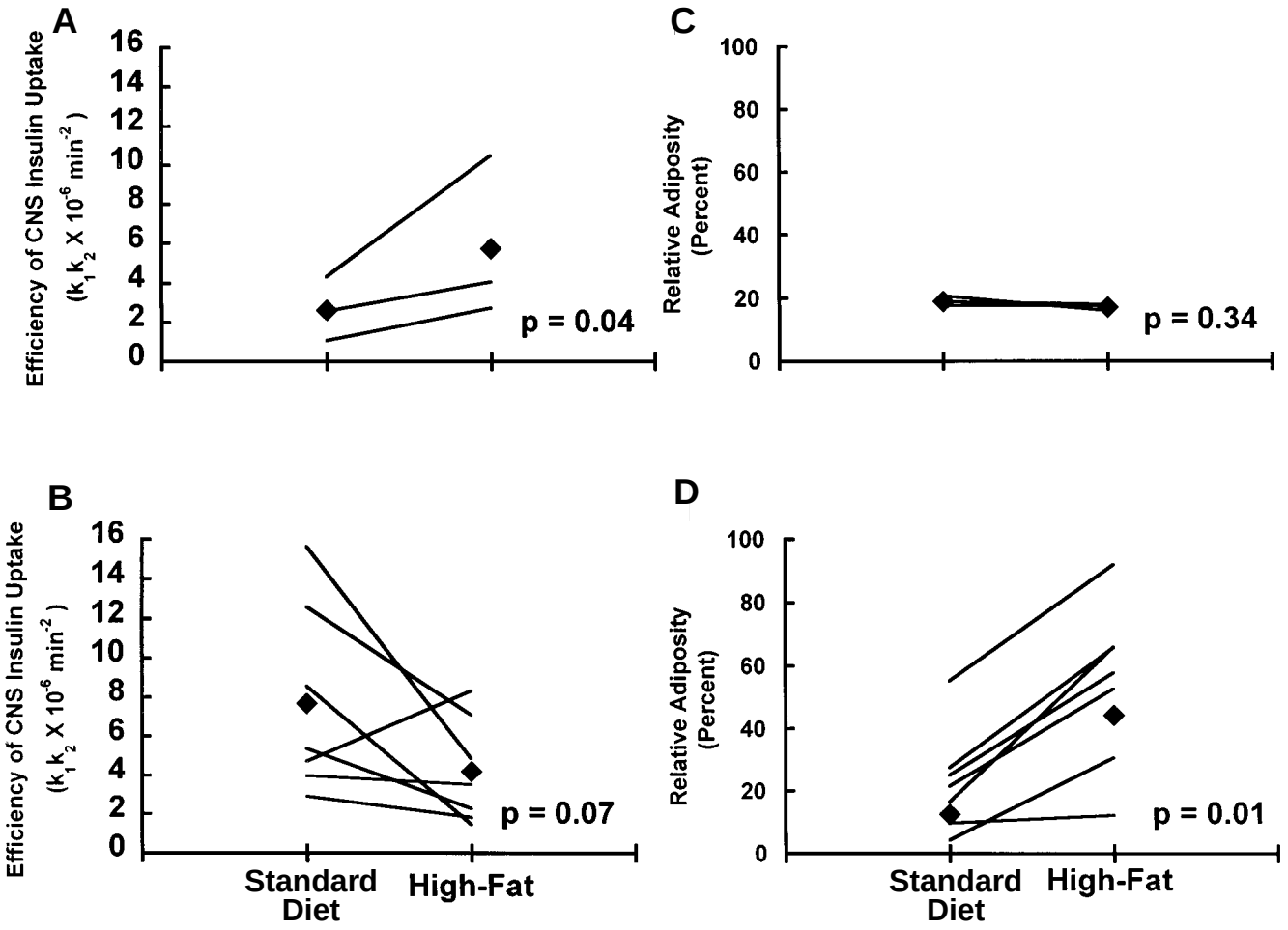

FIG. 3. Effects of high-fat feeding on k_1k_2 in relation to changes of relative adiposity (100 · FM/LBM). *A*: Changes of k_1k_2 for the 3 dogs that did not gain adiposity, as shown in panel *C*. *B*: Of the 7 dogs that gained adiposity (*D*), 6 exhibited decreases of k_1k_2.

changes of adiposity. Nonetheless, the change in k_4 was significantly associated with the change in k_1k_2 ($r = 0.73$, $P = 0.02$), and the percent change in k_4 was significantly associated with the percent change in k_1k_2 ($r = 0.84$, $P = 0.002$). Thus, high-fat feeding was associated with changes in the efficiency of CNS insulin uptake and clearance that were inversely related to changes of adiposity and energy intake, whereas changes of the ($k_2 + k_3$) transport parameter were not systematically related to changes of adiposity.

