

Energy balance of pregnant diabetic rats

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Pregnancy and diabetes lead to metabolic alterations in the energy balance that may not be completely independent. The objective of the present study was to look at the alterations induced by type 1 diabetes mellitus on the energy balance of pregnant rats and the offspring. Diabetes was induced by streptozotocin injection 15 d before the starting of pregnancy. The rats had their energy balance variables followed for 21 d. Protein, fat and energy content of dams was determined from samples of the carcasses. Pregnancy led to increased energy intake, energy gain and energy expenditure as well as higher gross food efficiency than non-pregnant counterparts. Diabetes increased metabolizable energy intake but not the energy gain of the animals: they had very high energy expenditure, so that diabetes blocked the improvement in gross food efficiency shown during pregnancy. Offspring from diabetic dams were born with lower body weight. Pregnant animals did not present the usual energy storage as seen by lower energy gain of diabetic dams as well as by the lower fat content in the carcasses of pregnant diabetic rats. It is concluded that diabetes impairs the energy variables usually enhanced by pregnancy alone.

Energy balance: Diabetes mellitus: Pregnancy: Energy expenditure

The regulation of energy balance involves the control of food intake and energy expenditure. During pregnancy, most animals increase their food intake (Chaves & Herrera, 1980; Luz & Griggio, 1990; Murphy & Abrams, 1993) as well as the gross food efficiency (Naismith & Brookes, 1983; Luz & Griggio, 1990). Part of the extra energy ingested is used to form the uterine contents and for uterus growth (Rosso, 1975; Hay, 1991; Luz & Griggio, 1992), and part is spent as heat as a consequence of the increased energy expenditure (Richard & Trayhurn 1985; Luz & Griggio, 1990). Furthermore, the major part of the extra intake is stored as fat to be used later during the lactation period when the energy demand is very high due to milk production (Andrews *et al.* 1986; Forsum *et al.* 1988; Trayhurn, 1989).

Diabetes mellitus (DM) is a chronic disorder of metabolism caused by an absolute or relative lack of insulin. Since insulin is the major storage hormone, minimal insulin deficiency results in diminished ability to increase the reservoir of body fuels. Major insulin deficiencies cause not only a decreased fuel accumulation in the fed state, but also an excessive mobilization of endogenous metabolic fuels in the fasting condition, leading to an elevated free fatty acid concentration, hyperglycaemia and hyperaminoacidaemia (Felig & Bergman, 1995).

Increased energy expenditure is a characteristic of DM (Nair *et al.* 1984; Molnár *et al.* 1989) that usually leads the subjects to a compensatory state of hyperphagia. Higher costs of protein synthesis (Nair *et al.* 1984; Charlton and Nair, 1998a) and gluconeogenesis (Nair *et al.* 1984), as well as increased thermogenic response to adrenaline (Shamoon *et al.* 1980; Müller *et al.* 1989) have been associated with the increase in energy expenditure during the diabetic state.

The catabolic state of poorly controlled type 1 DM has largely been attributed to insulin deficiency. Because the increase in protein breakdown is greater than the increase in protein synthesis, insulin deprivation results in a net release of amino acids in skeletal muscle (Charlton & Nair, 1998a), which suggests that insulin exerts an anti-catabolic effect in type 1 DM mainly through the inhibition of muscle protein breakdown.

Hyperglucagonaemia coexists with insulin deficiency or insulin resistance as occurs in many catabolic conditions. It has been shown that hyperglucagonaemia during insulin deficiency results in an increase in energy expenditure (Charlton & Nair, 1998b; Nair, 1987) and accelerated proteolysis (Nair *et al.* 1987).

Since both pregnancy and DM modify the energy balance, the purpose of the present study was to look at the

Abbreviation: DM, diabetes mellitus.

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alterations in the energy balance when pregnancy and DM are present and the repercussions on the offspring.

