Energy expenditure and diet-induced thermogenesis in presence and absence of hyperphagia induced by insulin

A. G. DULLOO AND L. GIRARDIER
Department of Physiology, Centre Medical Universitaire, University of Geneva, 1211 Geneva 4, Switzerland

DULLOO, A. G., AND L. GIRARDIER. *Energy expenditure and diet-induced thermogenesis in presence and absence of hyperphagia induced by insulin.* Am. J. Physiol. 257 (Regulatory Integrative Comp. Physiol. 26): R717–R725, 1989.—The influence of experimental hyperinsulinemia on energy intake, energy expenditure, and body composition was investigated in rats treated chronically with high doses of insulin. Energy balance studies, each of 2-wk duration, were conducted with two different long-acting insulins (Protamine and Monotard), administered in the morning (IM), the late afternoon (IA), or both (IMA) and in animals of three different ages, namely in 4-, 8-, and 12-wk-old rats. The results indicate that the level of hyperphagia induced by insulin was markedly influenced by the type of long-acting insulin ($P < 0.001$; Protamine > Monotard), by age ($P < 0.001$; 12 > 8 > 4 wk), as well as by the timing of insulin administration ($P < 0.002$, IMA > IM or IA). Body protein deposition was unaltered, but body fat and energy expenditure increased in parallel to the level of hyperphagia. Regression analysis shows a strong linear correlation ($r = 0.963$) between the change in energy expenditure and the change in energy intake in response to insulin and indicates that ~50% of the excess calories consumed was dissipated as heat. In the absence of hyperphagia, however, insulin administration had no effect on energy expenditure nor on energy partitioning. Similarly, the influence of altered meal pattern, induced by administering insulin at different times of the day, was also found to have no impact on energy expenditure. The current investigations therefore refute the notion that high doses of insulin via hyperinsulinemia and/or altered meal pattern have an inhibitory influence on whole body thermogenesis. In contrast, our data demonstrate that the adaptive phenomenon that tends to minimize the accumulation of excess caloric intake, i.e., diet-induced thermogenesis, persists in the hyperinsulinemic state.

energy balance; obesity; hyperinsulinemia

METABOLIC RATE is often reported to be elevated in response to increased food intake. This phenomenon, generally referred to as diet-induced thermogenesis (DIT), serves as a buffer against the accumulation of body fat (21, 28) and is to a large extent under the control of the sympathetic nervous system (19). While the signal(s) relating energy intake to the central activation of sympathetic activity and DIT remains highly speculative, a role for insulin-mediated glucose metabolism has been proposed (18, 23).

However, this notion that insulin plays an active role in the activation of DIT seems contradictory to its well-established function as an "energy storage" hormone and to the general association between hyperinsulinemia and obesity. In fact, insulin administration has been reported to slow weight loss in type II diabetic patients on a low caloric intake (14) and also to increase the efficiency of body weight gain in rats in which hyperphagia was apparently absent (2, 20). These findings, which have been interpreted as suggestive for an inhibitory effect of insulin on thermogenesis, are not supported by other reports, indicating that exogenous insulin either stimulates thermogenesis acutely or had no chronic influence on energy balance (1, 24).

Such conflicting results attributing stimulatory, inhibitory, or a mere lack of effect of insulin on energy expenditure are difficult to reconcile in the face of widely different experimental designs. In addition to differences in the dose of insulin administered, the age of the animals, and the duration of study, the lack of data on long-term energy expenditure and/or body composition limits the interpretation of most previous studies.

To gain further insight into the influence of hyperinsulinemia on energy expenditure, we have conducted a series of energy balance studies in rats receiving relatively high doses of exogenous insulin. Our main objectives were twofold: *1*) to assess whether, under conditions of experimental hyperinsulinemia, the phenomenon of DIT occurs in response to the hyperphagia induced by the insulin itself and *2*) to investigate whether, in the absence of hyperphagia, hyperinsulinemia alters energy expenditure. To this end, several experiments were conducted to examine energy balance and body composition in response to varying levels of insulin-induced hyperphagia that was achieved by utilizing two different long-acting insulin, animals aged 4, 8, and 12 wk, and the administration of insulin at different times of day.

MATERIALS AND METHODS

Animals, Diets, and Insulin

All studies were conducted on male Sprague-Dawley rats (CMU, Geneva) housed one per cage in a temperature-controlled room (23°C) with a 12:12 h light-dark cycle. The animals had free access to water and to a standard laboratory chow diet (Provimi-Lacta, Switzerland) consisting (by weight) of 20% protein, 60% carbohydrate, and 3% fat, with a metabolizable energy density of 12.1 kJ/g. Protamine zinc insulin (Hoechst-Basale, Hoechst-Pharma, Zurich) or Monotard insulin (Novo Industrie, Zurich) were injected subcutaneously in the

undiluted form. Control animals received daily injections of physiological saline.