We also examined the relationship between changes in CNS insulin transport parameters and in the 24-h insulin AUC in the 9 dogs for which both of these variables were measured. There was a significant inverse association between the percent change of k_1k_2 and that of 24-h insulin AUC ($r = –0.68$, $P = 0.045$), although 5 of the 9 dogs had reduced values of k_1k_2 concomitant with reduced 24-h circulating insulin levels. There was also a significant positive association between the percent change in the 24-h insulin level and both the percent change in body weight ($r = 0.81$, $P = 0.008$) and the change in relative FM ($r = –0.74$, $P = 0.024$).

Changes in the 24-h CNS insulin index. A representative example of the effect of high-fat feeding to reduce the 24-h insulin profile is shown in Fig. 5. This effect contributed to a marked decrease in the overall CNS insulin delivery estimated as the CNS insulin index (see RESEARCH DESIGN AND METHODS), the mean value of which was reduced during high-fat feeding by 60% from 187.62 to 75.08 pmol ($P = 0.04$, and $P = 0.009$ for the decrease of the mean log-transformed value of the CNS insulin index) (Fig. 6*A*). Regression analysis revealed a significant inverse association of the percent change in the CNS insulin index with the percent change in body weight ($r = –0.71$, $P = 0.03$) (Fig. 6*B*). Thus, all dogs had decreases in the 24-h CNS insulin index, but the greatest relative decreases occurred in dogs that had the largest relative gains in body weight.

Relationship of CNS insulin uptake to S_I. No significant correlations were observed between S_I and any of the CNS insulin transport parameters at baseline or during high-fat feeding. The strongest relationship observed was between the percent change in S_I and the percent change in k_1k_2 ($r = 0.36$, $P = 0.34$).

DISCUSSION

The delivery of insulin into the brain is determined by the interaction between the circulating insulin concentration and the efficiency of CNS insulin uptake. Based on the hypothesis that insulin is an important negative feedback signal that acts on CNS-effector pathways that regulate adiposity (1,3,5), factors that alter circulating insulin levels, the efficiency of CNS insulin uptake, or both could modify the amount of energy stored in the form of adipose tissue. By using a compartmen-

tal-modeling method developed in our laboratory to quantify parameters for CNS insulin transport in dogs (11,24), we found that the degree of weight gain induced by a high-fat diet is associated with a proportionate decrease in the efficiency of CNS insulin uptake. This model-derived result was recapitulated with a non–model-based method using the CSF-to–plasma insulin ratio achieved during the CNS insulin infusion protocol. Of the 7 dogs that responded to high-fat feeding with increased adiposity, 6 had reductions in the efficiency of insulin transport from plasma into CSF, as quantified by k_1k_2, whereas values of this parameter increased in the 3 dogs that did not gain fat. Furthermore, 54% of the variance in the relative change of k_1k_2 during high-fat feeding was accounted for by changes in body adiposity. The observation that mean caloric intake during high-fat feeding was also inversely related to the change of k_1k_2 is consistent with the hypothesis that insulin acts in the brain to reduce food intake. Alternatively, the effect of high-fat feeding to cause weight gain may have impaired the CNS insulin uptake process. The major finding of these studies, therefore, is that the effect of a high-fat diet to cause obesity was associated with reduced CNS uptake efficiency of insulin, a hormone strongly implicated in the central control of energy balance.