Material and methods

Fifty-three 3-month-old female Wistar EPM-1 rats with an initial body weight of 192.60 (SEM 1.74) g were used. The animal house was maintained at $23 \pm 2^\circ\text{C}$ on a 12 h light–dark cycle. During the experimental period, half of the rats were kept non-diabetic and the other half were diabetic. For each state, non-diabetic or diabetic, two groups were studied: non-pregnant and pregnant rats, giving four experimental groups as follows: non-pregnant–non-diabetic (n 14), pregnant–non-diabetic (n 13), non-pregnant–diabetic (n 12) and pregnant–diabetic (n 14).

DM was induced by a single intraperitoneal dose of streptozotocin (Sigma, St. Louis, MO, USA) (60 mg/kg) (Geiger *et al.* 1992; Anderson *et al.* 1993) diluted in a citrate buffer solution (0.2 M, pH 4.5) (Woongsurawat & Armbrecht, 1991; Grishan, 1993). Non-diabetic animals were injected with citrate buffer solution in a equivalent volume. In the first 48 h after the streptozotocin administration, the animals were allowed to choose between two glucose solutions (25 and 50 g/l) in order to compensate for hypoglycaemia. After this period, all animals received water and food *ad libitum*. Seventy-two hours after the streptozotocin administration, one drop of blood was taken from the tail of the awake animals to determine the extent of glycaemia by a blood glucose meter (Advantage; Roche Diagnostics, São Paulo, SP, Brazil). The animals were considered diabetic only when the glycaemia was 2.50 mg/ml or higher and at this time they started receiving 1 IU Neutral Protamine Hagedorn (NPH) insulin/d subcutaneously at 17.00 hours (Bibrás, Montes Claros, Brazil) until the last day of the experiment. Control animals received daily injections of saline of the same volume. Fifteen days after streptozotocin administration, one male rat was introduced into a cage with four females and kept there overnight. The presence of spermatozoa in vaginal smears taken in the next morning indicated that mating had taken place and that day was considered the first day of pregnancy.

Pregnant and non-pregnant rats were housed in individual metabolic cages for 21 d (pregnancy period). At the end of experimental period (21 d), the animals were anaesthetized with sodium thiopental (60 mg/kg) and the abdominal cavity was opened. Samples of blood were taken into a tube with heparin by an aortic pricking and the uterine contents (offspring and placentas) were extracted and separated. The uterus and ovaries were separated and weighed. The gut was removed, emptied and put back into the carcass. The carcass was weighed (wet weight) and homogenized in a blender with an equal volume of water. Two samples of 2 g each were collected from the homogenized carcass to determine the fat and protein content. Offspring and placentas from each dam were separated and the number and weight were recorded. Just after the killing by decapitation, the blood of all pups was pooled together into tubes with heparin and their carcasses were weighed (wet weight). Placentas and the offspring carcasses were separately homogenized.

The carcasses of non-pregnant animals were processed in the same way as the pregnant ones. The homogenized material was dried to constant weight in an oven at 60°C (dry weight), and the resulting powder was rehomogenized before a sample was burnt in an adiabatic calorimeter (IKA C-400; Janke and Kunkel, GmbH & Co., Breicgan, Germany). During the whole experimental period, faeces and urine were collected daily for energy content determination. Food intake was recorded daily and samples of pellets were frequently analysed in the calorimeter. Energy intake was calculated by multiplying the amount ingested (g) by the energy content of the diet. Absorbed energy was calculated as energy intake minus energy in the faeces. Metabolizable energy intake was considered as absorbed energy minus energy in the urine.

Body-energy gain was determined from a baseline group of sixty-five control and thirty-six diabetic rats killed on day 0 for each state studied (non-diabetic and diabetic). A regression of body weight *v.* body energy was calculated. The initial body energy of experimental animals was calculated from their body weight utilizing the regression equation calculated earlier. Body-energy gain corresponded to the energy in the carcasses minus the initial body energy. Energy expenditure over the 21 d experimental period was calculated as metabolizable energy intake minus body-energy gain. Gross food efficiency was calculated as energy gain divided by metabolizable energy intake and expressed as a percentage. To calculate energy expenditure and gross food efficiency the energy of offspring, placentas and uterus were added to maternal body-energy gain.