Energy Balance and Body Composition Measurements

Each experiment was conducted over a period of 2 wk, during which food intake was monitored continuously, and the metabolizable energy (ME) intake was determined by the method of Miller and Payne (22). At the end of the study, the animals were killed by decapitation, the skull, thorax, and abdominal cavity were incised, and the gut was cleaned of undigested food. The carcasses were then dried to constant weight in an oven maintained at 60°C and subsequently homogenized. Triplicate samples of each dried and homogenized carcass were analyzed for energy content by ballistic bomb calorimetry (22), and body fat content was measured on duplicate samples by the Soxhlet extraction method (12). Body protein was calculated from a general formula relating energy derived from fat, total energy value of the carcass, and energy derived from protein (9). Body energy gain was calculated from the difference between the final body energy content at the end of the experiment and the energy content of weight-matched animals killed on *day 1* of the experiment. Energy expenditure over the entire 2-wk experimental period was thus obtained from the difference between the total ME intake and the energy gained over that period.

Plasma Glucose and Insulin Determinations

Blood was drained from the tail vein, and after centrifugation plasma was frozen and stored at −20°C for later determinations of glucose and insulin. Plasma glucose was measured using a Beckman glucose analyzer (Beckman Instruments, Palo Alto, CA) (17). Plasma immunoreactive insulin was measured by radioimmunoassay according to the method of Herbert et al. (15).

General Experimental Design

Within each experiment, 30 rats were allocated to five groups ($n = 6$) with similar mean body weight. One group was killed on *day 1* to provide an estimate of body energy content at the start of the study. Another group served as controls, and the remaining groups received daily subcutaneous injections of insulin as follows: one group (IM) between 9:00 and 9:30 A.M.; another group (IA) between 5:00 and 5:30 P.M., and the last group (IMA) received insulin twice daily (one-third in the morning and two-thirds in the late afternoon). Insulin was administered in gradually increasing doses, starting at 2 U/100 g body wt with increments of 1 U/100 g every 2 days to reach a maximum of 8 $U \cdot 100\ g^{-1} \cdot day^{-1}$ at the end of the study. This same protocol was applied to animals within groups and between groups for all experiments. On *days 11–12*, food intake pattern was assessed by weighing the food before injections, namely between 9:30 A.M. and 5:30 P.M. (day food intake) and between 5:30 P.M. and 9:30 A.M. (night food intake).

Data Analysis

Data are presented as means ± SE unless otherwise noted. Statistical analyses were performed using analysis of variance (ANOVA). Within each experiment, post hoc comparisons between pairs of treatments were performed with the Newman-Keuls multiple sample comparison test after analysis of variance had established significant differences between treatments.

RESULTS

Effect of Insulin on Energy Balance and Body Composition in 4-Wk-Old Rats

Experiment 1. The effect of the Monotard insulin (MTI) on body weight, weight gain, and on the day-night food intake pattern are shown in Table 1. ANOVA shows no significant differences in the final body weight, body weight gain, nor body composition among the various groups. The data on food intake pattern on *days 11–12* indicate that insulin administration in the afternoon only (i.e., in the IA group) had no effect on day nor night food consumption. In contrast, the groups injected with insulin in the morning (i.e., the IM and IMA animals) consumed between 30 and 40% of the daily food during the day period compared with only 6–10% in the controls and IA group ($P < 0.001$). However, this increase in food intake in the IM and IMA groups was entirely compensated during the night period so that over 24 h the total food intake was not different from that of controls. Similarly, the cumulated energy intake over the entire 2-wk period was similar in all groups. Thus the chronic

TABLE 1. *Energy balance, food intake pattern, and body composition in 4-wk-old rats treated with Monotard insulin*

Group	Body Wt, g		Body Composition, g		24-h Food Intake Pattern, g		Energy Balance, kJ/2 wk		
	Final	Gain	Fat	Protein	Day	Night	Intake	Gain	Expenditure
C	202±3	89±6	15.5±0.4	36.3±1.7	1.9±0.6	22.5±1.1	3,579±102	674±35	2,905±79
IM	191±13	76±8	16.4±1.5	34.4±2.5	8.8±0.4	15.5±1.2	3,629±209	645±82	2,984±129
IA	203±8	88±7	13.7±0.8	37.5±1.8	1.1±0.2	22.3±0.7	3,763±124	620±47	3,143±98
IMA	191±5	77±5	16.8±0.9	37.0±1.0	6.9±0.4	16.8±0.4	3,684±80	733±32	2,951±65
Significance of F	NS	NS	NS	NS	$P < 0.001$	$P < 0.001$	NS	NS	NS
Post hoc comparison					IM, IMA vs. C, IA IM vs. IMA	IM, IMA vs. C, IA			

Values are means ± SE; $n = 6$; NS, not significant. Groups: C, control; IM, insulin injected in the morning; IA, insulin injected in the late afternoon; IMA, insulin injected both morning and afternoon.

