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D. P. Figlewicz

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Central insulin and macronutrient intake in the rat

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Chavez, Mark, Christine A. Riedy, Gertjan Van Dijk, and Stephen C. Woods. Central insulin and macronutrient intake in the rat. *Am. J. Physiol.* 271 (*Regulatory Integrative Comp. Physiol.* 40): R727–R731, 1996.—When rats are maintained on a standard laboratory diet, the infusion of low doses of insulin into the cerebroventricular system causes a reduction of food intake and body weight. It was recently reported that, if rats are maintained on a high-fat diet (56% calories as fat), they are insensitive to this action of insulin. To investigate further the effect of dietary composition on responsiveness to central insulin, we carried out two experiments. In *experiment 1*, rats were maintained on one of four equalcaloric diets (providing 7, 22, 39, or 54% of calories as fat) before and during a 6-day third-ventricular infusion (i3vt) of insulin (10 mU/day) or saline. Rats consuming 7 or 22% of calories as fat had a significant reduction of both food intake (-17.2 ± 2.9 and -14.6 ± 3.3 g, respectively) and body weight (-50 ± 5 and -41 ± 5 g, respectively) from baseline over the insulin-infusion period. Rats consuming 39 or 54% calories as fat did not reliably alter food intake (-4.0 ± 3.9 and -1.9 ± 3.7 g, respectively) or body weight (-10 ± 6 and -6 ± 4 g, respectively) in response to i3vt of insulin. In *experiment 2*, rats were offered a choice of three macronutrients (carbohydrates, fats, and proteins) in separate jars in their home cages. After they had adapted to the diets, they were infused i3vt with insulin or saline. Insulin caused a significant reduction of body weight relative to saline-infused controls (body wt: -23.1 ± 4 g) and a reduction in food intake that was selective for dietary fat. These data suggest that the effects of central insulin administration are highly dependent on the macronutrient content of the diet as well as the ability of rats to select their own diets.

body weight regulation; food intake; macronutrient diet; high-fat diet

THERE IS COMPELLING EVIDENCE that insulin provides a negative feedback signal to the brain in the control of food intake and the regulation of body weight (23). Insulin, secreted from the pancreas in direct proportion to adiposity (4), is passed through the blood-brain barrier via a receptor-mediated, saturable transport system, where it gains access to neurons with specific insulin receptors (6, 26, 28). Some of the highest concentrations of insulin receptor-expressing neurons are found in areas of the brain important in the control of food intake and energy metabolism (5). Many laboratories utilizing several mammalian species have reported that when low doses of insulin are infused into the brain, animals reduce their food intake and lose weight (8, 9, 17, 18, 20, 22, 34). The phenomenon is dose dependent, with larger doses of insulin resulting in an increased response (8, 22, 34). Recent studies from our laboratory indicate that the reduction in food intake and body weight after infusion of insulin into the brain is not secondary to nonspecific effects (i.e., malaise or incapacitation). Instead, insulin entering the central

nervous system (CNS) may represent a natural feedback system important in the regulation of energy balance (9, 10).

Little is known about possible interactions between central insulin infusion and dietary nutrient content. For most of the previous experiments in which insulin was infused into the CNS of rats, the animals were maintained on standard laboratory chow, a diet containing a relatively high amount of carbohydrate (CHO) and a low amount of fat. In the only published experiment to consider the effect of diet composition, Arase et al. (3) reported that rats maintained on a diet relatively high in CHO and low in fat respond to third-ventricular infusions (i3vt) of insulin by reducing their food intake and body weight. Conversely, rats maintained on a diet relatively high in fat and low in CHO do not change their intake in response to i3vt of insulin. To investigate this observation further, we carried out two experiments exploring the relationship between dietary nutrient content and the central infusion of insulin. In the first experiment, we replicated and extended the findings of Arase et al. (3) by maintaining rats on one of four diets varying systematically in their relative fat and CHO contents [7, 22, 39, and 54%, respectively, in the present study vs. 10 and 62% in the study by Arase et al. (3)] during i3vt infusion of insulin or vehicle. In a second experiment, the effect of i3vt insulin was determined in rats offered a choice of pure macronutrients.