Although 24-h insulin profiles declined uniformly during high-fat feeding (likely due to reduced carbohydrate stimulation of pancreatic β-cells [17]), insulin levels achieved during intravenous insulin infusion were 50% higher in this setting. This outcome likely reflects decreased insulin clearance from plasma, because the rate of insulin infusion was identical for each dog before and during the high-fat diet. Despite this increase of plasma insulin during the CNS insulin uptake study, the mean CSF insulin profile did not change among the 7 dogs that gained weight (Fig. 2). Consequently, the CSF-to–plasma insulin ratio among these animals decreased by 45%. By comparison, during high-fat feeding, CSF insulin increased more rapidly and reached higher values among the dogs that did not gain FM, despite plasma insulin levels that were comparable to those among the animals that did gain fat (Fig. 2*A*). Consequently, the CSF-to–plasma insulin ratio decreased during high-fat feeding and was inversely related to the change of adiposity across the entire group. Combined with the strong association between this ratio and k_1k_2 (Fig. 4*A*), our results support the model-derived parameter estimates obtained in this study and suggest that reduced insulin uptake efficiency accompanies weight gain induced by consumption of a high-fat diet.

Because plasma insulin levels were higher during the insulin infusion after high-fat feeding, and because CNS insulin uptake is saturable (6), the possibility that decreases of k_1k_2 associated with weight gain were due to saturation of the uptake mechanism can be considered. This possibility is unlikely for several reasons. First, it is incompatible with the findings of a previous study (6) that placed the K_m of the saturation curve at 4,450 pmol/l. Accordingly, the kinetics of this insulin uptake process predict that the rate of CNS insulin uptake should be a linear function of the plasma insulin level with no changes in k_1 until plasma insulin concentrations are greater than those observed in the current study. Consistent with this prediction, previous work using the same experimental model found that increasing plasma insulin ~3-fold from mean values of ~540 to ~1,660 pmol/l resulted in 3-fold increases of both the rate of rise and peak values of insulin in the CSF (33). The mean increase of plasma insulin detected in the current study (from 786 to 1,182 pmol/l) is therefore unlikely to explain the decrease of k_1k_2. This interpretation is strengthened by our finding that changes of k_1k_2 were not associated with changes of insulin in plasma (r = 0.3, P = 0.36), and that the 3 dogs that did not gain weight exhibited a mean increase of k_1k_2 of 120% despite a 21% increase of mean insulin. Thus, the changes we detected in k_1k_2 are unlikely to have resulted from saturation of the insulin uptake mechanism.

