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Insulin Increases Energy Expenditure and Respiratory Quotient in the Rat

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MENÉNDEZ, J. A. AND D. M. ATRENS. *Insulin increases energy expenditure and respiratory quotient in the rat.* PHARMACOL BIOCHEM BEHAV **34**(4) 765–768, 1989.—The effects of insulin on energy expenditure are a matter of dispute. Various authors have reported increases or decreases. Irrespective of their nature, it is not clear whether the effects of insulin on energy expenditure are secondary to insulin-induced hypoglycemia or changes in motor activity. The present study investigated the acute effects of insulin on energy expenditure, energy substrate utilisation, motor activity and blood glucose levels. Four U/kg of fast acting insulin had no effect on any of the metabolic or motor activity measures in spite of producing a 30% reduction in blood glucose levels. In contrast, 8 U/kg of insulin increased energy expenditure and respiratory quotient, with the latter effect indicating increased reliance on carbohydrates as a source of energy. This dose reduced blood glucose levels by 68%, yet had no significant effect on motor activity. Insulin, therefore, enhances thermogenesis and carbohydrate utilisation in a manner that can be dissociated from any effects on motor activity. These effects occur at a high dose and they are not counteracted by even massive hypoglycemia. It, therefore, appears that in terms of energy expenditure insulin may be characterised as catabolic, whereas in terms of substrate utilisation it may be characterised as anabolic.

Insulin Energy expenditure Respiratory quotient Thermogenesis Energy substrate utilisation Indirect calorimetry Rat

ENERGY balance is maintained by regulating both energy intake and expenditure. There is an extensive literature on the regulation of energy intake [for references see (12)], but there are relatively few data on the regulation of energy expenditure (2–4, 17, 21). It is widely agreed that the hormone insulin has major effects on both energy intake and expenditure, but the nature of the latter effect remains obscure (10). For example, in a recent review, Rothwell and Stock (19) cite data suggesting that low doses of insulin increase thermogenesis, whereas higher doses may decrease thermogenesis. They speculate that the decreased thermogenesis seen at higher doses may be due to the fact that insulin-induced hypoglycemia inhibits thermogenesis. In contrast, a number of studies have reported that insulin increases thermogenesis [for references see (10)].

One reason for the disparities in this literature is the existence of data on the relations between insulin and energy expenditure that are in varying ways indirect (14–16, 18). For example, some have inferred changes in thermogenesis from changes in the activity of brown adipose tissue (14). However, it is not clear whether such changes are indicative of overall thermogenesis. Alternatively, increased thermogenesis has been calculated through recording increased oxygen (O_2) consumption (14–16, 18). Although this procedure is correct, it is preferable to calculate thermogenesis by simultaneous measurements of carbon dioxide (CO_2) production as well as O_2 consumption (7,9). By measuring CO_2 production, changes in energy expenditure can be more precisely calculated. In addition, this procedure permits the determination of the respiratory quotient (CO_2 production/O_2 consumption) and, by inference, energy substrate utilisation (7,9). Since insulin markedly affects carbohydrate utilisation, one would expect it to affect respiratory quotient as well. Indeed, the present study shows that insulin has major effects on respiratory quotient.

There is a further interpretational difficulty with the existing data. None of the studies to date has measured motor activity (14–16, 18). This means that the apparent changes in thermogenesis could merely reflect changes in motor activity. Again, there is both theoretical and empirical support for such an effect (20,21). Addressing this problem by selecting data from resting periods (14–16, 18) may be misleading, particularly since how an inactive period is measured or defined is not stated. On the other hand, eliminating activity by using anesthetized rats (19) introduces the confounding effects of anaesthesia (21).

The present study was undertaken in order to determine the effects of acute subcutaneous insulin injections on energy expenditure (thermogenesis) in unanesthetized rats. By measuring both O_2 consumption and CO_2 production it is possible to measure thermogenesis more precisely. By concurrently measuring motor activity it is possible to determine the extent to which any thermogenic changes may be secondary to changes in motor activity. Further, the measurement of blood glucose concentration allows the determination of the relation between thermic and glycemic changes. The present data indicate that insulin increases thermogenesis and that this effect can be dissociated from any effect on motor activity, and is not antagonised by even extreme hypoglycemia.

METHOD

Subjects

Sixteen adult male Wistar rats (Castle Hill, Sydney) weighing

between 300 and 400 grams were used. They were pair-housed in a colony room maintained at 22 ± 2°C, with lights on from 6.00 a.m. to 8.00 p.m. All experiments were conducted during the light phase, approximately between 9.00 a.m.–3.00 p.m. Food (Allied Rat & Mouse Kubes, Sydney) and water were freely available, except during food deprivation when only water was provided. The rats were handled daily by the experimenters for two weeks before any experimental procedure, in order to reduce the stress of human contact.

