



# Thermodynamic assessment of allocation of energy and exergy of the nutrients for the life processes during pregnancy

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## Abstract

Thermodynamic analyses are performed to quantify allocation of the nutritional energy and exergy to most of the life processes by pregnant mice. In these analyses, 'internal work performance' is calculated for the first time in the literature for metabolism during pregnancy and found substantially higher than the 'external work performance'. Variation of the daily entropy generation rates and the daily internal work performance rates during the course of pregnancy showed a highly similar phasic behaviour. With the progression of the pregnancy, external work performance decreased and second law efficiency increased significantly. On the 13th day of pregnancy, net energy extracted from the food at the cellular energy metabolism subsystem was 15.0 kJ; approximately 3 kJ of it was employed for daily internal work performance, 0.8 kJ was allocated to daily external work performance and 0.8 kJ was stored in the adipose tissue without entering into the cellular energy metabolism subsystem. Heat generation in association with internal and external work performance was 9.1 and 2.2 kJ, respectively. Energy, pertinent to the first law, and exergy (useful energy), pertinent to the second law, balances are described graphically, and comparison of these plots showed that the total exergy of the nutrients allocated to internal and external work performance and heat generation is substantially smaller in magnitude when compared with those of energy balance.

**Key words:** Pregnant mice: Thermodynamic analyses: Allocation of nutrient energy: Life processes

Thermodynamic analyses have proved to be one of the most important tools for assessment of nutrition<sup>(1–6)</sup>. Movement, reproduction, sensation, growth, respiration, excretion and nutrition are referred to as the life processes in the literature<sup>(7)</sup>. Nutrition and respiration mean energy and oxygen intake to maintain metabolic activity. Excretion describes how the body gets rid of the metabolic waste through faeces, urine and by other means. Movement, reproduction and growth are the life processes, where most of the energy uptake from the foods is consumed. Thermodynamic interaction between the six of the seven life processes will be considered in this manuscript. Sensation is not a major energy sink like the others<sup>(8)</sup>, therefore will not be considered here.

Organisms maintain their living far from equilibrium with their surroundings by establishing mass and energy gradients between themselves and the environment<sup>(9)</sup>. A living system behaves as a unified whole made of elements that interact with each other<sup>(10)</sup>. Bioenergy extracted from the nutrients is converted from one form to another or work and heat in the process of living. Multiple and complex mechanisms have evolved in the body to regulate the interrelation between the energy intake with nutrients and its allocation to different life processes and storage

as fat<sup>(11)</sup>. Entropy, which is the measure of the unusable energy, is generated inevitably in these processes. Animals may tend to respond to increased energy demand in any of these life processes both via increasing energy intake and or by reducing energy allocation to the other life processes<sup>(12)</sup>. For instance, when the RMR of the birds increases upon immunisation, they may tend to increase consumption of nutrients, reduce the amount of the work performed<sup>(13–15)</sup> or reduce the energy expenditure for growth of regenerated feathers<sup>(16)</sup>.

Second law analyses evaluate useful energy, that is, exergy, within the context of the quality of the energy, and give an idea about its dissipation in terms of exergy destruction or entropy generation<sup>(17)</sup>. Exergy destruction analyses have been employed to provide valuable information about the quality of the energy conversions in metabolic processes<sup>(18,19)</sup>, understanding the effect of the externally imposed conditions on ageing<sup>(20,21)</sup>, comparing the efficiency of competing metabolic pathways in the brain<sup>(22,23)</sup>, muscle work performance<sup>(24–26)</sup>, thermodynamic analyses of nutrition<sup>(27)</sup> and anticancer therapy<sup>(28)</sup>. The energy cost of a reproductive event and the associated morphological changes is considered as the direct physiological cost of reproduction. Energy cost of thermogenesis, immune function and

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physical activity, hyperthermia, bone loss, disruption of sleep patterns, oxidative stress, etc. may be regarded among the consequential indirect costs<sup>(29)</sup>. Animals may reduce these costs to minimise the resource input for reproduction.

This study focuses on evaluating how the energy and exergy of nutrients are allocated to the life processes during pregnancy. With this purpose, the first and second laws of thermodynamics will be employed, internal and external work performance and entropy generation will be assessed and the variation of the second law efficiency of the fetal growth processes will be determined. Energy and exergy charts will be constructed and compared to evaluate the effect of entropy generation on the allocation of exergy of the nutrients to the life processes.