The percentage of water in the carcass was calculated by the difference between wet and dry weight and expressed as a percentage. Fat and protein content in the carcass was measured in the fresh samples of homogenized carcasses by chloroform–methanol (Folch *et al.* 1957) and Lowry (Leshner & Litwin, 1972) methods respectively. Glycaemia in dams and pups was measured by an enzymatic colorimetric method (Glucose E KIT-CELM, São Paulo, SP, Brazil).

Statistical analysis was performed by two-way ANOVA. When significant, the Newman-Keuls test was applied to detect which means were different from each other. Glycaemia and variables at birth of offspring from non-pregnant and pregnant rats at different states (diabetic and non-diabetic) were analysed by *t* test. The significance level to reject the null hypothesis was always set at 0.05 (Hirsch & Riegelman, 1992).

All the procedures and methods with the animals were examined and approved by the ethics committee of the Federal University of São Paulo (UNIFESP-EPM).

Results

Table 1 shows that pregnant rats always had a higher body-weight gain (total and net) than the corresponding non-pregnant ones ($P < 0.05$). DM led to a decrease in body-weight gain (total and net) of pregnant animals.

Table 2 shows the variables of energy balance as well as gross food efficiency and percentage of absorbed energy values. Pregnancy and DM led to an increase in metabolizable

Table 1. Body-weight gain of non-pregnant and pregnant rats in different states (diabetic and non-diabetic)*†
(Mean values with their standard errors)

State...	Body weight (g)								
	Non-pregnant			Pregnant					
	n	Mean	SEM	Total			Net		
n				Mean	SEM	n	Mean	SEM	
Non-diabetic	14	17.0 ^a	2.6	13	116.6 ^b	5.3	13	54.1 ^d	2.4
Diabetic	12	12.8 ^a	3.5	14	94.79 ^c	4.6	14	45.0 ^e	3.0

^{a,b,c,d,e}Mean values with unlike superscript letters were significantly different ($P<0.05$).

* For details of diets and procedures, see p. 510.

† Net body-weight gain corresponds to the total weight gain of the pregnant rats minus the weight of the conceptuses.

energy intake ($P<0.05$). Pregnant diabetic animals showed a significant reduction ($P<0.05$) in body-energy gain. The pregnant non-diabetic rats showed higher body-energy gain than the non-pregnant ones ($P<0.05$). The energy expenditure was significantly increased by pregnancy as well as DM ($P<0.05$). As expected, the pregnant non-diabetic rats showed higher gross food efficiency ($P<0.05$) than the respective non-pregnant ones, and decreased gross food efficiency ($P<0.05$) was observed in pregnant diabetic animals. No significant differences ($P>0.05$) for percentage of absorbed energy were detected.

The variables of body composition and glycaemia are shown in Table 3. Pregnancy did not affect ($P>0.05$) these variables in both states (diabetic and non-diabetic), except that pregnant non-diabetic animals had higher protein content than non-pregnant ones. DM led to a decrease in fat ($P<0.05$), but not in water content ($P>0.05$). Obviously glycaemia was increased in diabetic rats ($P<0.05$).

Table 4 shows the variables of the uterus from non-pregnant and pregnant animals. Uterus weight and energy content were significantly higher ($P<0.05$) for pregnant rats when compared with the respective non-pregnant animals. DM led to a reduction in uterus weight in both non-pregnant and pregnant animals ($P<0.05$). Uterus energy content was not altered by DM ($P>0.05$).

Table 5 shows the variables of urine from non-pregnant and pregnant animals. Pregnancy did not lead to significant differences ($P>0.05$) in urinary volume, but increased ($P<0.05$) urinary energy in non-diabetic rats. DM increased urinary volume and energy in both pregnant and non-pregnant animals ($P<0.05$).