METHODS

General Methods

Male Long-Evans rats obtained from the vivarium maintained by the Department of Psychology, University of Washington, were individually housed in hanging stainless steel cages in a temperature- (21–24°C) and light-controlled (12:12-h light-dark) room. The rats had free access to water and food throughout the experiment. In both experiments, the rats were fed pelleted chow before receiving the experimental diet.

All rats received third-ventricular cannulas before being given the experimental diets. As described previously (9), rats were anesthetized (3 ml/kg ip) with Equithesin (42.5 mg chloral hydrate, 95.7 mg pentobarbital sodium, and 21.3 mg magnesium sulfate solubilized in 1 ml containing 44.3% propylene glycol, 11.5% ethyl alcohol, and 44.2% water), and gentamycin (0.2 ml im) was administered prophylactically in a single dose. Then rats were placed in a stereotaxic instrument, and the dura was exposed by a small (2 × 2 mm) craniotomy. The tip of a vertically mounted 22-gauge stainless steel guide cannula was lowered 7.8 mm ventral to the dura at a coordinate 2.4 mm posterior to bregma and directly on midline, with lambda and bregma having the same vertical coordinate. The midsagittal sinus was briefly displaced laterally while the guide cannula was lowered. The cannula system was fixed in place by use of dental acrylic and anchor screws. Each guide cannula was fitted with a 26-gauge

Table 1. *Composition of diets in experiment 1*

Constituents	Diet			
	7% Fat	22% Fat	39% Fat	54% Fat
Casein	13.0	13.0	13.0	13.0
Corn starch	76.7	67.6	46.0	29.7
Soybean oil	3.3	3.4	3.4	3.5
Beef tallow		2.7	9.4	13
Lard		4.8	5.2	7.5
Mineral mixture	4.0	4.0	4.0	4.0
Vitamin mixture	3.0	3.0	3.0	3.0
Methyl cellulose		1.5	16.0	26.3
1% DL-Methionine				

Values are expressed as grams per 100 g. All constituents were obtained from ICN Nutritional Biochemicals (Cleveland, OH).

obturator that extended 0.5 mm beyond the tip of the guide cannula. Verification of cannula placement was performed several days later by monitoring water consumption in non-water-deprived rats after a 1- μ l i3vt injection of angiotensin II (20 ng/ μ l). Animals that did not consume at least 5 ml of water within 1 h were excluded from the experiment. All operated rats in *experiment 2* met this criterion, and two rats in *experiment 1* were excluded.

At the beginning of the experiments, each rat was briefly anesthetized with halothane. The obturator was removed and replaced with a 26-gauge infusion cannula that extended 1.0 mm beyond the tip of the guide cannula. The infusion cannula was attached with polyethylene tubing to a 14-day osmotic minipump (Alzet, 2002) filled to deliver the experimental solutions at a rate of 0.5 μ l/h. The pumps and tubing were then implanted subcutaneously into the interscapular pocket.

Protocols

Experiment 1. At the end of at least 10 days of recuperation from the i3vt cannulation, the animals weighed an average of 317 ± 4 g ($n = 72$). Following this period, the animals were ranked by body weight, and rats in each cohort of four were randomly assigned to one of the weight-matched groups. The four groups (18/group) were given nutritionally complete diets providing 54, 39, 22, or 7% of total calories as fat (Table 1). The diets were derived from the recipes of Langhans and Scharrer (15). Protein content was constant for all diets (12.5–13.9% of total calories), and all diets provided 3.45–3.83 kcal/g. The rats were maintained on their respective diets for 2 wk before the minipumps were implanted and the infusions begun. Animals in each dietary condition were then ranked by body weight, and the members of each pair were assigned to one of two weight-matched subgroups (i.e., making a total of 8 groups, 9 animals/group), one receiving i3vt insulin (10 mU/day regular porcine insulin, Lilly, 100 mU/ml) dissolved in saline, and the other receiving saline. Food intake and body weight were measured daily for 1 wk before the infusion period and for the final 4 days of the 6-day infusion period.