TABLE 2. *Energy balance, food intake pattern, and body composition in 4-wk-old rats treated with Protamine insulin*

Group	Body Wt, g		Body Composition, g		24-h Food Intake Pattern, g		Energy Balance, kJ/2 wk		
	Final	Gain	Fat	Protein	Day	Night	Intake	Gain	Expenditure
C	201±8	73±9	16.5±0.9	36.7±1.0	1.5±0.6	22.1±1.3	3,550±150	632±75	2,918±79
IM	210±9	83±9	23.7±1.4	37.7±0.9	12.5±0.4	19.9±3.0	4,265±184	917±86	3,348±125
IA	215±7	88±5	20.7±1.1	37.0±1.2	2.5±1.0	25.4±1.5	4,031±138	774±67	3,257±71
IMA	231±4	103±6	28.6±0.9	39.0±1.0	9.0±0.8	27.8±1.8	4,521±97	1,134±37	3,387±57
Significance of F	NS ($P = 0.07$)	NS ($P = 0.07$)	$P < 0.001$	NS	$P < 0.001$	NS ($P = 0.06$)	$P < 0.01$	$P < 0.01$	$P < 0.01$
Post hoc comparison	IMA vs. C	IMA vs. C	IM, IA, IMA vs. C IMA vs. IM, IA		IM, IMA vs. C, IA IM vs. IMA	IMA vs. IM	IM, IA, IMA vs. C	IM, IMA, vs. C IMA vs. IA	IM, IA, IMA vs. C

Values are means ± SE; $n = 6$; NS, not significant. Groups: C, control; IM, insulin injected in the morning; IA, insulin injected in the late afternoon; IMA, insulin injected both morning and afternoon.

administration of the MTI in 4-wk-old rats failed to induce hyperphagia and had no influence on their energy expenditure nor on body composition.

Experiment 2. In an attempt to induce hyperphagia with insulin in young rats, the experiment in 4-wk-old rat was repeated with another long-acting insulin, the protamine insulin (PZI). The results of this study are shown in Table 2. Insulin treatment tended to increase the mean body weight ($P = 0.07$), but pairwise comparison showed significant differences only between the IMA and control groups. The data on day-night food intake pattern indicate that the PZI, like the MTI, also increased the day food consumption in the IM and IMA groups. However, no compensatory decrease in night food consumption was observed in the latter groups. On the other hand, night food consumption in the IA and IMA groups, although not statistically significant from that of controls on that particular day, was nevertheless elevated by 15 and 25%, respectively; however, the 40% increase in night food intake in the IMA group relative to the IM group was significantly different ($P < 0.05$). As shown in Table 2, the cumulated energy intake over 2 wk was significantly elevated above controls in all insulin-treated groups, with the level of hyperphagia reaching 20, 14, and 27% in the IM, IA, and IMA groups respectively ($P < 0.01$). However, only part of this increase in food intake resulted in fat deposition, since energy expenditure was also elevated by 12–16% in the insulin-treated groups (Table 2).

Effect of Chronic Exogenous Insulin Administration on Plasma Insulin and Plasma Glucose Concentrations

Experiment 3. Given the differential effects of the two types of insulin on food intake, a third experiment was conducted in 4-wk-old rats that directly compared the effect of these two types of insulin on plasma levels of glucose and insulin. In both cases, insulin was administered twice daily (as described above for the IMA groups above). After 7 days, blood was collected from the tail vein at times indicated in Fig. 1 (food and water was available ad libitum, including between injection and sampling times). In both insulin-treated groups, plasma insulin levels were similar to controls before the morning treatment. After exogenous insulin administration, plasma insulin concentrations were elevated by fourfold 4 h later and remained two-to-threefold above control values 8 h posttreatment. Figure 1 also shows the individual values for plasma glucose concentrations, which were higher in both insulin-treated groups than in controls before morning insulin treatment. Four hours after insulin administration, plasma glucose levels fell markedly to values ranging from 25 to 43 mg/100 ml. Another 4 h later, the plasma glucose concentration in both insulin-treated groups tended to be either lower or higher than in controls.

Thus, at the times that plasma was sampled, both insulin-treated groups showed similar levels of hyperinsulinemia, with concomitant hypoglycemia 4 h later, followed by phases of hypoglycemia or hyperglycemia.

Effect of Insulin on Energy Balance and Body Composition in 8-Wk-Old Rats

Since in 4-wk-old rats insulin-induced hyperphagia was either absent or relatively mild, further studies investigated the possibility that insulin administration in older (8-wk-old) animals would induce a greater degree of hyperphagia. Similar experimental protocols as in *experiments 1* and *2* were used.

Experiment 4. The results of this study, which examined the effect of the MTI in 8-wk-old rats, are shown in Table 3. There were no differences in body weight nor in body protein among the various groups. Body fat content tended to be higher in all insulin-treated groups compared with the control group, but ANOVA showed no statistically significant differences among the groups. Data on food consumption pattern on *days 11–12* indicate that food intake was significantly elevated during the day period in the IM and IMA groups. However, compensatory reduction in night food intake occurred only in the IM-treated animals but not in the IMA-treated group. Total ME intake over the entire 2 wk was elevated by 9, 10, and 15% in the IM, IA, and IMA groups, respectively, and this was accompanied by increases in 4, 7, and 11% in energy expenditure. However,