**Methodology and approaches**

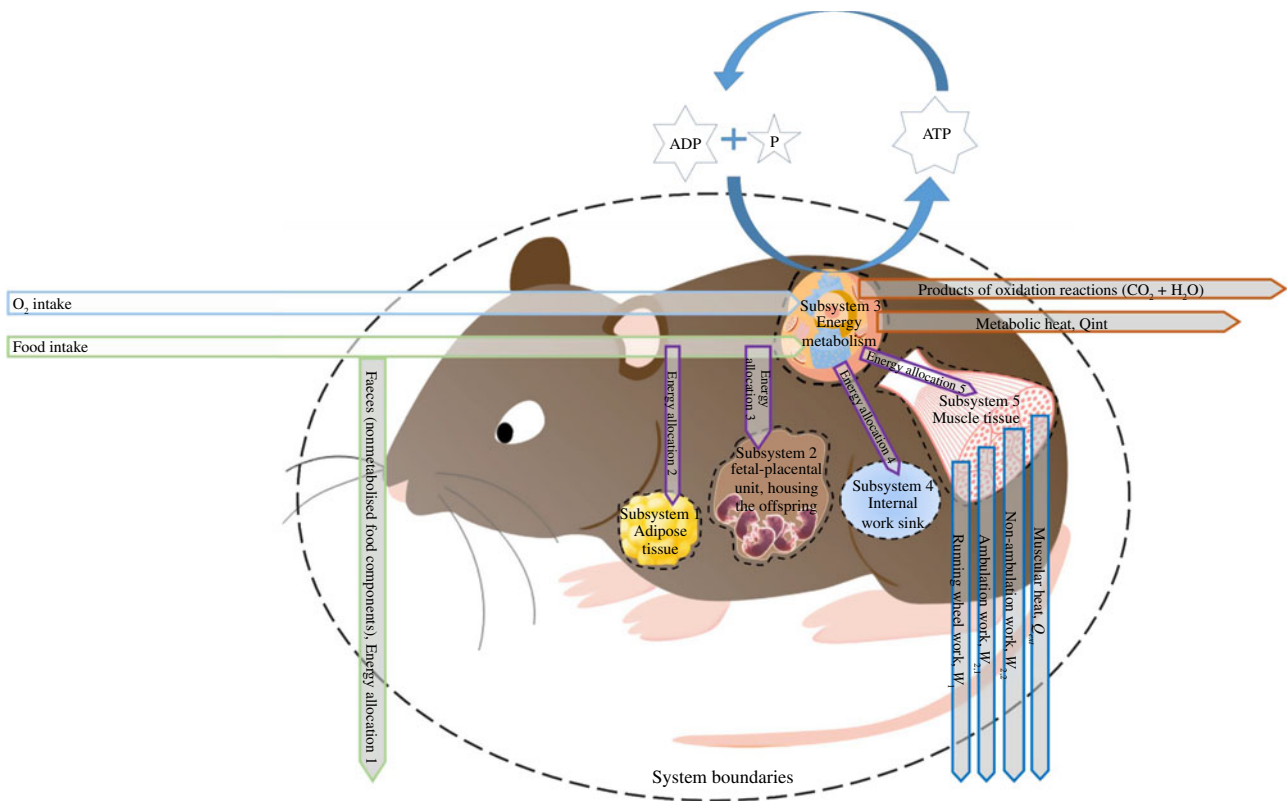
*Data and system boundaries*

The pregnant mouse is the thermodynamic system; its life processes are analysed after dividing it into conceptually distinct subsystems: the adipose tissue, fetal-placental unit, housing the offspring, the amniotic fluid, energy metabolism, internal work sink and the muscle tissue (Fig. 1). Experimental data obtained with the C57BL/6J mice were adapted from Ladyman *et al.*<sup>(30)</sup>. Data obtained for the first 18 days of pregnancy included the body weight, daily food intake, average RER, daily running wheel distance, ambulation and non-ambulatory movement distances. Cumulative incremental body weight data were interpolated to cover the entire 18 days of pregnancy by curve fitting as explained in the online Supplementary material.

*Mass and energy balances*

Each day of pregnancy was assessed individually in terms of mass, energy and exergy balances. Nutrients enter into the system with their inherent energy. A non-metabolised fraction of the food is excreted with the faeces<sup>(27)</sup>. The faeces are assumed to contain 5.2% of fat, 7.9% of protein and 2.4% of carbohydrate up-taken in the diet based on the observations of Romo-Vaquero *et al.*<sup>(31)</sup> and shown with the arrow labelled as ‘faeces, energy allocation 1’ in Fig. 1. Weight-related data, such as the daily body weight of the mice, weight increments of the adipose tissue, offspring, placenta and the amniotic fluid, are presented in the online Supplementary material. In addition, chemical energy-related data, such as the waste with faeces, accumulation with the abdominal fat and the expense for manufacturing the offspring and the placenta, are presented in online Supplementary Table S1, together with the other thermodynamic data.

*Mass and energy flow around subsystem 1.* Cumulative incremental body weight data were used to calculate energy accumulation within system boundaries in the adipose tissue ( $m_1$ ), in the fetal-placental unit ( $m_2$ ) which houses placenta, offspring and amniotic fluid. The ‘total weight gain’ during the pregnancy including the litter, placenta, adipose tissue, etc. was 19.3 g, as estimated from the fitted empirical model (data are presented in the online Supplementary material). The ‘total maternal body weight gain’ was 3.7 g at the end of the pregnancy<sup>(30)</sup> and assumed to be stored in the adipose tissue ( $m_1$ ). The percentage ratio of the total maternal body weight



**Fig. 1.** Schematic description of a mouse as an open thermodynamic system.

gain:total weight gain was calculated as 19 % and used to predict the daily maternal body weight gain ( $m_1$ ), which was assumed to be occurring in the adipose tissue (subsystem 1), as given in online Supplementary Table S1. The following assumptions were done while developing the model:

- Temperature, pressure and concentration distributions are uniform throughout the system;
- Aerobic cellular metabolism prevails;
- Both the internal and the external work performance and heat generation are fuelled only by the energy extracted from the cellular metabolism, not by adipose tissue or organ utilisation;
- Sucrose is hydrolysed to glucose and fructose; glucose enters into glycolytic pathway directly; fructose enters the same pathway as fructose 6-P after coming from the liver;
- Glucose degradation occurs through the glycolytic pathway only, which means that all the glucose in the food is oxidised in the cellular metabolism;
- There is not any depletion of food or starvation situation. Only carbohydrate and fat in the food are used as the major energy sources to fuel the cellular energy metabolism. Correspondingly, amino acids obtained from the food proteins (except the amount excreted in the faeces) are used in the synthesis pathways to produce new proteins for both the mother and the offspring and not used as an energy source;
- Daily amount of the faeces is proportional to food consumption, but its composition is always the same;
- Mass accumulation occurs in the adipose tissue and in the fetal-placental unit. Placenta, offspring and the amniotic fluid are located in the fetal-placental unit; incremental weight in various maternal tissues is neglected;
- Fat, protein, water and ash contents of the offspring were assumed to be the same as that of the U.B.C. strain Swiss Albino male mice as reported by Bailey *et al.*<sup>(32)</sup>;
- The fatty acid composition of the fat part of the offspring was assumed as the same as that of the adipose tissue reported by Kassem *et al.*<sup>(33)</sup>. The species and age differences of rodents were ignored. The proteins of the offspring were assumed to have the average theoretical amino acid composition given by Mady & Oliveira<sup>(19)</sup>;
- Exergy destruction is calculated after performing an exergy balance around the cellular metabolism subsystem. Exergy flow occurs via incoming food components and oxygen, leaving products of the oxidation reactions, heat transfer and work flow. Contribution of the other cellular activities to exergy flow is neglected.

As presented in the online Supplementary Table S6, specific enthalpy of formation of the fatty acid molecules at 298.15 K ( $\Delta h^\circ_{f,298-15}$ ) was obtained from the NIST webbook database<sup>(34)</sup> or calculated with equation 1 adapted from Green & Perry<sup>(35)</sup>.

$$\Delta h^\circ_{f,298.15} = 68 \cdot 29 + \sum_{i=1}^{n_{\text{groups}}} n_{i,\text{group}} \Delta h^\circ_{f,i,\text{group}} \quad (1)$$

where  $n_{\text{groups}}$  is the number of the atomic groups contained in the molecule;  $n_{i,\text{group}}$  is the number of the atoms  $i$  contained

in a molecule;  $\Delta h^\circ_{f,i,\text{group}}$  is the standard enthalpy of formation of that atomic group. All values were re-calculated for physiological conditions with equation 2<sup>(35)</sup>.

$$\Delta h^\circ_{f,T} = \Delta h_{f,298.15} + c_{p,298.15} \times \Delta T \quad (2)$$

Some of the  $c_p$  values are given in literature, but the others are calculated by using the modified Kopp's rule, as described by Hurst & Harrison<sup>(36)</sup> and presented in the online Supplementary material.

$$c_p = \sum_{i=1}^{N_i} n_E c_E \quad (3)$$

Energy accumulation in the adipose tissue is described in Fig. 1 as 'energy allocation 2', and daily numerical values are given in online Supplementary Table S1.