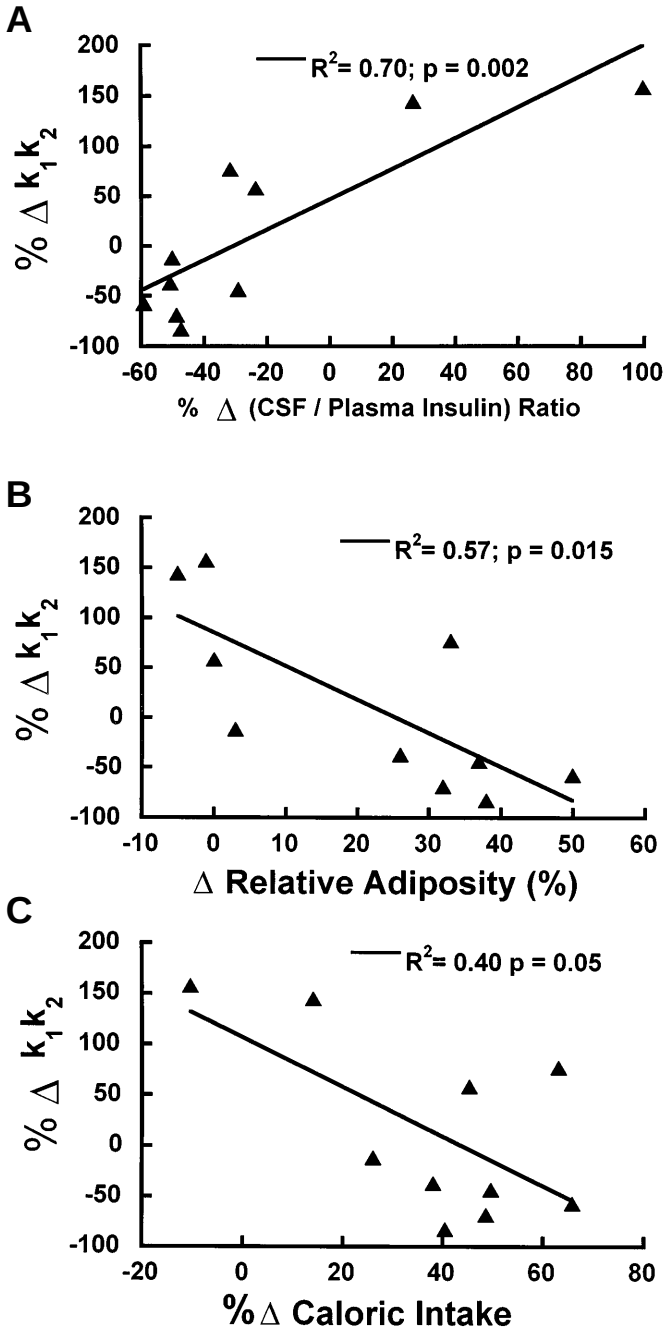

FIG. 4. Regression analyses of the associations of the percent change of k_1k_2 with the percent change in the CSF-to–plasma insulin ratio (*A*), the change in relative adiposity (*B*), and the percent change in caloric intake (*C*).

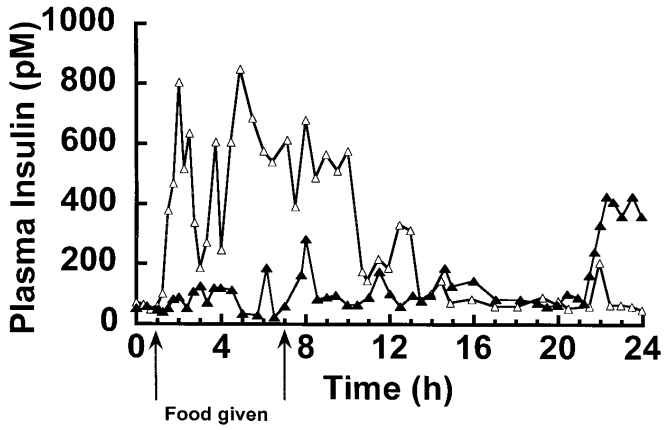

FIG. 5. A representative example of 24-h circulating insulin profiles obtained during standard-diet feeding (△) and during high-fat feeding (▲). During high-fat feeding, the time-weighted mean 24-h insulin level was significantly reduced from 254 ± 33 to 140 ± 22 pmol/l (17) (P = 0.004). The rise of plasma insulin in the representative dog during the latter part of the 24-h high-fat diet study was not representative of the dogs as a group (P = 0.25 for Student's t test of insulin levels in the last 2 h of 24-h monitoring period vs. the first 2 h).

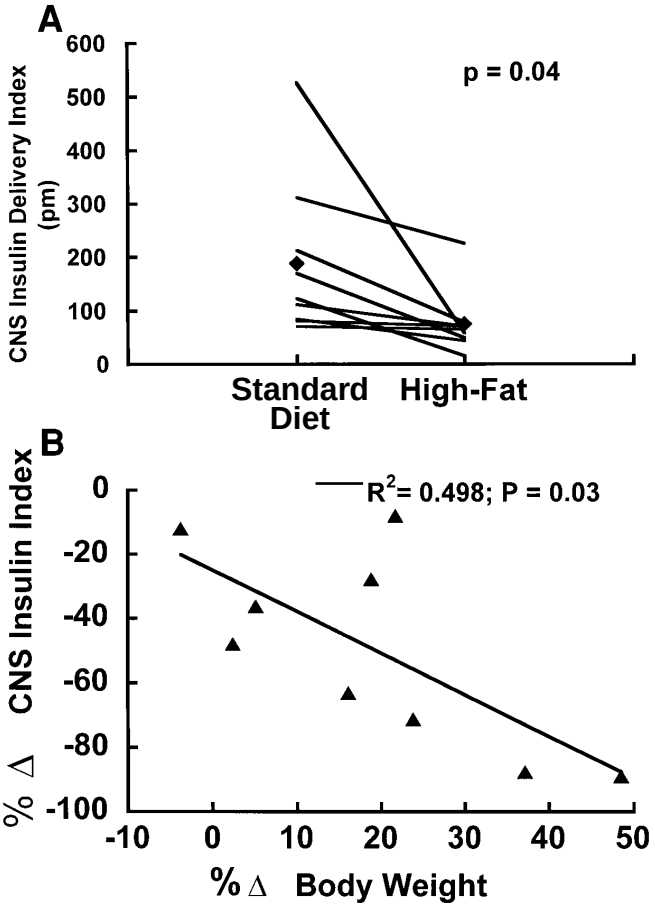

FIG. 6. *A*: CNS insulin levels measured as the CNS insulin index were all reduced after 7 weeks of high-fat feeding. *B*: Regression of the percent change in the CNS insulin index on the percent change in body weight.