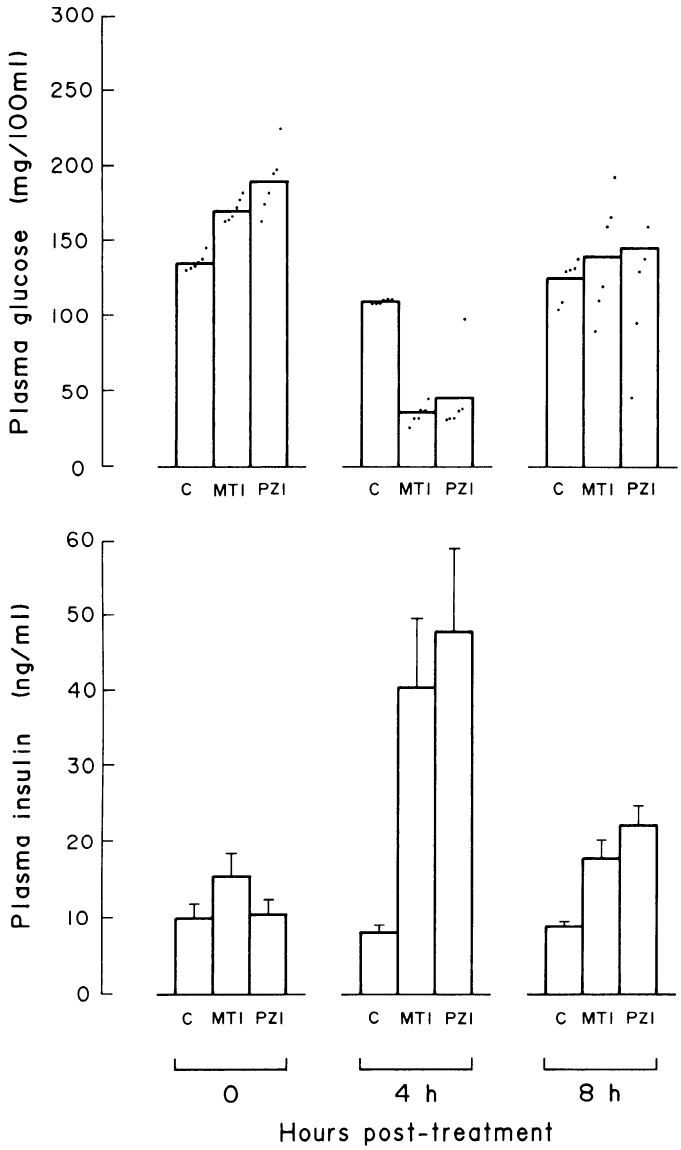

FIG. 1. Plasma levels of glucose and insulin before insulin treatment and at 4 h and 8 h after subcutaneous injection of insulin (1.7 U/100 g body wt). For plasma glucose, distribution of individual values are shown as dots plotted above or below their respective mean values. Data for insulin concentrations are means ± SE. Body weights (means ± SE) for saline-injected controls (C), Monotard insulin-treated group (MTI), and Protamine zinc insulin-treated group (PZI) were 185 ± 4, 179 ± 3, and 186 ± 2 g, respectively.

ANOVA indicates that in these 8-wk-old rats the effects of MTI in altering energy balance were of marginal statistical significance, since $P = 0.05$ and $P = 0.09$ for energy intake and energy expenditure, respectively.

Experiment 5. In this study examining the effect of the PZI in 8-wk-old rats, body weight was slightly higher (though not significantly) in the insulin-treated groups compared with controls (Table 4). However, body fat was significantly elevated in all insulin-treated groups (IM 40%, IA 18%, and IMA 35% above controls, $P < 0.001$), but body protein was unaffected. The data on food intake pattern indicate that food consumption in the IM and IMA groups were elevated during the day period, followed by partial compensatory decrease in night food intake. In contrast, the IA-treated animals had similar day food intake as the controls, but night food consumption was significantly higher by 28%. Over the entire experimental period, the cumulated ME intake was elevated by 26, 24, and 29% in the IM, IA, and IMA groups, respectively (Table 4), and was associated with increases in energy expenditure of 15, 18, and 21%, respectively. Thus, as in the 4-wk-old rats, chronic treatment with PZI resulted both in hyperphagia and in DIT in the 8-wk-old animals.

Effect of Insulin on Energy Balance and Body Composition of 12-Wk-Old Rats

In an attempt to assess energy expenditure at higher levels of insulin-induced hyperphagia, the studies were extended to 12-wk-old animals using the same experimental protocol as before.

Experiment 6. The results of the study examining the effect of the MTI on energy balance of 12-wk-old rats are shown in Table 5. ANOVA indicates a significant difference ($P < 0.01$) between groups in body weight gain. However, post hoc comparison indicates that only the IMA group gained significantly more weight than the control group. On the other hand, all insulin-treated groups had higher fat content than controls ($P < 0.001$), but body protein was unaltered. Food intake measured on *days 11–12* indicate that in the IM group food consumption was elevated during the day period only and contrasted with the IA group, in which food consumption increased only during the night periods. On the other hand, the IMA group had higher food intake during both

TABLE 3. *Energy balance, food intake pattern, and body composition in 8-wk-old rats treated with Monotard insulin*