**Mass and energy flow around subsystem 2.** In the model, after excluding the percentage of the weight gain referred to as the 'total maternal body weight gain', the remaining 81 % of the body weight increment was associated with the daily weight increase of the fetal-placental unit ( $m_2$ ) including those of the offspring ( $m_{2,1}$ ), placenta ( $m_{2,2}$ ) and amniotic fluid ( $m_{2,3}$ ). Energetic value (26 384 J/g), weight (0.6 g) and the energy (16 754 J) of the placenta were adapted from the experimental data presented by Myrcha *et al.*<sup>(37)</sup>; the total weight of the placenta plus that of the amniotic fluid was reported in the same study as 1.6 g. The average litter weight was 5.6 g<sup>(30)</sup>, and its energetic value was 4.0 kJ/g<sup>(37)</sup>. These data were used in the current study to estimate  $m_2$ ,  $m_{2,1}$ ,  $m_{2,2}$  and  $m_{2,3}$ . At the last day of pregnancy, each of  $m_{2,2}$  and  $m_{2,3}$  was estimated as 4.0 and 6.0 g, respectively. Daily increments of each mass constituent were predicted from the last day of pregnancy data in proportion to the weight of mice. These given energy content of the biomass and placenta were used to calculate energetic costs of offspring and placenta which are presented as 'energy allocation 3' in Fig. 1. The daily numerical values are given in online Supplementary Table S1. This energy allocation was assumed to be the same as chemical energy consumed in the production processes within subsystem 2 boundaries. In this assessment, energy cost of the amniotic fluid was ignored.

**Mass and energy flow around subsystem 3.** The daily food intake data, collected from a control group of pregnant C57BL/6J mice fed with standard rodent chow diet and water *ad libitum*, were adapted from Ladyman *et al.*<sup>(30)</sup>. It was assumed that the nutrients ended up in the faeces left the system boundaries without entering metabolism; therefore, their chemical energies or exergies were not used in any balance. Since there were sufficient carbohydrates and fats in the medium, the model is based on the consideration that the amino acids were not employed in the energy metabolism. The net amount of the food entering energy metabolism, that is, subsystem 3, was calculated after correcting for the amounts diverted to faeces, adipose tissue and the fetal-placental unit, that is, subsystem 2. Food protein was assumed to be utilised in the fetal-placental unit processes, and any remainder was excreted in the urine. The remaining amount of the food was oxidised within the cellular

metabolism subsystem boundaries. RER (the ratio of the volume or mole of CO<sub>2</sub> produced:the volume of O<sub>2</sub> used) was employed like an efficiency factor of the oxidation process<sup>(38)</sup> to determine the proportion of the carbohydrates utilised in metabolism. Ladyman *et al.*<sup>(30)</sup> reported the average daily RER values at 2nd–3rd, 4th–6th, 7th–9th, 10th–12th, 13th–15th and 15th–18th days of pregnancy as 0.90, 0.90, 0.92, 0.93, 0.96 and 0.96, respectively. These values were multiplied by the energy extracted from nutrients during cellular metabolism ( $\Delta h_{rxn}$ ). Energy of the food entering into subsystem 3 was calculated for 310.15 K by using the energies of its major components in the online Supplementary material. Major food components of the standard control diet as provided by the manufacturer (Altromin) are presented in the online Supplementary material.

In order to calculate 'energy allocation 5' given in Fig. 1, numerical values for daily external (muscular) work performance ( $W_{ext}$ ) and the corresponding heat generation ( $Q_{ext}$ ) were needed. Daily running wheel distance data presented by Ladyman *et al.*<sup>(30)</sup> were used for the calculation of the work done on the running wheel ( $W_1$ ). It is the sum of the displacement work ( $W_x$ ) on the running wheel plus the work done against friction ( $W_f$ ) of the material of the wheel (equations 4–8).

$$W_1 = W_x + W_f \tag{4}$$

$$W_x = F_x \Delta_x \tag{5}$$

$$F_x = m g \tag{6}$$

$$W_f = F_f \Delta_x \tag{7}$$

$$F_f = m g \mu_f \tag{8}$$

where  $F$  is the force,  $m$  is the mass of a mouse,  $g$  is the gravitational acceleration,  $\Delta_x$  is the running distance, and  $\mu_f$  is the friction factor of running wheel material. Ladyman *et al.*<sup>(30)</sup> distinguished the active ambulation and the non-ambulatory movements on the basis of travelling >1 cm or <1 cm of distances, based on  $x$  and  $y$  beam breaks. Ambulation work ( $W_{2,1}$ ) and the non-ambulatory work ( $W_{2,2}$ ) were calculated after neglecting friction (equation 5). These ambulatory and non-ambulatory work components were added up to calculate the spontaneous activity ( $W_2$ ). Both voluntary ( $W_1$ ) and spontaneous activity ( $W_2$ ) were used to calculate external work,  $W_{ext}$ .

$$W_2 = W_{2,1} + W_{2,2} \tag{9}$$

$$W_{ext} = W_1 + W_2 \tag{10}$$

The first law of the thermodynamics after neglecting the potential and kinetic energy is

$$\sum_{in} [\dot{N} h]_{in} - \sum_{out} [\dot{N} h]_{out} - \dot{Q}_{out} - \dot{W}_{out} = d[\dot{N} h]_{system}/dt \tag{11}$$

where subscripts 'in' and 'out' indicate the entering and leaving quantities through the system boundaries, respectively,  $\dot{N}$  refers

to the molar flow rate,  $h$  indicates specific enthalpy,  $\dot{Q}$  and  $\dot{W}$  indicate heat and work flow rates, respectively. These quantities refer to the 'numerical amounts' and are positive<sup>(39)</sup>. Therefore, both heat and work components which are released from subsystem 3 had 'minus' signs in equation 11. It was assumed that if the ingested food undergoes oxidation reactions, then internal energy hidden in their chemical structures becomes available for the metabolism within subsystem 3 boundaries<sup>(9)</sup>. The term ' $d[\dot{N} h]_{system}/dt$ ' on the right hand of equation 11 refers to the energy accumulation within the system boundaries; since there would be no accumulation in any organ in a healthy organism, it was neglected. Thus, the enthalpy of oxidation reaction ( $\Delta H_{rxn}$ ) of the food components could be considered as the fuelling energy for work performance and heat generation<sup>(40)</sup>. Total daily heat and work outputs, external and internal work are presented in Table 1.