The number, weight, energy content and glycaemia of the offspring are shown in Table 6. All values represent the average of pooled conceptuses. Maternal DM did not affect the number of pups or the placentas ($P>0.05$). The offspring energy content was also not affected by maternal

Table 2. Variables of energy balance, gross food efficiency and absorbed energy in non-pregnant and pregnant rats in different states (diabetic and non-diabetic)*
(Mean values with their standard errors)

State...	Non-pregnant			Pregnant		
	n	Mean	SEM	n	Mean	SEM
Metabolizable energy intake (kJ/21 d)						
Non-diabetic	14	4209.1 ^a	210.4	13	5685.6 ^c	176.3
Diabetic	12	5375.6 ^{bc}	236.6	14	6374.6 ^d	234.3
Energy gain (kJ/21 d)						
Non-diabetic	14	214.2 ^a	59.8	13	765.6 ^c	105.4
Diabetic	12	-10.5 ^{ab}	62.6	14	119.6 ^b	65.3
Energy expenditure (kJ/21 d)†						
Non-diabetic	14	3994.8 ^a	198.0	13	4920.2 ^b	176.0
Diabetic	12	5386.1 ^b	269.0	14	6255.5 ^c	263.7
Gross food efficiency (%)						
Non-diabetic	14	4.96 ^a	1.33	13	13.36 ^b	1.69
Diabetic	12	1.91 ^a	1.00	14	2.11 ^a	1.11
Absorbed energy (%)						
Non-diabetic	14	74.27 ^a	0.93	13	74.89 ^a	0.42
Diabetic	12	73.63 ^a	0.81	14	74.32 ^a	1.26

^{a,b,c,d}Mean values with unlike superscript letters were significantly different: $P<0.05$.

* For details of diets and procedures, see p. 510.

† To calculate energy expenditure, the energy content of fetuses and placentas were added to maternal energy gain.

Table 3. Variables of body composition (fat, protein and water in the carcass) and glycaemia of non-pregnant and pregnant rats in different states (diabetic and non-diabetic)*
(Mean values with their standard errors)

State...	Non-pregnant			Pregnant		
	<i>n</i>	Mean	SEM	<i>n</i>	Mean	SEM
Protein content (g/kg)						
Non-diabetic	14	254.35 ^a	11.45	13	288.28 ^b	12.30
Diabetic	12	300.38 ^b	7.16	14	291.01 ^b	9.66
Fat content (g/kg)						
Non-diabetic	14	126.01 ^a	7.89	13	137.28 ^a	10.72
Diabetic	12	75.15 ^b	5.43	14	84.81 ^b	6.19
Water content (%)						
Non-diabetic	14	64.41 ^a	0.36	13	62.71 ^a	0.38
Diabetic	12	68.55 ^a	0.55	14	69.39 ^a	0.67
Glycaemia (mg/ml)						
Non-diabetic	14	1.440 ^a	0.080	13	1.030 ^a	0.030
Diabetic	12	4.370 ^b	0.345	14	4.763 ^b	0.310

^{a,b}Mean values with unlike superscript letters were significantly different ($P < 0.05$).

* For details of procedures, see p. 510.

DM besides the decreased body weight ($P < 0.05$) noted in pups from diabetic mothers. Maternal DM led to a significant increase ($P < 0.05$) in pups glycaemia.

Discussion

The current study demonstrated that DM led to an increase of the energy expenditure not only in non-pregnant, but also in pregnant animals. All of our diabetic animals were treated daily with insulin in order to avoid large differences in the individual glycaemia values among diabetic animals, as well as to keep the glycaemia levels into a range that allowed the animals survival.

The pregnancy, as expected, increased the energy expenditure as a consequence development of conceptuses and placentas (Rosso, 1975; Luz & Griggio, 1990; Hay, 1991). In order to compensate for this increased energy expenditure, the animals enhanced their metabolizable energy intake and showed increase in the net body-weight gain (Luz & Griggio, 1992, 1998).