Experiment 2. At the end of at least 10 days of recuperation from i3vt surgery, the animals weighed an average of 390 ± 4 g ($n = 20$). At this time, pelleted chow was removed, and rats were given ad libitum access to three pure macronutrient sources. The diets (CHO = 3.7 kcal/g, protein = 3.7 kcal/g, and fat = 7.8 kcal/g) were available in three separate jars within the home cage. Each jar was weighed daily, and its location was rotated to avoid biases resulting from place preferences. The precise diet formulas were taken from Tepper and Kanarek (30). The principal sources of CHO, fat, and protein were corn starch, hydrogenated vegetable oil, and

casein, respectively. Each macronutrient was supplemented equally with requisite vitamins and minerals. At the beginning of the infusion period, the animals were assigned to one of two groups (10 rats/group) with approximately equivalent relative consumptions of the different macronutrient sources (see Table 3). One group received i3vt insulin (6 mU/day regular porcine insulin, Lilly, 100 mU/ml) dissolved in saline, and the other group received saline. Food intake and body weight were measured daily for 1 wk before and for 6 days during the infusion period. As in *experiment 1*, baseline for each rat was calculated as the mean intake (in kcal) of each macronutrient over the 2 days preceding the beginning of the infusion period, and infusion period values were based on the final 4 days of the 6-day infusion period.

For both *experiments 1* and *2*, changes in food intake and body weight over the first 2 infusion days were not included in the analyses because animals in all groups had variable food intake and body weight because of the implantation surgery of injectors and minipumps.

Analyses of Data

All data are presented as means \pm SE. In *experiment 1*, a three-way analysis of variance (ANOVA) with one repeated measure over time (i.e., baseline and infusion period) was used for the analysis of food intake and body weight data. For comparisons of individual means, *t*-tests using Bonferroni's correction were applied. In *experiment 2*, a multivariate analysis of variance (MANOVA) with repeated measures was used to assess differences among macronutrient sources (CHO, fat, protein), drug (insulin, vehicle), and time (baseline, infusion period). Diet and time were designated as repeated-measures variables. Between- and within-subjects comparisons of individual means were made across the same macronutrient sources (i.e., fat vs. fat), but not across different macronutrient sources (i.e., fat vs. protein). Post hoc *t*-tests with Bonferroni's correction were applied for this analysis. Analyses of body weights were made using *t*-tests with α set at 0.01.

RESULTS

Experiment 1

Food intake and body weight data are presented in Table 2. ANOVA revealed that changes in food intake,

Table 2. *Food intake and body weight of rats in experiment 1 during the baseline period and during infusion of either insulin (10 mU/day) or saline*

	Food Intake, kcal		Body Weight, g	
	Baseline	Infusion	Baseline	Infusion
i3vt ins				
54% fat	79.1 \pm 4.1	77.2 \pm 4.2	339 \pm 3	334 \pm 5
39% fat	83.4 \pm 3.2	79.4 \pm 3.7	347 \pm 5	340 \pm 4
22% fat	75.8 \pm 5.6	61.2 \pm 3.3*	333 \pm 4	292 \pm 4*
7% fat	81.0 \pm 3.9	63.8 \pm 4.7*	349 \pm 4	299 \pm 7*
i3vt sal				
54% fat	76.9 \pm 3.8	74.8 \pm 4.9	335 \pm 6	330 \pm 5
39% fat	86.0 \pm 3.2	82.3 \pm 3.5	343 \pm 5	334 \pm 4
22% fat	81.8 \pm 4.9	78.4 \pm 4.6	334 \pm 4	329 \pm 5
7% fat	81.9 \pm 3.1	79.7 \pm 3.5	346 \pm 5	339 \pm 4

Values are means \pm SE. Baseline values are averages of the final 2 days of the baseline period. Infusion values are averages of the final 4 days of the 6-day infusion period. i3vt ins, 3rd ventricular infusion of insulin; i3vt sal, 3rd ventricular infusion of saline. * $P < 0.01$.