The term 'internal work performance' was defined previously as the unseen part of the muscle work performance<sup>(41,42)</sup>. In Table 2, based on the observations of Sherwood<sup>(43)</sup> and Guyton & Hall<sup>(44)</sup>, 25 % of the energy extracted from the nutrients were allocated for work and the remaining to heat generation. Daily external work performance was calculated with equation 10, and then, the internal work,  $W_{int}$ , was calculated after subtracting the external work from the total work output. It was assumed that the internal work includes that of the heart beating, breathing, formation of new molecules, maintenance of the body, replacement of the dead cells, muscle contraction–relaxation process, signal transmission in neurons and active transport of some molecules<sup>(44–46)</sup>. Daily internal and external work performance rates and energy accumulation in the adipose tissue are drawn *v. time* in the same graph (Fig. 2) for easy comparison, and multiple linear regression analyses were done after considering the internal work performance as the dependent variable, and energy accumulation in the adipose tissue and the external work performance as independent variables. After excluding the energies of products of the metabolic oxidation, heat generation and internal work performance,

**Table 1.** Daily heat and work outputs during pregnancy\*

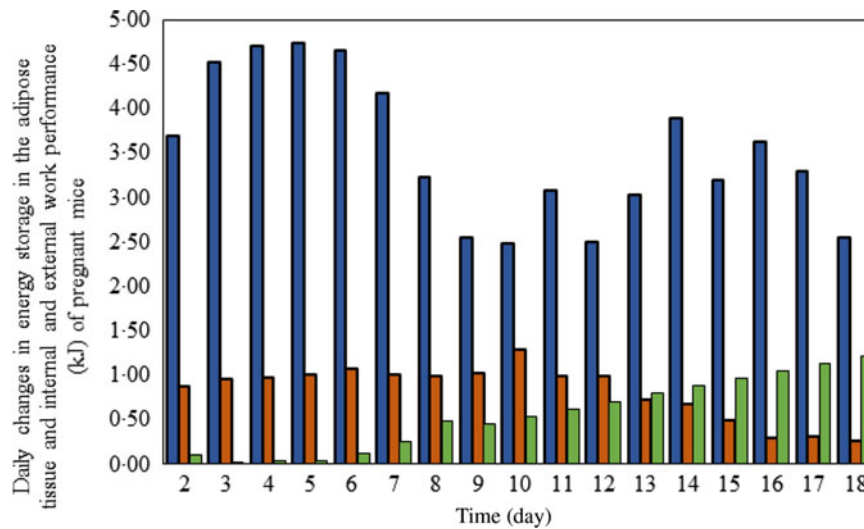
Days	$Q_{out} + W_{out}$ (kJ)	$W_{ext}$ (kJ)	$W_{int}$ (kJ)	$Q_{ext}$ (kJ)	$Q_{int}$ (kJ)
2	18.2	0.9	3.7	2.6	11.1
3	21.9	1.0	4.5	2.9	13.6
4	22.7	1.0	4.7	2.9	14.1
5	23.0	1.0	4.7	3.0	14.2
6	22.9	1.1	4.7	3.2	14.0
7	20.8	1.0	4.2	3.0	12.5
8	16.9	1.0	3.2	3.0	9.7
9	14.3	1.0	2.5	3.1	7.6
10	15.1	1.3	2.5	3.8	7.5
11	16.3	1.0	3.1	3.0	9.2
12	14.0	1.0	2.5	3.0	7.5
13	15.0	0.7	3.0	2.2	9.1
14	18.3	0.7	3.9	2.0	11.7
15	14.8	0.5	3.2	1.5	9.6
16	15.7	0.3	3.6	0.9	10.9
17	14.4	0.3	3.3	0.9	9.9
18	11.2	0.3	2.5	0.8	7.6

\* All quantities represent 'amounts'; thus, they are positive quantities. In the energy balance of equation 11, their direction was considered.

**Table 2.** Constituents of the cellular metabolism subsystem exergy balance

Days	Exergy entering with the food (kJ)	Exergy of the oxygen inhaled (kJ)	$Mex_{in}$ values after being multiplied with RER (kJ)	Exergy of the carbon dioxide exhaled (kJ)	Exergy of the metabolically generated water (kJ)	Exergy destruction (kJ)*	$\eta_{II}$ (%)*	Exergy destruction per mole of ATP produced (kJ/mol)
2	23.5	0.2	21.3	0.9	0.5	15.1	20.4	50.2
3	28.3	0.2	25.6	1.1	0.6	18.2	20.4	50.2
4	29.1	0.2	26.5	1.1	0.6	18.8	20.6	50.0
5	29.5	0.2	26.9	1.1	0.6	19.1	20.6	50.5
6	29.4	0.2	26.8	1.1	0.6	19.0	20.6	50.5
7	26.2	0.2	24.2	1.0	0.5	17.2	20.9	51.4
8	21.3	0.2	19.7	0.8	0.4	14.0	20.9	51.4
9	18.0	0.2	16.7	0.7	0.4	11.9	20.9	51.4
10	18.8	0.2	17.6	0.7	0.4	12.5	21.2	52.2
11	20.3	0.2	19.0	0.8	0.4	13.5	21.2	52.2
12	17.4	0.1	16.3	0.7	0.3	11.6	21.2	52.2
13	18.1	0.2	17.5	0.7	0.4	12.5	21.8	53.2
14	22.0	0.2	21.3	0.9	0.4	15.2	21.8	53.9
15	17.8	0.2	17.2	0.7	0.3	12.3	21.8	53.9
16	18.9	0.2	18.3	0.7	0.4	13.1	21.9	54.0
17	17.4	0.1	16.8	0.7	0.3	12.0	21.9	54.0
18	13.5	0.1	13.1	0.5	0.3	9.3	21.9	54.0

\* Exergy destruction and second law efficiencies were calculated with equations 16 and 17, respectively.



**Fig. 2.** Variation of the internal and external work and accumulation in the adipose tissue during pregnancy. ■,  $W_{int}$  (kJ); ■,  $W_{ext}$  (kJ); ■, energetic increment in adipose tissue (kJ).

the remaining energy outputs of subsystem 3 are allocated to subsystem 5, as described in Fig. 1.

### Exergy balance

Similar to the energy flow throughout the system, exergy flow is also associated with mass, heat and work flow.