It has been found that type 1 DM usually leads to an increase in energy expenditure in human subjects and animals (Nair *et al.* 1984; Molnár *et al.* 1989). This increase is attributed mainly to a higher protein catabolism (Bennet

et al. 1991; Charlton & Nair 1998a), besides hyperglucagonaemia (Salter, 1960; Boden *et al.* 1977; Nair *et al.* 1987) and higher thermogenic response to adrenaline (Shamoon *et al.* 1980; Müller *et al.* 1989). Our present results, in agreement with this literature, showed an increased energy expenditure in diabetic animals in both pregnant and non-pregnant (about 16 and 23 % respectively) compared with the respective non-diabetic animals. In order to compensate for the increased energy expenditure, diabetic animals enhanced their metabolizable energy intake. Besides the hyperphagia, pregnant diabetic animals were not able to maintain the body-weight and body-energy gain at the same levels shown by control animals which can be explained by the lower gross food efficiency presented by them. Impairment of gross food efficiency is probably due to higher costs of protein synthesis (Nair *et al.* 1984) and gluconeogenesis (Nair *et al.* 1984) present in diabetic subjects. It is interesting to notice that DM even led to an inhibition of the characteristic increase of gross food efficiency during pregnancy as previously reported (Luz & Griggio, 1990). DM can lead to gastrointestinal alterations such as dysphagia, abdominal pain, nausea,

Table 4. Weight and energy content of uteruses from non-pregnant and pregnant rats in different states (diabetic and non-diabetic)*
(Mean values with their standard errors)

State...	Non-pregnant			Pregnant		
	<i>n</i>	Mean	SEM	<i>n</i>	Mean	SEM
Uterus weight (g)						
Non-diabetic	14	0.63 ^a	0.03	13	3.75 ^c	0.12
Diabetic	12	0.55 ^b	0.04	14	3.50 ^d	0.12
Uterus energy content (kJ)						
Non-diabetic	14	2.81 ^a	0.32	13	15.76 ^b	1.37
Diabetic	12	3.16 ^a	0.24	14	14.17 ^b	0.60

^{a,b,c,d}Mean values with unlike superscript letters were significantly different ($P < 0.05$).

* For details of procedures, see p. 510.

Table 5. Urine volume and energy content from non-pregnant and pregnant rats in different states (diabetic and non-diabetic)*
(Mean values with their standard errors)

State...	Non-pregnant			Pregnant		
	<i>n</i>	Mean	SEM	<i>n</i>	Mean	SEM
Urinary volume (ml/d)†						
Non-diabetic	14	8.68 ^a	0.37	13	11.88 ^a	0.64
Diabetic	12	56.23 ^b	6.66	14	64.00 ^b	4.62
Urinary energy (kJ/g)‡						
Non-diabetic	14	5.91 ^a	1.02	13	6.88 ^c	0.54
Diabetic	12	21.62 ^b	3.89	14	18.21 ^{bd}	1.82

^{a,b,c,d}Mean values with unlike superscript letters were significantly different ($P < 0.05$).

* For details of diets and procedures, see p. 510.

† Daily urinary volume was averaged over the 21 d of the experiment.

‡ Urinary energy was obtained from a sample of the urine collected over 21 d of the experiment.

Table 6. Plasma glucose (mg/ml) and variables at birth of offspring from non-pregnant and pregnant rats in different states (diabetic and non-diabetic)†‡ (Mean values with their standard errors)

State...	Non-diabetic (n 13)		Diabetic (n 14)	
	Mean	SEM	Mean	SEM
Pups (n)	11.1	0.7	10.6	0.6
Weight (g)				
Pups	4.92	0.19	4.00*	0.26
Placentas	0.65	0.02	0.70	0.05
Energy (kJ)				
Pups	12.53	0.59	10.65	1.04
Placentas	2.00	0.06	2.16	0.13
Glycaemia of pups (mg/ml)	1.090	0.130	3.852*	0.291

Mean values were significantly different from those of the non-diabetic group: * $P < 0.05$.

† For details of diets and procedures, see p. 510.

‡ Each individual body weight, body energy value and glycaemia value corresponded to the average of the pooled body weight and energy value of the offspring from each dam.

vomiting, diarrhoea and malabsorption. These symptoms can be associated with altered gastrointestinal muscarinic response (Altan *et al.* 1987) and functional alterations in gastrointestinal gap junctions, which could result in a decreased gastrointestinal motility (Öztürk *et al.* 1997), and reduction in nutrient absorption. Nevertheless, our diabetic animals did not show any alterations of their absorptive capacity represented in the present study as the percentage of absorbed energy.