Table 3. Food intake of animals in experiment 2 during the baseline period or during intraventricular infusion of either insulin (6 mU/day) or saline

Diets	i3vt ins		i3vt sal	
	Baseline	Infusion	Baseline	Infusion
CHO	32.1 ± 4.1	33.6 ± 3.7	33.8 ± 4.1	32.8 ± 3.4
Protein	22.9 ± 3.4	21.8 ± 3.2	24.4 ± 5.8	23.2 ± 5.4
Fat	41.8 ± 4.3	21.5 ± 5.1*	38.0 ± 3.8	36.2 ± 4.4

Values are means ± SE in kilocalories. Baseline values are averages of the final 2 days of the baseline period. Infusion values are averages of the final 4 days of the 6-day infusion period. CHO, carbohydrate diet. * $P < 0.01$.

when averaged over the final 4 days of the infusion, were significantly different for both diet ($F_{3,64} = 3.16$, $P < 0.05$) and infusion ($F_{1,64} = 6.41$, $P < 0.01$). The repeated-measure variable "time" was significant ($F_{1,64} = 43.6$, $P < 0.01$), as were the interactions infusion × time ($F_{1,64} = 24.3$, $P < 0.01$), diet × time ($F_{3,64} = 3.5$, $P < 0.02$), and infusion × diet × time ($F_{3,64} = 12.9$, $P < 0.01$). There was a significant reduction in food intake for the insulin-treated animals consuming the 7% ($t_{64} = 8.8$, $P < 0.01$) and 22% fat diets ($t_{64} = 7.5$, $P < 0.01$) relative to their baseline intakes. There was also a significant reduction in food intake of rats infused with insulin compared with saline-treated rats consuming the 7% ($t_{64} = 10.2$, $P < 0.01$) and 22% ($t_{64} = 8.9$, $P < 0.01$) fat diets. No other comparisons were significant.

ANOVA revealed that body weight of animals was significantly affected by diet ($F_{3,64} = 9.4$, $P < 0.01$), but not by infusion ($F_{1,64} = 3.7$, $P = \text{NS}$). The interaction between these variables was also significant ($F_{3,64} = 3.6$, $P < 0.05$). The repeated measure variable "time" was significant ($F_{1,64} = 152.9$, $P < 0.01$), as was its interaction with infusion (infusion × time: $F_{1,64} = 50.8$, $P < 0.01$) and diet (dietary group × time: $F_{3,64} = 16.5$, $P < 0.000$). Additionally, the interaction of all three variables (time × infusion × diet: $F_{3,64} = 14.9$, $P < 0.01$) was significant. There was a significant reduction in body weight of insulin-infused animals consuming 7% ($t_{64} = 6.7$, $P < 0.01$) and 22% ($t_{64} = 10.8$, $P < 0.01$) fat compared with their baseline body weights. Infusion of insulin also produced a significant reduction in body weight compared with the vehicle-infused controls of rats fed the 7% ($t_{64} = 10.5$, $P < 0.01$) and 22% fat diets ($t_{64} = 9.7$, $P < 0.01$). No other comparisons were significant.

Experiment 2

Food intakes during baseline and infusion-period conditions are presented in Table 3. A MANOVA with repeated measures was used to assess differences among diet (CHO, protein, and fat), infusion (insulin and vehicle), and time (baseline and infusion period). The between-subjects effect of infusion was not significant ($F_{1,18} = 0.52$, $P = \text{NS}$). The main effects for diet ($F_{2,36} = 5.62$, $P < 0.01$) and time ($F_{1,18} = 7.5$, $P < 0.01$) were significant. The variable "time" interacted significantly