The percentage of the nutrients ending up in the faeces was adapted from Romo-Vaquero *et al.*<sup>(31)</sup>. Chemical exergy,  $ex^{\circ}_{chem}$ , of the nutrients is generally available in the literature, but in cases when such data were not available, calculations were based on equation 12<sup>(40)</sup>:

$$ex = ex^{\circ}_{chem} = \Delta g^{\circ}_{f,T} + \sum_{k=1}^{n_{elements}} n_{k,element} ex^{\circ}_{chem,k,element} \quad (12)$$

The standard Gibbs free energy of formation,  $\Delta g^{\circ}_{f,298.15}$ , included in specific exergy calculations refers to the formation energy of one mole of a compound at its standard state. Joback & Reid<sup>(47)</sup> proposed equation 13 to calculate the standard Gibbs free energy of formation with the group contribution method:

$$\Delta g^{\circ}_{f,298.15} = 53.88 + \sum_{i=1}^{n_{groups}} n_{i,group} \Delta g^{\circ}_{f,i,group} \quad (13)$$

Under constant ionic strength and pressure, the Gibbs free energy of formation of a molecule was calculated as:

$$\Delta g^{\circ}_{f,T} = \left( [T/T_0] \Delta g^{\circ}_{f,298.15} + [1 - (T/T_0)] \Delta h^{\circ}_{f,298.15} \right) \quad (14)$$

After combining equations 13 and 14, we will obtain:

$$ex^{\circ}_{chem,T} = \left( [T/T_0] \Delta g^{\circ}_{f,298-15} + [1 - (T/T_0)] \Delta h^{\circ}_{f,298-15} \right) + \left( \sum_{k=1}^{n_{elements}} n_{k,element} ex^{\circ}_{chem,k,element} \right) \quad (15)$$

where standard temperature,  $T_0$ , is 298 K and the physiological system temperature,  $T$ , is 310 K. Molar chemical exergies of the food components and the daily exergy waste with faeces at the physiological system temperature are presented in the online Supplementary material.

**Exergy flow around subsystem 1.** The daily exergy accumulation in the adipose tissue (subsystem 1) was calculated by using the predicted daily maternal body weight gain ( $m_1$ ), the main fatty acid composition of the adipose tissue<sup>(33)</sup> and the daily chemical exergies of the fatty acids as presented in the online Supplementary material.

**Exergy flow around subsystem 2.** The daily chemical exergy cost of manufacturing the offspring was calculated based on the chemical exergies of the fatty acids and that of the average amino acid molecule, 21 784 kJ/kg, as reported by Mady & Oliveira<sup>(19)</sup>. The daily exergy:energy ratios of the offspring were used to estimate the daily exergy values of the placenta as presented in online Supplementary Table S8.

**Exergy flow around subsystem 3.** In the energy balance, the  $\Delta H_{rxn}$  of the metabolic oxidation reactions was regarded as the total energy fuelling the work performance and the heat generation by the pregnant mice. Exergy is not a conserved thermodynamic property. Exergy destruction in subsystem 3 was calculated as the difference between the exergy of the input foods plus oxygen, and that of the oxidation products plus heat and work. The net amount of chemical exergy of the food entering subsystem 3 was the residual obtained after subtracting the mass flows of faeces, subsystems 1 and 2 inputs and protein part of the food consumed by the mouse and used to calculate the daily exergy uptake by the cellular metabolism (Table 2). Similar to that of the energetic approach, the RER values were used to introduce the efficiency of the oxidation of nutrients to the cellular metabolism calculations; therefore, values of  $\sum (N ex)_{in}$  were multiplied by the values of the RER in Table 2. Chemical exergy of the products of the oxidation reactions is presented in online Supplementary Table S6, and the daily exergy leaving the body by means of these products via exhalation is given in Table 2.

External and internal work and associated heat generation are the other outputs of subsystem 3. As a general approach, the exergy of work equals its energy and the exergy of heat was calculated from the work performance of the Carnot engine operating between the system and the environment temperatures as ' $Q [1 - (T_s/T_{b,i})]$ '. Exergies pertinent to the system components were calculated, and exergy balance around the subsystem 3 was evaluated by equation 16 for each day;

$$\sum_{in} [\dot{N} ex]_{in} - \sum_{out} [\dot{N} ex]_{out} - \sum_i [1 - (T_s/T_{b,i})] \dot{Q}_i - \dot{W} - \dot{E}x_{dest} = d[N ex]_{system}/dt \quad (16)$$

where  $ex$  is the specific exergy,  $T_b$  is 310·15 K, that is, the body temperature on its surface and  $T_s$  is the temperature of the immediate surrounding and taken as the average of the body surface (310·15 K) and the room (298·15 K) temperatures. Exergy accumulation term for cellular metabolism at the right-hand side of equation 16 was neglected. Daily exergy destruction rates in the cellular metabolism, subsystem 3, of the pregnant mice are presented in Table 2. The second law efficiency was calculated by dividing the actual work output to the maximum possible work output<sup>(39)</sup>. The daily second law efficiencies were calculated for subsystem 3 by equation 17:

$$\eta_{II}(\%) = \left( \dot{W}_{out} / \left[ \sum (\dot{N} ex)_{in} - \sum (\dot{N} ex)_{out} \right] \right) \times 100 \quad (17)$$

The second law exergy efficiencies and the exergy destruction rates were also expressed based on per mole of ATP produced in subsystem 3 and then presented in Table 2.

There may be small fluctuations in the amounts of the daily food intake; therefore, 5% uncertainty was estimated for the exergy of the inputs. The exergy of the faeces has higher uncertainty than those of the inputs, due to potential changes in the time spent or amount absorbed in the digestive tract; therefore, 7% uncertainty was estimated for the exergies of outputs. External work was calculated based on the data of Ladyman *et al.*<sup>(30)</sup>; internal work was calculated after subtracting the external work from the estimated total work performance. Thus, a higher uncertainty (10%) was estimated in association with the total work output component of the exergy balance. Since heat generation was estimated as a constant fold of the work performance, uncertainties involved in heat generation were assumed to be the same as that of the work performance. Estimated daily uncertainties (kJ) associated with each component of exergy balance are presented in online Supplementary Table S9. The daily uncertainty values of exergy destruction (kJ) were calculated according to these estimations and error propagation technique and averagely found as 1·1 kJ which is corresponding about 7·7 in percentage (online Supplementary Table S9).

## Results and discussion

### Mass and energy balances

Nutrient metabolism in the present study was under the influence of the hormonal changes and fetus demand. There is 27·8% difference between the inputs and the outputs in mass balance around subsystem 3, which may be caused due to the allocation of some of the metabolites of the tricarboxylic acid cycle to the synthesis of the cellular components, including those of the fetus. Energy accumulation in the maternal body, that is, in the adipose tissue, was assumed as a constant fraction of the total weight, 19%, and its daily values are listed in online Supplementary Table S1. Fraction of the chemicals stored in the adipose tissue has vital effects on health during pregnancy.