Insulin deficiency is usually accompanied by elevation of plasma fatty free acid concentration as a result of increased free fatty acid outflow from fat depots, where the balance of the free fatty acid esterification–tracylglycerol lipolysis cycle is displaced in favour of lipolysis (Felig & Bergman, 1995). Thus, as expected, our diabetic animals, pregnant or not, showed a significant reduction in their carcass fat content.

Besides fat metabolism alterations, diabetic subjects can show a negative N balance and marked substrate wasting which is not surprising, since insulin, when present in normal amounts, stimulates protein synthesis and amino acid uptake and inhibits protein catabolism and the output of amino acids from muscle (Felig & Bergman, 1995). In fact, we did not detect significant differences between carcass protein content for pregnant diabetic animals, probably because of insulin administration, but non-pregnant diabetic rats had an increased protein content in the carcass.

Considering that pregnancy leads to uterus hypertrophy, an increase in the weight and energy content in this tissue is expected during this period (Luz & Griggio, 1998). On the other hand, a reduction in uterus weight could be expected in diabetic animals since DM can alter the hypothalamus–hypophysis–ovary axis leading to a reduction in plasma concentrations of the luteinizing hormone, follicle-stimulating hormone, prolactin and oestrogen (La Marca *et al.* 1999), and it is reported that a decrease in oestrogen levels can reduce uterus size (Merry & Holehan, 1994). Accordingly, we observed a reduction in uterus weight in diabetic animals.

It is widely known that polyuria, glucosuria and proteinuria occur in diabetic subjects (Felig & Bergman, 1995).

Therefore, the results obtained for urinary variables were expected, since the glucose and protein content present in urine from diabetic animals must raise their urinary energy losses. The urinary energy losses represented 2.3 and 21.3% absorbed energy for control and diabetic animals respectively, while pregnancy did not affect these variables.

The effects of DM on the development of fetuses and placentas are controversial. A high incidence of congenital malformation (Diamant, 1991; Giavini *et al.* 1993; Menebola *et al.* 1995; Siman & Ericksson, 1997), abortion, and fetal reabsorption (Giavini *et al.* 1993; Siman & Ericksson, 1997) have been reported during diabetic pregnancies. Some authors have reported increased fetal and placental weights (Hod *et al.* 1991; Mayhew *et al.* 1993; Boden, 1996; Fu *et al.* 1996) from diabetic mothers while others have reported decreased (Atkins *et al.* 1994; Takenaka & Toyoda, 1995; Claubat *et al.* 1997) or no alteration (Ezekwe *et al.* 1984; Clarson *et al.* 1989) in weight. Our present results show that the maternal DM affected only the fetuses' weight, whereas there were no differences in the number of fetuses and placentas or their energy content nor in placental weight.

It is known that fetal glycaemic status tracks that of the mother, with fetal hyperglycaemia and hyperinsulinaemia subsequent to maternal hyperglycaemia (Illisley, 2000) and, considering the fetuses' capacity for insulin release, it is expected that glycaemia would be lower in fetuses than their mothers (Rudge *et al.* 1995). In accordance with Uvena-Celebrezze & Catalano (2000), fetal blood glucose levels *in utero* are approximately 70–80% of maternal glucose levels. Consistent with these previous observations, we found that fetuses showed had glycaemia levels lower than those of their mothers (80.9%).

The present study shows that DM impaired energy deposition in pregnant animals due to high energy expenditure. Furthermore, in pregnant animals, DM blocked the improvement in food efficiency and fat deposition usually seen during pregnancy, leading to a loss of weight or energy of the dams and the offspring, which were born with lower body weight than controls.

Several of the DM-related impairments described are

also shown in human subjects, which suggests that the rat is a good experimental model for DM. Differences described may be related to insulin therapy imposed on human subjects, which is quite different from the present experimental procedure. Insulin was given to rats to keep glycaemia at equally high levels among the group and to avoid interruption of gestation that otherwise would occur, rather than as a therapeutic procedure to control it.

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