Apparatus

Energy expenditure (EE) and respiratory quotient (RQ) were obtained after recording the O_2 consumption and the CO_2 production by indirect calorimetry (13,21). The rats were placed in a clear acrylic cylinder (6.28 litres), with a stainless steel grid floor. Atmospheric air was passed through the chamber at a flow rate of 1600 ml/min and a pressure of 8000 N/m^2 (8 kPa) above atmospheric. A 110 ml/min sample of air leaving the chamber was directed through a permeation drier (PermaPure PD-750-12PP) and passed through an S-3A O_2 analyzer and a CD-3A CO_2 analyzer (Applied Electrochemistry, USA). Primary gravimetric standards (Commonwealth Industrial Gases, Sydney) were used for the daily calibration of both analyzers. Locomotor activity was recorded continuously by placing the chamber on an electronic balance (Metler PE 2000) and using the unintegrated signal from the strain gauge (13,21). The monitoring of the apparatus was done through a Z-80 based, S-100 bus microcomputer system, which provided a minute by minute recording of O_2 consumption, CO_2 production and activity counts. The following calculations were made: EE (kJ) = moles O_2 (364 + 113 RQ) (7,9), expressed in J/g/min; and RQ = vol. CO_2 produced/vol. O_2 consumed (9).

Blood Glucose Determination

Blood samples were taken by "milking" the tail after removing 0.5–1.0 mm of its tip (5). The blood was stored at 5°C in heparinized vials and analyzed in a glucose analyzer (Yellow Springs Instruments, Model 23AM, USA) at the end of the experimental session. Two samples were taken from every rat, one immediately before the experiment and the other one immediately after. Blood glucose concentration was expressed as mmol/l.

Drugs

Insulin Actrapid® MC, neutral solution of porcine insulin (Commonwealth Serum Laboratories, Novo Pty. Ltd., Sydney), was used in doses of 4 U/kg and 8 U/kg, both in a volume of 1 ml/kg. This type of insulin belongs to the group of "fast insulins" which are characterised by rapid absorption, prompt onset and relatively short duration (11). NaCl 0.9% in an equivalent volume was used as control.

Data Analysis

Two-way analysis of variance was used for comparisons between insulin and saline treatments (the two variables being the treatment condition, insulin and saline, and the time after the injections). Two-tailed Student's *t*-test was used for the comparison between blood glucose concentrations before and after the injections.

Procedure

The experiments were performed after 2 hours of food deprivation. Following a 15-minute period of acclimatization inside the chamber, each rat was removed and injected subcutaneously with either insulin or saline, then tested for 60 minutes. A 17-minute equilibration period (not included in the testing time) was automatically set by the computer after closing the chamber. Both insulin and saline injections were given in a counterbalanced order, with at least a four-day interval between them. Six rats were injected with the 4 U/kg dose and ten with the 8 U/kg dose.

TABLE 1

EFFECT OF 4 U/kg INSULIN ON ENERGY EXPENDITURE, RESPIRATORY QUOTIENT AND ACTIVITY

	Insulin	Saline	ANOVA	
EE[a]	0.195	0.186	Treatment	F(1,5) = 4.29
	(0.004)	(0.003)	Time	F(59,295) = 25.05*
			Interaction	F(59,295) = 0.66
RQ[b]	0.87	0.87	Treatment	F(1,5) = 5.14
	(0.02)	(0.02)	Time	F(59,295) = 4.64*
			Interaction	F(59,295) = 0.22
AC[c]	22.2	22.4	Treatment	F(1,5) = 1.06
	(2.4)	(2.7)	Time	F(59,295) = 9.78*
			Interaction	F(59,295) = 0.86

Values in the INSULIN and SALINE columns are expressed as mean ± SEM (in parentheses).
[a]EE = Energy Expenditure, in joules/gram/minute.
[b]RQ = Respiratory Quotient.
[c]AC = Activity, in counts/minute.
*$p<0.001$.

RESULTS

No significant effects ($p>0.05$) were obtained after the 4 U/kg insulin injection (Table 1). Blood glucose comparison between pre- and postinjection values showed an average decrease of 29.7%, $t(5) = 6.75 < 0.01$ (Table 3).

The 8 U/kg injection produced significant increases in EE and RQ, without any effect on activity (Table 2 and Fig. 1). Blood glucose comparison showed an average decrease of 67.8%, $t(9) = 9.82 < 0.001$ (Table 3).