Modified lipid metabolism and redistribution of the adipose tissue are important for providing steady nutrition to the fetus<sup>(48)</sup>. Therefore, it is suggested that storing a fraction of the maternal body incremental weight in the adipose tissue is a kind of adaptive reaction to pregnancy<sup>(49)</sup>. Increase of the weight of the adipose tissue during pregnancy implies that the energy input to the system was higher than what was needed by the pregnant mouse plus the growing fetus, drawing attention to the positive balance in the energy metabolism. In the present study, a continuous mass accumulation in adipose tissue during the pregnancy was observed with variabilities in the generally increasing trend, as presented in online Supplementary Table S1. Decreasing muscle work performance, as described in Fig. 2, might be a result of the difficulty in carrying around the increased weight of the adipose tissue.

Some of the food components are employed in the fetal-placental unit as construction materials for the manufacturing of the offspring, placenta and the amniotic fluid elements. In the present study, mass and energy expenses were considered for the construction of the offspring and placenta only. The placenta provides nutrients and oxygen for the fetus, and the demand for oxygen and nutrition increases as pregnancy goes on<sup>(50,51)</sup>. Therefore, as the fetus grows up, the mass of the placenta increases proportionally. The mechanism of this relationship is complicated and depends on numerous maternal and fetal factors<sup>(51)</sup>. In the present study, the incremental mass and energy of the placenta are calculated by using the values reported by Myrcha *et al.*<sup>(37)</sup>. It is shown in the online Supplementary material that both the mass and energy contents of the placenta are higher at the last two-thirds of the pregnancy as argued by Şen & Önder<sup>(52)</sup>.

Food intake generally increases as pregnancy continues<sup>(30,53)</sup>. After subtracting the mass leaving with the faeces, material stored in the adipose tissue, the constituents of the fetal-placental unit and the food proteins, the remaining food goes to cellular metabolism to extract its energy hidden in its chemical bonds. Amino acids are actively transported across the placenta to the developing fetus.

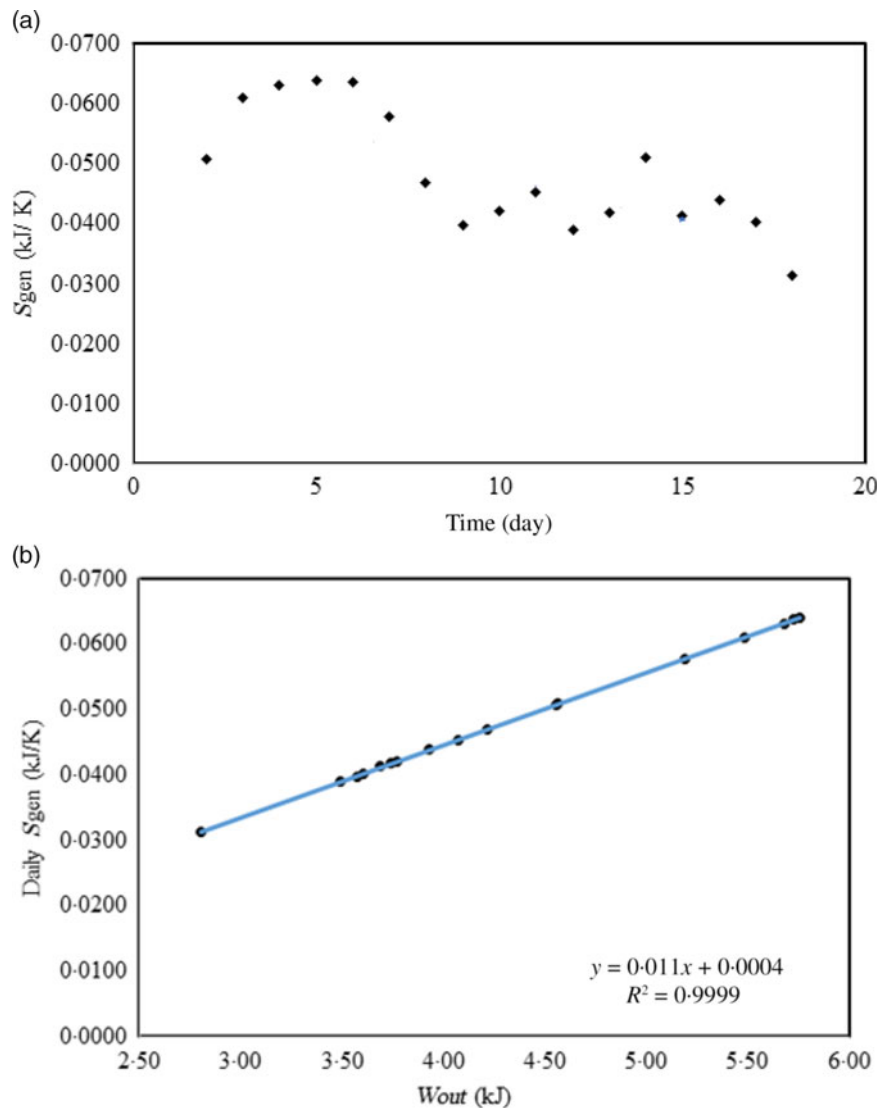
Metabolic activity utilises the chemical components of the food with the contribution of oxygen and produces carbon dioxide and water. The energies of the products and the reactants of the oxidation reactions were calculated by considering the mole numbers at the physiological temperatures in the online Supplementary material. The net enthalpy of the oxidation reaction values,  $\Delta H_{rxn}$ , are multiplied with the RER and listed in the online Supplementary material. It is observed that, although daily total food intake weight generally has an increasing profile ( $P < 0.05$ ), energy extracted from the oxidation of the net food input in the cellular metabolism does not. When the second and final dates of the pregnancy are compared, it is observed that in the cellular metabolism, the catabolised energy fraction from the total food intake, which is consumed for  $W_{imb}$ ,  $W_{ext}$ ,  $Q_{int}$  and  $Q_{ext}$ , decreases ( $P < 0.05$ ). This variable profile might be related to other outputs or subsystem energy expenses, such as the faecal output, storage in the adipose tissue and the energetic expenses of the fetal-placental unit subsystems. These kind of energy costs may increase, depending on the progress of the pregnancy.