with diet (diet × time: $F_{2,36} = 10.0$, $P < 0.01$) and infusion (infusion × time: $F_{1,18} = 18.1$, $P < 0.01$), revealing that total caloric intake was differentially changed by the insulin infusion. In addition, the interaction of all three variables (infusion × time × diet: $F_{2,36} = 16.3$, $P < 0.01$) was significant. The interaction of infusion and diet was not significant ($F_{2,36} = 0.17$, $P = \text{NS}$). Comparisons of individual means revealed a significant reduction of fat intake by animals treated with insulin compared with their own baseline intake ($t_{36} = 10.6$, $P < 0.01$), but not for the other macronutrients. Fat consumption was also significantly reduced in the insulin-infused rats relative to the vehicle-infused controls ($t_{36} = 7.5$, $P < 0.01$) during the infusion period. All other comparisons were not significant.

Before the infusions, body weights of animals in the insulin- and vehicle-infused groups were not significantly different (387.3 ± 6.6 vs. 382.6 ± 6.3 ; $t_{18} = 0.98$, $P = \text{NS}$). At the end of the infusion period, body weights of the insulin-infused rats were significantly lower ($t_{18} = 8.0$, $P < 0.001$) than those of the vehicle-treated controls (355.6 ± 5.7 vs. 378.7 ± 6.8 g).

DISCUSSION

The results of the present experiments demonstrate that the effects of i3vt insulin administration on food intake and body weight can be modified with alterations in dietary nutrient composition. *Experiment 1* revealed that central insulin loses its ability to reduce food intake and body weight when a certain amount of fat is consumed. *Experiment 2* demonstrated that when animals are able to compose their diet from separate macronutrient sources (i.e., CHO, fat, and protein), they reduce total intake by selectively reducing their intake of fat in response to central insulin administration, and this is concomitant with a reduction in body weight.

The results of *experiment 1* are in agreement with a report by Arase et al. (3), who used a more limited series of diets (two rather than four). That group interpreted their finding to indicate that elevated fatty acid oxidation caused by consumption of a high fat diet causes the brain to become less sensitive to insulin. The results from *experiment 1* are not inconsistent with this interpretation, and there are several related reports that support the same conclusion. First, when rats are fasted, they become increasingly lipolytic as they rely to a greater extent on stored fat as a fuel source. We have previously found that fasted rats have a blunted anorexic response to intraventricular insulin relative to free-feeding controls (9). Second, Plata-Salamán et al. (20) reported that acute intraventricular insulin administration was relatively ineffective in reducing food intake during the light compared with the dark in 24-h food-deprived rats. This means that central insulin was less effective in reducing food intake during times when rats are normally oxidizing more fat (i.e., when they are lipolytic) relative to periods when they are relatively lipogenic. Third, the yellow-bellied marmot, a mammal that hibernates in the winter, reduces its food intake in response to intraventricular insulin

administration during the summer, when its metabolic status is predominately lipogenic (14). Conversely, marmots become insensitive to the effects of central insulin during the winter, when their metabolic status is primarily lipolytic (13). Taken together, the data in the literature combined with the data from *experiment 1* suggest that insulin is less efficacious at reducing food intake when animals are relying on relatively more fat to fuel their metabolism.

Caution must be exercised when using diet selection paradigms. A number of reports have demonstrated that many factors affect dietary self-selection, including prior preferences, feeding stimuli, and type of diet presented (21, 33). In *experiment 2*, however, when rats were allowed to compose their individual diets from separate macronutrient sources, the outcome was unambiguous. Rats receiving central insulin selectively reduced their intake of dietary fat compared with their preinfusion baseline intakes and with those of saline-infused controls (cf. Table 3). These results are consistent with data of D. A. VanderWeele (personal communication), who found that rats allowed to self-select their own diet from three macronutrient sources preferentially reduced their intake of fat in response to a chronic subcutaneous insulin infusion at a dose that did not produce hypoglycemia. Several reports suggest that when insulin levels are experimentally lowered either by food restriction or streptozotocin-induced diabetes mellitus, rats preferentially increase their consumption of fat (2, 7, 16, 19, 25, 30, 31); however, conflicting results have been reported for the food-deprivation condition (33). Hence, one conclusion that can be made is that when rats have a choice, dietary fat intake varies inversely and dietary CHO and protein do not vary with experimentally induced elevations of insulin in the brain.