Group	Body Wt, g		Body Composition, g		24-h Food Intake Pattern, g		Energy Balance, kJ/2 wk		
	Final	Gain	Fat	Protein	Day	Night	Intake	Gain	Expenditure
C	370±6	60±8	35.2±1.8	72.0±1.0	6.1±1.1	25.3±1.8	5,182±146	477±43	4,705±163
IM	371±5	64±3	43.1±1.7	69.5±0.8	16.7±1.3	17.3±2.2	5,643±87	728±71	4,915±78
IA	367±7	56±6	42.8±1.4	68.6±1.3	4.6±0.2	29.6±0.6	5,714±193	686±38	5,028±208
IMA	362±15	53±5	43.7±4.4	69.4±2.0	15.6±2.2	24.2±2.1	5,961±264	745±181	5,216±113
Significance of F	NS	NS	NS ($P = 0.07$)	NS	$P < 0.001$	$P < 0.002$	NS ($P = 0.05$)	NS ($P = 0.09$)	NS ($P = 0.09$)
Post hoc comparison					IM, IMA vs. C, IA	IA, IMA, C vs. IM	IMA vs. C		

Values are means ± SE; $n = 6$; NS, not significant. Groups: C, control; IM, insulin injected in the morning; IA, insulin injected in the late afternoon; IMA, insulin injected both morning and afternoon.

TABLE 4. *Energy balance, food intake pattern, and body composition in 8-wk-old rats treated with Protamine insulin*

Group	Body Wt, g		Body Composition, g		24-h Food Intake Pattern, g		Energy Balance, kJ/2 wk		
	Final	Gain	Fat	Protein	Day	Night	Intake	Gain	Expenditure
C	362±5	47±8	40.6±2.3	67.8±0.9	0.62±0.22	30.0±0.6	5,504±173	573±76	4,931±127
IM	376±4	58±7	57.0±2.0	69.1±1.2	16.4±2.4	23.8±1.4	6,924±224	1,277±98	5,647±218
IA	374±14	57±8	48.1±3.0	70.1±2.7	0.72±0.22	38.5±1.7	6,798±340	958±155	5,840±274
IMA	373±10	58±6	54.9±2.6	67.3±1.7	16.4±1.8	23.7±1.7	7,125±108	1,172±95	5,953±113
Significance of F	NS	NS	$P < 0.001$	NS	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.002$	$P < 0.01$
Post hoc comparison			IM, IMA, IA vs. C		IM, IMA vs. C, IA	IM, IMA vs. C, IA IA vs. C	IM, IA, IMA vs. C	IM, IA, IMA vs. C	IM, IA, IMA vs. C

Values are means ± SE; $n = 6$; NS, not significant. Groups: C, control; IM, insulin injected in the morning; IA, insulin injected in the late afternoon; IMA, insulin injected both morning and afternoon.

TABLE 5. *Energy balance, food intake pattern, and body composition of 12-wk-old rats treated with Monotard insulin*

Group	Body Wt, g		Body Composition, g		24-h Food Intake Pattern, g		Energy Balance, kJ/2 wk		
	Final	Gain	Fat	Protein	Day	Night	Intake	Gain	Expenditure
C	403±11	10±2	40.2±1.1	85.2±2.0	0.75±0.36	30.2±0.9	5,032±105	100±28	4,932±103
IM	424±10	29±5	55.9±1.8	85.6±2.2	16.7±1.2	26.1±3.8	6,224±161	712±21	5,512±165
IA	419±15	25±9	49.6±4.5	84.4±2.3	1.2±0.9	39.1±3.5	5,827±270	435±178	5,392±195
IMA	433±9	41±8	63.0±2.5	87.0±2.3	14.9±1.1	36.4±3.2	6,777±98	1,021±57	5,756±99
Significance of F	NS	$P < 0.01$	$P < 0.001$	NS	$P < 0.001$	$P < 0.02$	$P < 0.001$	$P < 0.001$	$P < 0.01$
Post hoc comparison		IMA vs. C	IM, IA, IMA vs. C IMA vs. IA		IM, IMA vs. C, IA	IA, IMA vs. IM	IM, IA, IMA vs. C IMA vs. IM, IA	IM, IA, IMA vs. C IMA, IA vs. IM IMA vs. IA	IM, IA, IMA vs. C

Values are means ± SE; $n = 6$; NS, not significant. Groups: C, control; IM, insulin injected in the morning; IA, insulin injected in the late afternoon; IMA, insulin injected both morning and afternoon.

TABLE 6. *Energy balance, food intake pattern, and body composition in 12-wk-old rats treated with Protamine insulin*

Group	Body Wt, g		Body Composition, g		24-h Food Intake Pattern, g		Energy Balance, kJ/2 wk		
	Final	Gain	Fat	Protein	Day	Night	Intake	Gain	Expenditure
C	404±11	12±10	41.2±1.7	83.9±2.2	0.30±0.07	25.5±1.0	4,504±178	172±59	4,332±184
IM	440±16	44±7	62.0±7.1	85.0±2.0	15.8±1.5	24.0±4.2	5,923±307	875±293	5,048±106
IA	450±24	58±14	67.9±4.3	87.5±5.1	0.57±0.2	34.4±2.0	6,309±420	1,189±246	5,120±205
IMA	466±10	72±18	74.5±4.0	85.0±2.6	15.7±0.8	43.7±3.7	7,079±77	1,385±137	5,694±179
Significance of F	NS ($P = 0.09$)	$P < 0.05$	$P < 0.001$	NS	$P < 0.001$	$P < 0.01$	$P < 0.001$	$P < 0.01$	$P < 0.01$
Post hoc comparison		IMA vs. C	IM, IA, IMA vs. C		IM, IMA vs. C, IA	IA, IMA vs. C IMA vs. IM	IM, IA, IMA vs. C IMA vs. IM	IM, IA, IMA vs. C	IM, IA, IMA vs. C