Because the transport mechanism that underlies CNS insulin uptake is hypothesized to use the insulin receptor as the transendothelial carrier of insulin (8,10,34), our data are consistent with the hypothesis that becoming obese on a high-fat diet impairs this receptor-mediated process. The findings that rats with diet-induced obesity (35) or genetic obesity due to the *fa/fa* mutation (the fatty Zucker rat) (36) have disproportionately low CSF insulin concentrations relative to plasma levels and that brain capillary endothelial cells from obese *fa/fa* rats exhibit reduced insulin binding (37) provide additional support for this hypothesis. Thus, reduced CNS insulin delivery may be a feature of several different forms of obesity.

The hypothesized impairment of receptor-mediated insulin uptake across the BBB appears to have a counterpart in the periphery because insulin resistance associated with obesity is associated with reduced clearance of plasma insulin (38), a process dependent on insulin receptors expressed in the liver. Indeed, we observed a significant positive association between the relative changes of body weight and plasma insulin levels (r = 0.78, P = 0.008) achieved during the 90-min insulin infusion period, which supports a decrease in receptor-mediated insulin clearance from plasma.

Our study was not designed to determine whether the effect of high-fat feeding to increase food intake and body weight was responsible for or attributable to reduced efficiency of CNS insulin uptake. If reduced CNS insulin uptake efficiency was a direct early consequence of high-fat feeding that caused weight gain, then this relationship could have important implications for the pathogenesis of obesity induced by dietary factors. It would suggest that the ability of high-fat feeding to reduce the efficiency of CNS insulin uptake can lead to weight gain. This hypothesis, while untested, is in agreement with our observation that the animals that demonstrated an increase, rather than a decrease, of k_1k_2 did not gain weight on the high-fat diet. The alternative hypothesis, that reduced CNS insulin uptake efficiency is a consequence of weight gain, also has potentially important implications for energy homeostasis. Specifically, an effect of weight gain to diminish the CNS uptake efficiency of an adiposity signal implies that, for a given increase in its peripheral concentration (due to increased adiposity), the brain would be exposed to a proportionately smaller signal than that before the increase in weight. Thus, even if the fall in k_1k_2 observed in our study was caused by weight gain, such an effect may predispose to the defense of the elevated weight or even to further expansion of the adipose depot.

The decrease in CNS insulin delivery that we observed during high-fat feeding entailed both reduced CNS insulin uptake efficiency and reduced circulating insulin levels. When measured after 7 weeks on the high-fat diet, the mean 24-h plasma insulin profile was reduced by 44% relative to that measured during chow feeding (17), despite higher values obtained during the intravenous insulin infusion protocol (Fig. 5). Because weight gain was also associated with decreased CNS insulin uptake efficiency, CNS insulin levels estimated as the CNS insulin index decreased in proportion to weight gain. Thus, the dogs that exhibited the largest relative increases of body weight were those with the greatest relative decreases of estimated CNS insulin levels. It should be noted that 2 dogs that gained little weight nonetheless had substantial reductions of

the CNS insulin index (because circulating insulin levels were reduced), whereas 1 animal that did gain weight had only a minor reduction (Fig. 6). This variability is consistent with the hypothesis that factors additional to insulin participate in the negative feedback control of adiposity, and several of these may also be affected by changes in diet composition. For example, leptin (39) is an adiposity-related hormone that could affect the propensity for weight gain on a high-fat diet, especially in light of the recent human study showing that high-fat feeding reduced 24-h leptin profiles by 38% in comparison with equicaloric high-carbohydrate feeding (40).

The finding that the effect of high-fat feeding on k_1k_2 was inversely and significantly related to its effect on adiposity but not to its effect on S_I suggests that insulin sensitivity of peripheral tissues and the efficiency of brain insulin uptake are not regulated in parallel. Thus, the effect of a high-fat diet to impair insulin sensitivity in skeletal muscle and adipose tissue during high-fat feeding may involve mechanisms different from those that affect CNS insulin transport.