The internal work activities were observed consuming more energy than the muscular activity during the whole pregnancy. Fig. 2 is new to the literature and describes how growth of the offspring inside the fetal-placental unit may contribute to the internal work activity. Multiple linear regression analysis ( $P < 0.05$ ) carried out on the data presented in Fig. 2 has confirmed that energy utilisation was correlated with both internal and external work performances and energy accumulation in adipose tissue. Incremental increases in the mass of metabolically active tissue; maternal cardiovascular or renal or respiratory work and tissue synthesis utilise a fraction of the basal metabolic energy<sup>(54)</sup> or in other words – the fraction of the internal work – in this study. Also, the increase in difficulty of movement, due to higher body mass, and discomfort due to morphological and physiological changes resulting from pregnancy might be another reason for the decreased physical activity<sup>(55)</sup>. Variation of the internal work performance with time in Fig. 2 and variation of the entropy generation with time in Fig. 3(a) follow exactly the same behaviour. Considering that the increase in entropy generation describes waste of exergy involved in a process, these figures show that there are phases in pregnancy, where energy and exergy are used less efficiently in comparison with the other phases. During the first 5 d of the pregnancy period, although there is a slight gradual increase in the external work performance, increase in internal work output is higher. Morphological changes occurring in this period probably contributed to this demand. Then, the difference between internal and external work performance starts decreasing at mid-pregnancy due to a decrease in the internal work activity. Entropy generation increases with work performance as described in Fig. 3(b).

In the present study, fetal-placental unit was regarded as a separate system; therefore, synthesis of the offspring and the placenta is not included in the internal work, but there may be indirect interaction of that activity, such as metabolic activity, between them. Also, the complex hormonal network and changing secretion profile of some hormones can affect the physiological cost of reproduction and dependently internal work<sup>(56–59)</sup>. Ladyman *et al.*<sup>(30)</sup> observed an increase in spontaneous activity in the cage and a decrease in the voluntary activity output during mid-pregnancy; therefore, no big difference was observed in the total external work performance except 10th day. Melzer *et al.*<sup>(55)</sup> while reviewing the process of pregnancy reported that pregnant women generally prefer less intense and more comfortable activities involving lower risk of maternal and fetal injury. In Fig. 2, internal work appears to be performed in two phases described by consecutive peaks. The ratio of the internal:external work energy expenses is higher during the last trimester comparing to mid-pregnancy with an obvious decrease in muscular activity. Correspondingly, the gap between internal and external work gets larger since the fetus becomes bigger and needs more energy for its further growth. Similarly, Borodulin *et al.*<sup>(60)</sup> argued that pregnant individuals decrease their overall physical activity, but the most noticeable change in the length and intensity of their activity occurs in the third trimester of pregnancy.

As previously argued by Sherwood<sup>(43)</sup>, not more than 25 % of the energy of the nutrients becomes available for either external





**Fig. 3.** (a) Daily entropy generation and (b) variation of the entropy generation rate with work output during pregnancy (data points are represented with  $\blacklozenge$  and  $\bullet$ , lines represent linear fitting).

or internal work performance in human physiology. This author stated that the remaining part turns to heat during the sequential energy. Guyton & Hall<sup>(44)</sup> rephrase this work performance ratio 'as not more than 27%'. Kopelman *et al.*<sup>(61)</sup> attributes this low efficiency to the incompetence of intermediary metabolism, where transformation of the energy of the food is required for internal work performance to sustain internal structure and to perform external physical work. In the current study, the ratio of 25–75% is used to portion the energy of the nutrients into total work and heat as described in Table 2.

#### Exergy balance and entropy generation rate

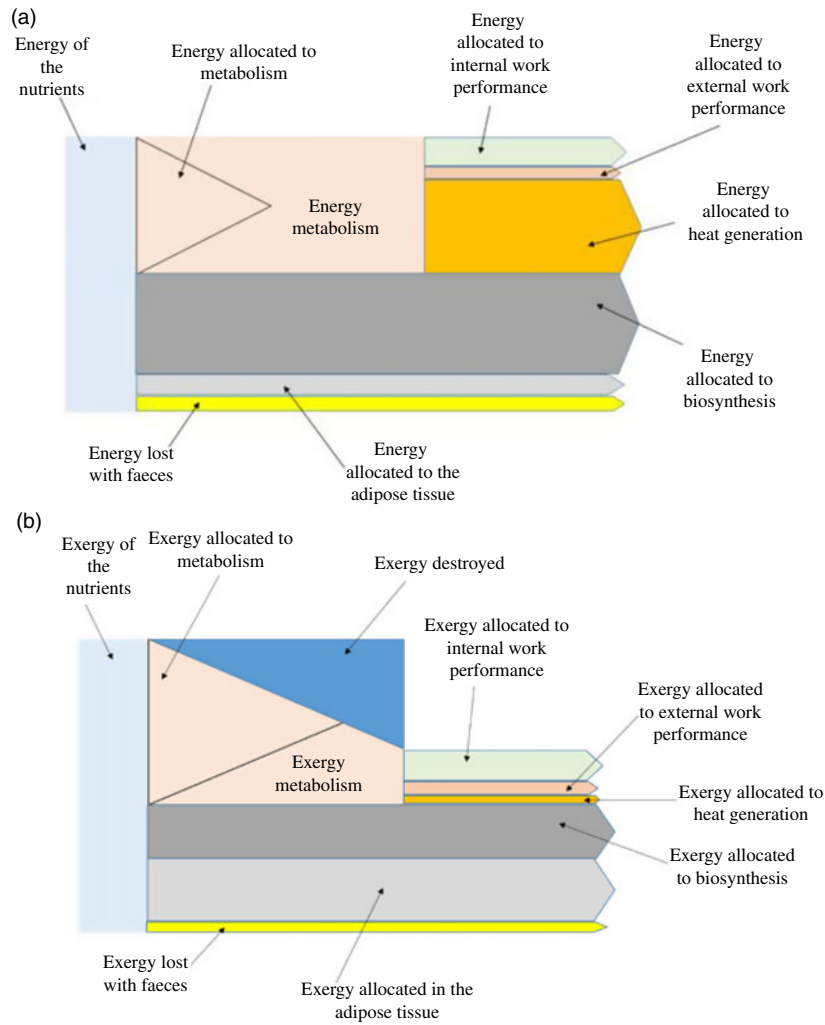
Exergy destruction and entropy generation rates are related by equation 18.

$$\dot{E}x_{dest} = T_0 \dot{S}_{gen} \quad (18)$$

where  $T_0$  is the standard temperature, 298.15 K. Equation 18 has a theoretical basis as explained by Çengel & Boles<sup>(39)</sup> and has been used in numerous publications, including Çatak *et al.*<sup>(24)</sup>. Exergy destruction rate,  $\dot{E}x_{dest}$ , was calculated with equation 16; and then the daily entropy generation rates were calculated from equation 18. The daily entropy generation rates were plotted *v.* the days of pregnancy in Fig. 3(a), where it was observed that the daily entropy generation showed a phasic behaviour with the progression of the pregnancy. Besides, a linear relation between entropy generation and the work output was observed ( $P < 0.05$ ,  $R^2: 0.99$ ) in Fig. 3(b). Allocation of the energy and exergy of the nutrients to life processes is described graphically in Fig. 4.