Values are means ± SE; $n = 6$; NS, not significant. Groups: C, control; IM, insulin injected in the morning; IA, insulin injected in the late afternoon; IMA, insulin injected both morning and afternoon.

day and night periods. Cumulative energy intake was elevated above control values in all insulin-treated groups, namely by 24, 16, and 35% in the IM, IA, and IMA groups, respectively ($P < 0.001$), and was associated with increases in energy expenditure of 13, 9, and 17%, respectively ($P < 0.01$).

Experiment 7. As in the 12-wk-old rats treated with the MTI, treatment with PZI also resulted in significantly greater gain in body weight in the IMA group compared with controls (Table 6). Similarly, body fat was elevated in all insulin-treated groups, but body protein was unaffected. The data on the pattern of food intake indicate that elevated food intake occurred during the day period only in the IM group, during the night period only in the IA group, but during both day and night in the IMA group. Over the 2-wk experimental period, the cumulative food intake was found to be elevated by 30–36% in the IM and IA groups and by more

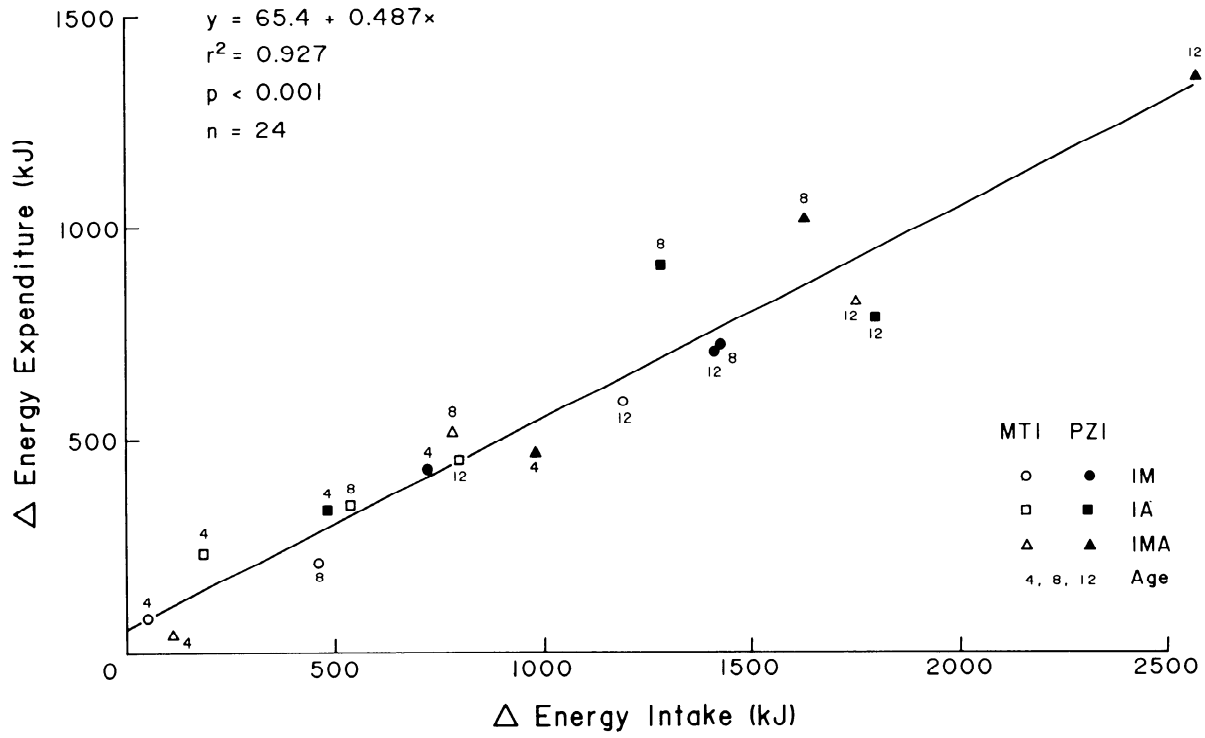

FIG. 2. Linear regression of change in energy expenditure on change in energy intake induced by insulin. Each point represents mean value for groups injected with insulin in the morning (IM), the late afternoon (IA), or both (IMA). Open symbols represent those treated with Monotard insulin (MTI) and closed symbols for groups treated with Protamine zinc insulin (PZI). Age of groups (4, 8, and 12 wk) are indicated by numbers next to symbols.

than 50% in the IMA group. Similarly, energy expenditure was increased in all insulin-treated groups by ~15% in the IM and IA groups and by more than 30% in the IMA group.