There is both empirical and theoretical support for the different results of these two experiments. Taken together, however, the results and their interpretations seem to be conflicting at two points. First, in *experiment 2*, the percentage of calories consumed as fat by the animals before the start of the insulin infusions was ~43%. Compared with the fat intakes of rats in the different groups in *experiment 1*, this would be considered a relatively high-fat diet and the rats might have been predicted to be insensitive to insulin's anorexigenic effect (i.e., rats given no choice and consuming diets containing 39 or 54% calories as fat were unresponsive to i3vt insulin). However, the rats in *experiment 2* did reduce food intake and body weight in response to i3vt insulin. Second, in *experiment 1*, rats consuming the diets containing 7 or 22% calories as fat responded to central insulin infusion by reducing food intake and body weight, but rats on the relatively high-fat/low-CHO diet did not. Hence insulin was effective only when it caused a relatively greater reduction of dietary CHO than of dietary fat. One prediction from *experiment 1* pertinent to the interpretation of *experiment 2* might therefore have been that, if rats had a selective

reduction of any macronutrient, it would have been CHO.

An explanation that is consistent with both experiments is based on the observation of Plata-Salamán et al. (20) that insulin is not effective at all times of the daily cycle, i.e., i3vt insulin is more effective at reducing food intake in the dark phase than it is in the light phase. It may in fact be the case that insulin has a limited temporal window during the dark phase when it can affect food intake. It is known that rats allowed to select macronutrients or enriched diets consume relatively large amounts of CHO at dusk, whereas fat is mainly consumed at dawn (27, 29). Thus, assuming the rats in *experiment 2* were selecting macronutrients in this same pattern, it may be that i3vt insulin was most efficacious in causing a reduction in food intake at the dawn phase, i.e., when fat consumption predominated. This possibility is in agreement with the observation that the level of plasma corticosterone [which may reduce insulin sensitivity in a number of brain areas involved in the control of feeding (11)] is relatively low at dawn compared with the levels observed at dusk (1, 12). On the other hand, any tendency of insulin to cause reductions in CHO or protein consumption may be opposed by peak levels of plasma corticosterone at dusk, when animals are most likely to consume CHO and proteins (1, 12).

Although different fat sources were used in *experiments 1* and *2* (in *experiment 1*, the fat sources were beef tallow, soybean oil, and lard, whereas in *experiment 2* the fat sources were vegetable shortening and corn oil), this difference is unlikely to account for the apparent discrepancy. In the Arase et al. (3) study, vegetable shortening and corn oil were the sources of fat. Thus comparable paradigms with different fat sources [Arase et al. (3) and our *experiment 2*] make it unlikely that the current results obtained in *experiment 2* are due to the use of the particular fat sources. Parenthetically, differences in the amount of protein are also unlikely explanations. The protein level in *experiment 1* was lower than that used in the Arase et al. (3) study (i.e., 12.5–13.9% vs. 20%). However, the fundamental findings and conclusions of the two experiments are in agreement, and the rats in all of the conditions grew during the baseline period. In fact, the rats in *experiment 1* gained an average of 25.5 g of weight on the experimental diets during the 2-wk interval before the infusions, implying that the protein content of the diets was sufficient for normal growth.

In summary, a unifying interpretation of the results of *experiments 1* and *2* is that there is a window in the late dark phase in which endogenous or exogenously administered insulin is able to reduce food intake. In *experiment 1*, the systematically increased fat-to-CHO ratio in the diet pushed the bulk of overnight food intake toward the earlier dark phase when insulin is less efficacious at suppressing food intake. In *experiment 2*, the window in which insulin suppresses food intake coincides with the normal elevated consumption of fat.

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