In summary, we have provided evidence that, in dogs, increased adiposity induced by high-fat feeding is associated with reduced CNS insulin delivery that results from reductions in both the efficiency of CNS insulin uptake and the circulating insulin level. These results suggest that, in some individuals, high-fat feeding may impair the CNS delivery of a circulating hormone involved in negative feedback control of energy balance, an acquired defect that could contribute to the pathogenesis and/or maintenance of weight gain associated with high-fat feeding.

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REFERENCES

1. Woods SC, Stein LJ, McKay LD, Porte D Jr: Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature* 282:503–505, 1979
2. Park C, Chavez M, Woods SC: IV T insulin decreases respiratory quotient in rats (Abstract). In *Abstracts of the Annual Meeting of the Society for Neuroscience, 1992*. p. 939
3. Schwartz MW, Figlewicz DP, Baskin DG, Woods SC, Porte D Jr: Insulin in the brain: a hormonal regulator of energy balance. *Endocr Rev* 13:387–414, 1992
4. Schwartz MW, Figlewicz DP, Baskin DG, Woods SC, Porte D Jr: Insulin and the central regulator of energy balance: update 1994. *Endocr Rev Monogr* 2: 109–113, 1994
5. Kaiyala KJ, Woods SC, Schwartz MW: New model for the regulation of energy balance by the central nervous system. *Am J Clin Nutr* 62 (Suppl. 5): 1123S–1134S, 1995
6. Baura G, Foster D, Porte D Jr, Kahn SE, Bergman RN, Cobelli C, Schwartz MW: Saturable transport of insulin from plasma into the central nervous system of dogs in vivo: a mechanism for regulated insulin delivery to the brain. *J Clin Invest* 92:1824–1830, 1993
7. King GL, Johnson SM: Receptor-mediated transport of insulin across endothelial cells. *Science* 227:1583–1586, 1985
8. Pardridge WM: Receptor-mediated peptide transport through the blood-brain barrier. *Endocr Rev* 7:314–330, 1986
9. Duffy KR, Pardridge WM: Blood-brain barrier transcytosis of insulin in developing rabbits. *Brain Res* 420:32–38, 1987
10. Wu D, Yang J, Pardridge WM: Drug targeting of a peptide radiopharmaceutical through the primate blood-brain barrier in vivo with a monoclonal antibody to the human insulin receptor. *J Clin Invest* 100:1804–1812, 1997
11. Baura G, Foster DM, Kaiyala K, Porte D Jr, Kahn SE, Schwartz MW: Insulin transport from plasma into the central nervous system is inhibited by dexamethasone in dogs. *Diabetes* 45:86–90, 1996
12. Van Heek M, Compton DS, France CF, Tedesco RP, Fawzi A, Graziano MP, Sybertz EJ, Strader CD, Davis HD Jr: Diet-induced obese mice develop peripheral, but not central, resistance to leptin. *J Clin Invest* 99:385–390, 1997
13. Ramsey JJ, Kemnitz JW, Colman RJ, Cunningham D, Swick AG: Different central and peripheral responses to leptin in rhesus monkeys: brain transport may be limited. *J Clin Endocrinol Metab* 83:3230–3235, 1998
14. Chen M, Bergman RN, Porte D Jr: Insulin resistance and β-cell dysfunction in aging: the importance of dietary carbohydrate. *J Clin Endocrinol Metab* 67:951–957, 1988
15. Storlien LH, James DE, Burleigh KM, Chisholm DJ, Kraegen EW: Fat feeding causes widespread in vivo insulin resistance, decreased energy expenditure, and obesity in rats. *Am J Physiol* 251:E576–E583, 1986
16. Storlein LH, Jenkins AB, Chisholm DJ: Influence of dietary fat composition on development of insulin resistance in rats. *Diabetes* 40:280–289, 1991
17. Kaiyala KJ, Prigeon RL, Kahn SE, Woods SC, Porte D Jr, Schwartz MW: Reduced β-cell function contributes to impaired glucose tolerance in dogs made obese by high-fat feeding. *Am J Physiol* 277:E659–E667, 1999