DISCUSSION

Influence of Insulin on Energy Expenditure

The main findings of the current investigations, based on the measurements of energy balance and body composition, indicate that *1*) in the absence of hyperphagia chronic insulin administration has no influence on energy expenditure nor on energy partitioning and *2*) the effect of exogenous insulin on energy expenditure is directly related to the level of hyperphagia.

In fact, one of the major implications of the present studies concerns the adaptive role of energy expenditure in the regulation of energy balance. Although the existence of DIT has been challenged by some, several laboratories have demonstrated that DIT occurs in response to hyperphagia induced by dietary modifications (28). The studies reported here therefore extend this concept of DIT as an adaptive phenomenon that is also demonstrable when hyperphagia is induced by insulin. In Fig. 2, a regression plot of the increase in energy intake against the increase in energy expenditure shows a good linear fit and that ~50% of the extra energy consumed was dissipated as heat, i.e., in DIT. Part of this increase in energy expenditure could be attributed to the energy cost of depositing the undissipated excess calories. However, body composition analysis indicates that this increased energy stores was only in the form of fat, with an energy cost of deposition (~0.35kJ/kJ gained) that accounted only for a minor component of the total DIT.

On the other hand, the proportion of excess calories dissipated as heat was uninfluenced by the timing of insulin administration despite the fact that food consumption pattern was markedly altered. For example, within each experiment, the marked alteration in the day-night food consumption in the IA groups compared with the IM groups resulted in no significant difference in energy balance nor in body composition. It therefore seems that energy expenditure is mainly influenced by the overall level of hyperphagia and that changes in meal pattern induced by insulin have little or no impact on energy expenditure.

Similarly, our data indicate that in the range of 4- to 12-wk-old animals age had no significant effect on the proportion of excess calories dissipated as heat: DIT ranged between 50 and 70%, 50 and 77%, and 44 and 60% in the 4-, 8-, and 12-wk-old rats, respectively. Several reasons could underlie this apparent discrepancy between the data presented here and previous work indicating that the capacity for DIT declines with age (25). First, age-related differences in DIT have been demonstrated in rats with wider differences in age (5–26 wk old) than those utilized in the current studies (4–12 wk old). Second, differences in diet composition, environmental temperature, and animal strains, all factors that have been shown to influence the capacity for DIT (28), make comparison difficult between the level of DIT obtained in this present study and that previously reported in response to dietary-induced hyperphagia. Thus, although it is not known whether the magnitude of DIT in response to hyperphagia would be different in the

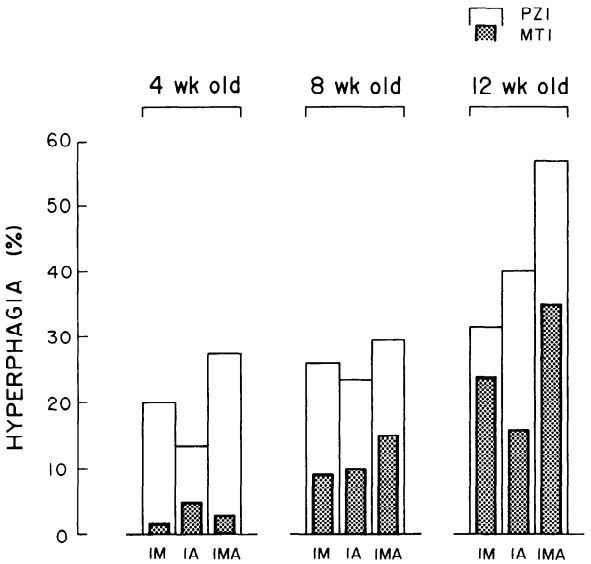

FIG. 3. Insulin-induced hyperphagia (expressed as %increase in caloric intake above controls) in 4-, 8-, and 12-wk-old rats treated for 2 wk with either Monotard insulin (MTI) or Protamine zinc insulin (PZI). IM, IA, and IMA, groups injected with insulin in the morning, the late afternoon, or both, respectively. Three-way analysis of variance indicates significant main effects of age ($P < 0.001$), type of long-acting insulin ($P < 0.001$), and timing of insulin administration ($P < 0.002$). Interactions of age × type, age × time, type × time, and age × type × time were not statistically significant.

absence of insulin, it is nonetheless clear from the current studies that DIT persists over the range of age tested even in the presence of chronic hyperinsulinemia.

During the past decade, similarities between DIT and cold-induced thermogenesis have been emphasized inasmuch as both stimuli increase the functional activity of the sympathetic nervous system in brown adipose tissue (19, 26), but in contrast sympathetic neural activity in skeletal muscle responds only minimally to cold exposure and not at all to alterations in dietary intake (11). However, given the multiple central and peripheral effects of insulin, the mechanisms mediating DIT in response to insulin-induced hyperphagia are likely to be more complex. For example, in response to acute insulin-induced hypoglycemia, a decrease in blood flow to brown adipose tissue, probably through a central inhibition of the sympathetic nervous system (4), may be counteracted by an increase in thermogenesis resulting from an increase in nerve impulse traffic in peripheral sympathetic nerves to skeletal muscle (13) and from an elevated plasma catecholamine (7). On the other hand, chronic insulin administration has been shown to elevate both sympathetic activity and thermogenic capacity in brown adipose tissue (27, 30), but it is unclear whether the coupling of insulin to sympathetic activity and DIT results from a direct effect insulin per se or from the accompanying hyperphagia. However, it is evident from our studies that in the absence of hyperphagia a state of hyperinsulinemia does not inhibit nor enhance whole body energy expenditure.