TABLE 2

EFFECT OF 8 U/kg INSULIN ON ENERGY EXPENDITURE, RESPIRATORY QUOTIENT AND ACTIVITY

	Insulin	Saline	ANOVA	
EE[a]	0.208	0.192	Treatment	F(1,9) = 18.75†
	(0.004)	(0.003)	Time	F(59,531) = 13.68‡
			Interaction	F(59,531) = 1.01
RQ[b]	0.92	0.86	Treatment	F(1,9) = 9.04*
	(0.02)	(0.01)	Time	F(59,531) = 2.88‡
			Interaction	F(59,531) = 0.91
AC[c]	29.1	29.9	Treatment	F(1,9) = 0.01
	(4.9)	(4.8)	Time	F(59,531) = 4.29‡
			Interaction	F(59,531) = 0.81

Values in the INSULIN and SALINE columns are expressed as mean ± SEM (in parentheses).
[a]EE = Energy Expenditure, in joules/gram/minute.
[b]RQ = Respiratory Quotient.
[c]AC = Activity, in counts/minute.
*$p<0.05$; †$p<0.01$; ‡$p<0.001$.

TABLE 3

EFFECT OF INSULIN ON BLOOD GLUCOSE CONCENTRATION

	Pre[a]	Post[b]	Effect (%)[c]
4 U/kg	3.17 (0.26)	2.23 (0.37)	−29.7*
Saline (1)	3.15 (0.20)	3.14 (0.25)	−0.3
8 U/kg	3.04 (0.24)	0.98 (0.10)	−67.8†
Saline (2)	3.08 (0.25)	3.10 (0.18)	+0.6

Blood glucose concentration values (in mmol/l) are expressed as mean ± SEM (in parentheses).

[a]Pre = immediately before the experiment.

[b]Post = immediately after the experiment.

[c]Effect (%) = [(post/pre) × 100] − 100.

Saline (1) = saline injections in the 4 U/kg insulin group.

Saline (2) = saline injections in the 8 U/kg insulin group.

*$p<0.01$; †$p<0.001$.

Blood glucose concentration did not change after the saline injections ($p>0.05$), demonstrating that the injection procedure itself has little effect on this parameter (Table 3).

In spite of a 33% mean difference between both groups in motor activity values following saline injections (Tables 1 and 2), there were no significant differences ($p>0.05$). This is in agreement with previous reports (13,21) which have shown even greater mean differences that failed to reach significance. This reflects the very large variability that characterises motor activity measures.

DISCUSSION

The present study examined the acute effects of subcutaneous injections of insulin on energy expenditure and energy substrate utilisation in unanesthetized rats. Although it is certainly an oversimplification, for the purposes of the present experiment, these two effects may be referred to as the metabolic effects of insulin. The technique of indirect calorimetry along with concurrent measurement of motor activity allows the determination of the relation between these metabolic changes and changes in motor activity. Measuring blood glucose concentration before and after the treatments allows the determination of the relation between metabolic and glycemic changes. Whereas continuous measurement of CO_2 production and the calculation of respiratory quotients allows a more precise determination of thermogenesis, its main value is to permit the determination of the substrate that the animal is using for its energy (9). Decreased respiratory quotient indicates increased reliance on fats as a source of energy, whereas increased respiratory quotient indicates increased reliance on carbohydrates (9).

The 4 U/kg dose of insulin had no effect on energy expenditure, respiratory quotient or motor activity. The fact that it produced an almost 30% reduction in blood glucose levels indicates that this was an effective dose. This shows that a moderately high dose of insulin and the resulting hypoglycemia are not sufficient to alter either energy expenditure or substrate utilisation. The dissociation between the thermic and glycemic effects also shows that thermogenesis is not affected by even a very substantial hypoglycemia. This contrasts sharply with Rothwell and Stock's statement that hypoglycemia may inhibit thermogenesis (19).