18. Levin BE, Hogan S, Sullivan AC: Initiation and perpetuation of obesity resistance in rats. *Am J Physiol* 256:R766–R771, 1989
19. Levin BE, Sullivan AC: Glucose-induced norepinephrine levels and obesity resistance. *Am J Physiol* 253:R475–R481, 1987
20. Rocchini AP, Moorehead C, Wentz E, Deremer S: Obesity-induced hypertension in the dog. *Hypertension* 9:III64–III68, 1987
21. Tucker LA, Kano MJ: Dietary fat and body fat: a multivariate study of 205 adult females. *Am J Clin Nutr* 56:616–622, 1992
22. Miller WC, Niederpruem MG, Wallace JP, Lindeman AK: Dietary fat, sugar, and fiber predict body fat content. *J Am Diet Assoc* 94:612–615, 1994
23. Prosperpi C, Sparti A, Scutz Y, DiVetta V, Milon H, Jequier E: Ad libitum intake of a high-carbohydrate or high-fat diet in young men: effects on nutrient balances. *Am J Clin Nutr* 66:539–545, 1997
24. Schwartz MW, Bergman RN, Kahn SE, Taborsky GJ Jr, Fisher LD, Sipols AJ, Woods SC, Steil GM, Porte D Jr: Evidence for uptake of plasma insulin into cerebrospinal fluid through an intermediate compartment in dogs. *J Clin Invest* 88:1272–1281, 1991
25. Wehberg K, West D, Kieswetter C, Granger J: Baroreflex sensitivity in the canine model of obesity-induced hypertension. *Am J Physiol* 259:R981–R985, 1990
26. Jebb SA, Elia M: Techniques for the measurement of body composition: a practical guide. *Int J Obes* 17:611–621, 1993
27. Widdowson EM, Dickerson JWT: Chemical composition of the body. In *Mineral Metabolism: An Advanced Treatise, Vol. 2. The Elements*. Comar CL, Bronner F, Eds. New York, Academic Press, 1964, p. 1–247
28. Beard JC, Bergman RN, Ward WK, Porte D Jr: The insulin sensitivity index in nondiabetic man: correlation between clamp-derived and IVGTT-derived values. *Diabetes* 35:362–369, 1986
29. Bergman RN, Ider YZ, Bowden CR, Cobelli C: Quantitative estimation of insulin sensitivity. *Am J Physiol* 236:E667–E677, 1979
30. Bergman RN: Toward physiological understanding of glucose tolerance. *Diabetes* 38:1512–1527, 1989
31. Bergman RN, Finegood DT, Ader M: Assessment of insulin sensitivity in vivo. *Endocr Rev* 6:45–86, 1987
32. Berman M, Weiss MF: *The SAAM Manual*. Washington, DC, U.S. Government Printing Office, 1978
33. Schwartz MW, Sipols AJ, Kahn SE, Lattemann DP, Taborsky GJ Jr, Bergman RN, Woods SC, Porte D Jr: Kinetics and specificity of insulin uptake from plasma into cerebrospinal fluid. *Am J Physiol* 259:E378–E383, 1990
34. Frank HJ, Pardridge WM: A direct in vitro demonstration of insulin binding to isolated brain microvessels. *Diabetes* 30:757–761, 1981
35. Israel PA, Park CR, Schwartz MW, Green PK, Sipols AJ, Woods SC, Porte D Jr, Figewicz DP: Effect of diet-induced obesity and experimental hyperinsulinemia on insulin uptake into CSF of the rat. *Brain Res Bull* 30:571–575, 1993
36. Stein LJ, Dorsa DM, Baskin DG, Figlewicz DP, Porte D Jr, Woods SC: Reduced effect of experimental peripheral hyperinsulinemia to elevate cerebrospinal fluid insulin concentrations of obese Zucker rats. *Endocrinology* 121:1611–1615, 1987
37. Schwartz MW, Figlewicz DP, Kahn SE, Baskin DG, Porte D Jr, Greenwood MRC: Insulin binding to brain capillaries is reduced in genetically obese hyperinsulinemic Zucker rats. *Peptides* 11:467–472, 1990
38. Jones C, Abbasi F, Carantoni M, Polonsky K, Reaven G: Roles of insulin resistance and obesity in regulation of plasma insulin concentrations. *Am J Physiol* 278:E501–E508, 2000
39. Zhang Y, Proenca R, Maffie M, Barone M, Leopold L, Friedman JM: Positional cloning of the mouse obese gene and its human homologue. *Nature* 372:425–432, 1994
40. Havel PJ, Townsend R, Chaump L, Teff K: High-fat meals reduce 24-h circulating leptin concentrations in women. *Diabetes* 48:334–341, 1999