Insulin and Defective Thermogenesis in Obesity

The current investigation also has implications for the current debate concerning the role of hyperinsulinemia in the etiology of obesity. It has been proposed that hyperinsulinemia and/or insulin resistance, induced by an overactive vagal activity, plays an important causative role in the thermogenic defect of the obese (5, 16). Other works, however, do not support this contention, since the defective thermogenesis precedes the development of hyperinsulinemia and in addition postobese human subjects maintaining their body weight on a low caloric intake show blunted DIT without any clinical signs of hyperinsulinemia or insulin resistance (10). Furthermore, surgical and pharmacological approaches to reduce insulin secretion do not improve the thermogenic defect in obesity (6, 8).

More recently, the proposal of an inhibitory effect of insulin on thermogenesis has been reemphasized following the report that insulin-treated animals show a greater efficiency of weight gain than controls (2). However, the results of these studies are unconvincing in and of themselves for several reasons. First, despite the fact that food intake was reported not to be significantly different from controls, it was elevated by 15–20% (2). In fact in a subsequent study (3), the authors reported that the same insulin administration protocol increased food intake significantly by 25% during the first few days of treatment. It seems likely therefore that hyperphagia occurring at some stage during treatment with insulin, rather than an effect of insulin dissociated from hyperphagia, contributed to the weight gain. Second, the efficiency of weight gain (often termed metabolic or food efficiency) is a poor and often a misleading index of changes in energy expenditure, since relatively small changes in body weight do not reflect changes in body composition.

By assessing both energy balance and body composition, the studies presented here overcome these limitations and indicate that in the absence of hyperphagia high doses of insulin do not influence energy expenditure. Our data therefore do not support the notion of an inhibitory influence of chronic hyperinsulinemia on thermogenesis, at least in animals fed ad libitum a standard chow (high-carbohydrate, low-fat) diet. On the other hand, it is uncertain whether the higher body fat content previously reported in insulin-treated rats tube fed isocaloric amounts of a high fat diet (29) resulted from diminished thermogenesis. Since body protein content also decreased concomitantly in this latter study (29), it seems that only energy partitioning, rather than energy expenditure, was altered. Further work is warranted to investigate the interaction between dietary composition and hyperinsulinemia on thermogenesis.

Influence of Insulin on Food Intake

Although the studies reported here were not specifically directed at examining the role of insulin in the control food intake, they nevertheless indicate that chronic food intake is markedly influenced by differences in the type of long-acting insulin and the timing of insulin administration as well as by relatively small differences in the age of the animals. As shown in Fig. 3, the level of hyperphagia was much more pronounced with PZI than with MTI, and to a lesser degree it increased with age and also tended to be more marked

when the same amount of insulin was administered twice a day compared with one larger dose daily.

A more sustained elevation in plasma insulin level probably underlies the general tendency for food intake to be higher in the IMA groups than in the IM or IA groups. In contrast, the differential effects of PZI and MTI on hyperphagia are not explained by gross differences in the level of hyperinsulinemia nor in the plasma glucose profile, which were similar with both insulin types. However, blood was collected at only three time points during 24 h, and hence it is likely that differences in the kinetics of these two types of insulin occurring between these sampling times are responsible for these marked differences in hyperphagia. In fact, a main difference between these two types of long-acting insulin lies in a slower onset of action of the MTI (2 h) compared with PZI (30 min), but whether this difference alone is sufficient to explain their differential effects on food intake requires further investigations.

Our findings that the level of hyperphagia increased with age is somehow surprising, since insulin resistance is known to develop with increasing age and older animals would consequently be expected to be less responsive to the effects of insulin. However, since the insulin dosage was calculated on the basis of body weight, the older and hence heavier animals exhibited greater levels of hyperphagia simply because they received a greater absolute amount of insulin. Although this explanation is plausible, it is not sufficient, since unpublished data in our laboratory indicate that the greater level of hyperphagia observed in 12-wk compared with 8-wk-old rats persists even when the same absolute amount of insulin was administered. Other aspects of age such as adiposity or stage of growth must also influence the level of hyperphagia induced by insulin.

In conclusion, the current investigations demonstrate that, in rats exhibiting hyperphagia on a high-carbohydrate, low-fat diet, the phenomenon of DIT persists in the hyperinsulinemic state. However, even though these studies refute the notion that chronic hyperinsulinemia per se altered meal pattern or that some other aspects of insulin action influence energy expenditure, the possibility remains that insulin, acting as a signal between diet and sympathetic activity, has a permissive role in the mediation of DIT.

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Address for correspondence: A. G. Dulloo, Centre Medicale Universitaire, Dept. of Physiology, University of Geneva, 9 Ave. de Champel, 1211 Geneva 4, Switzerland.

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