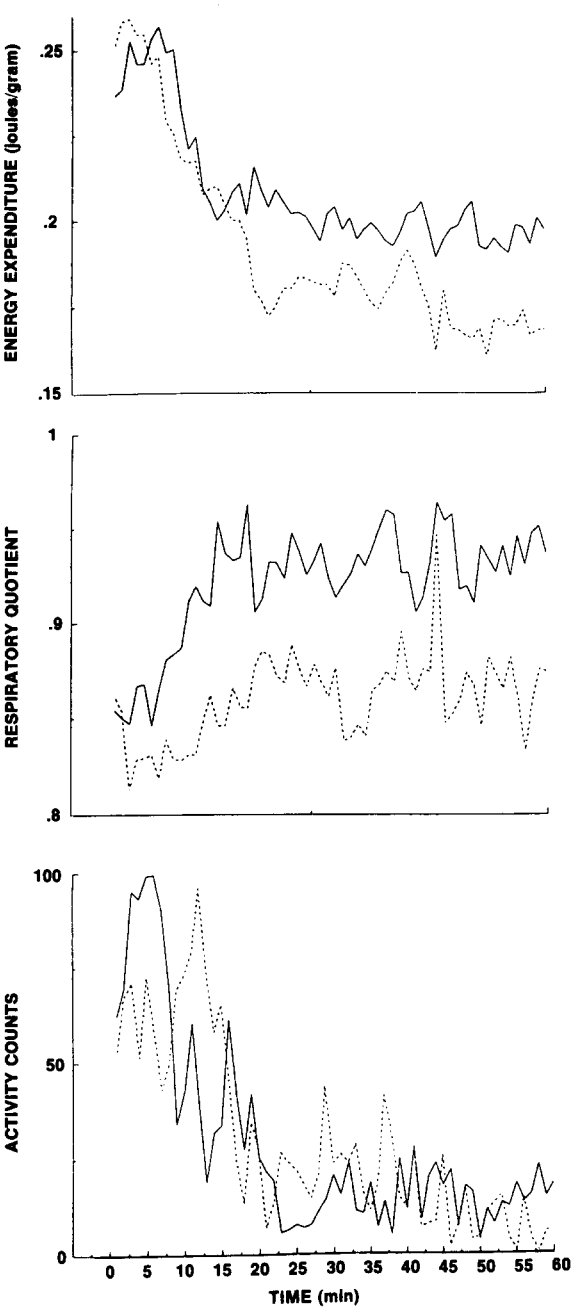

FIG. 1. Mean energy expenditure (top), respiratory quotient (middle) and activity (bottom) over a 60-minute test session following SC injections of either insulin 8 U/kg (solid line) or saline (dotted line). SEM are not represented because they are smaller than the scale.

The 8 U/kg dose of insulin produced a large and long lasting increase in both energy expenditure and respiratory quotient. The energy expenditure data show a substantial, but transient, elevation following the handling and injection procedure. This stress-induced thermogenesis is typical both in its magnitude and duration (13). The thermogenic effect of insulin does not become fully apparent until some 20 minutes of the recording session have elapsed, by which time the stress effect is no longer apparent. There was no sign of any diminution of the insulin effect after one hour of recording.

Both the initial and final values of energy expenditure after saline injections agree closely with previous findings from this laboratory (3, 4, 13, 20, 21) and others (6–8). However, they are below theoretical requirements calculated in one other report (1). This discrepancy remains to be resolved.

In contrast to the effect on energy expenditure, the effect of the high dose of insulin on respiratory quotient was apparent almost from the beginning of the recording session. As is typical in this paradigm, respiratory quotient is almost unaffected by the stress of handling and injection (13). Like the effect on energy expenditure, respiratory quotient appeared to remain elevated even one hour after starting the recording session. The increase in respiratory quotient indicates an increased reliance on carbohydrates for energy or even fat synthesis when RQ values are above 1.00 (9). Although the values in the present study did not generally reach such high levels, it may still be possible that a process of fat synthesis occurs as a direct consequence of the insulin treatment.

The present experiment shows that moderate doses of insulin have no effect on thermogenesis, whereas higher doses substantially increase thermogenesis. They provide no support for any insulin-induced inhibition of thermogenesis (19). These data along with the increase in respiratory quotient caused by insulin may help to resolve a longstanding paradox. As Rothwell and Stock have pointed out: "Because of its classical role as an anabolic hormone promoting accumulation of nutrients within the body, it would seem unlikely, or even contradictory to suggest that insulin could also act as a signal for thermogenesis (and hence fat loss)" [(19), p. 94]. High doses of insulin are certainly thermogenic, but the fact that they also produce a large increase in respiratory quotient indicates that they shift energy substrate utilisation toward increased dependence on carbohydrates. Thus, insulin would tend to conserve fat at the expense of depleting carbohydrate reserves. This, in combination with a facilitation of food intake (10,12), constitutes a strong anabolic force. Thus, in terms of thermogenesis, insulin appears to have a catabolic function, whereas in terms of energy intake and substrate utilisation it is clearly anabolic.

Consideration should be given to the fact that the study was conducted during the light phase of the daily cycle, when rats do the least of their eating. Accordingly, it remains to be determined whether similar metabolic effects are obtained at other points in the diurnal cycle.

The mode and site of action for the insulin's thermogenic effect is a matter of controversy. The ventromedial hypothalamus, serotonergic pathways and other catecholaminergic systems have been described as the centrally localized targets for insulin (19). However, attention has been also paid to peripheral actions of insulin, such as direct effects on brown adipose tissue (19). Furthermore, feeding conditions, injection procedures, and acute or chronic treatments, are other parameters to be considered when analysing insulin's effects (19). In the design employed here, the short food deprivation period and the single injections may well have played a role in the thermogenic effect, as well as the possibility of a hypoglycemic-induced shivering or an insulin-induced release of epinephrine. Thus, the present data do not address either the site or the mode of insulin's thermogenic effects. They do, however, clearly demonstrate that under certain conditions, at least, insulin increases thermogenesis and carbohydrate dependency.

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