Gibbs free energy and chemical exergy of formation of the food constituents under physiological conditions were calculated by using equations 13–15 and presented in the online Supplementary material. Gibbs free energy is a path-independent state function, whereas exergy is sensible to the





**Fig. 4.** Graphical representation of the allocation of nutrients' (a) energy and (b) exergy to life processes.

path used in biothermodynamic assessments<sup>(62)</sup>. Faecal material has its own chemical exergy, but it cannot be utilised for a useful purpose; therefore, it is considered as a waste output. In the present study, chemical exergy of the faeces was calculated at the physiological conditions for each day of pregnancy to see approximate exergy waste with the faecal output of the pregnant mouse. Calculations given in the online Supplementary material at online Supplementary Table S1 indicate that exergy output with faeces increased linearly ( $P < 0.05$ ,  $R^2: 0.85$ ) with the progression of pregnancy.

In the present study, daily exergy accumulation in the adipose tissue was calculated, as presented in the supplement at online Supplementary Table S1, based on the daily maternal body weight gain estimates and chemical exergies of the fatty acids of the adipose tissue by using the data reported by Kassem *et al.*<sup>(35)</sup>. Exergy potential of subsystem 1 serves as a reserve to be used in case of need, that is, under depletion of food, but in the current study, exergy of the food consumed is more than what is needed by the pregnant mice; therefore, exergy reserve stored in the adipose tissue was not used for any activity.

The ratio between the numerical values of the energy and the exergy of the offspring was assumed to be the same as that of the placenta. Exergy of the placenta was calculated after multiplying this ratio by its energy. These calculated exergetic costs were interpreted as an exergy accumulation component in the pregnant mouse system.

The first five columns of Table 2 describe the data adapted from Ladyman *et al.*<sup>(30)</sup>, converted on daily basis and processed with the help of the thermodynamic data presented in the online Supplementary material. The remaining three columns of this table are evaluated as a result of the thermodynamic analyses performed. Components of the exergy balance equation around subsystem 3 were calculated based on the net weight of the food entering the cellular metabolism and are presented in Table 2. Subsequently, daily exergy destruction in the cellular metabolism of the pregnant mouse was calculated with this exergy balance around subsystem 3. Daily entropy generation is calculated with the use of the daily exergy destruction as described in equation 18 (Fig. 3(a)). Although there are variations in entropy generation rates, it is observed that the pregnant mouse attained the maximum and minimum entropy generation rates at the 5th

and 18th days of the pregnancy, respectively. The daily entropy generation profile is very similar to that of the daily internal work output (Figs. 2 and 3(a)). Since the internal work is the major work output, the general profile of the entropy production is similar to the total work output trend ( $P < 0.05$ ,  $R^2: 0.99$ ) (Fig. 3(b)). Entropy generation by the mice is caused by irreversibilities involved in, many processes, including beating of heart, glucose degradation,  $\beta$ -oxidation of fatty acids and protein degradation,  $\text{Na}^+/\text{K}^+$  pump processes, the activities associated with gene expression, etc.<sup>(10,63)</sup> In thermodynamically irreversible processes, there is a degradation or loss of 'available energy' during exergy transformations. Because of this loss, the same process cannot be performed in the reverse direction anymore<sup>(10,64)</sup>. This loss of available exergy is referred to as exergy destruction or entropy generation within the context of the second law of thermodynamics. Aoki<sup>(65)</sup> concluded that entropy generation increases with the increasing intensity of exercise (external or muscular work in the current study) and related this observation to heat generation, one of the main irreversibilities in the living systems. Similarly, Rahman<sup>(66)</sup> concluded that heat generation is the main phenomenon that affects the entropy generation in the human body. In the current study, similar to work output, heat output from cellular energy metabolism subsystem also has a linear relationship with entropy generation ( $P < 0.05$ ,  $R^2: 0.99$ ).

A reversible process with no friction, no energy dissipation and no entropy generation may be regarded as an ideal system<sup>(10)</sup>. If the cellular metabolism of a pregnant mouse is regarded as an ideal system, the best performance of cellular metabolism will be the same as the net obtainable exergy. The denominator of the second law efficiency formula, that is, in equation 17, comprises the maximum exergy obtainable from cellular metabolism, which will be used for doing work without any destruction; the total external and internal work outputs are present in the numerator. Table 2 shows that the second law efficiency of the cellular metabolism of a pregnant mouse is about 21% which means this is the maximum conversion efficiency that the mouse can reach in terms of the second law. If the statement by Sherwood<sup>(43)</sup> and Guyton & Hall<sup>(44)</sup>, arguing that the total obtainable work equals approximately 25% of the energy of the food consumed, is substituted for the first law efficiency, it may be concluded that the second law efficiencies in their studies were lower than those of the current study.

Sorgüven & Özilgen<sup>(67)</sup> calculated the first and second law efficiencies of a muscle cell based on glucose consumption and compared their calculations with the results based on the data acquired in different biological muscle systems. According to Sorgüven & Özilgen<sup>(67)</sup>, the first law efficiencies were about 21% and the second law efficiencies were about 25%. In the current study, the system includes a substantially dynamic fetal growth process affecting the thermodynamic efficiencies of the system. Even under these prevailing conditions, the first and the second law efficiencies were very close to the average of the given values by Sorgüven & Özilgen<sup>(67)</sup>.

The increment in exergy destruction rate per mole ATP production during the progression of pregnancy ( $P < 0.05$ ), as described in Table 2, is similar to the incremental profile of the exergetic efficiency. The daily ratios of the exergy

destruction rate per mole of ATP production reported in the current study are higher than those calculated for mitochondria and lower than those calculated for cytoplasm by Genç *et al.*<sup>(22)</sup>. This difference might be due to the selection of different systems, their inputs and outputs.

### Comparison of the energy and exergy balances

Energy, pertinent to the first law, and exergy (useful energy), pertinent to the second law, balances are described graphically in Fig. 4(a) and (b), respectively. In these figures, thickness of the input and output streams to or from metabolism is proportional to their numerical magnitude. Energy and exergy allocated to biosynthesis, faeces and the adipose tissue do not enter to metabolism. Exergy of the nutrients, faeces and those of the fatty acids stored in the adipose tissue are calculated as the magnitudes above the dead state, for example, that of ash, which would be obtained upon combustion of their constituents. Metabolic heat generation is described as a thick stream in Fig. 4(a), while it is a very thin stream in Fig. 4(b). Exergy is defined as the 'useful work' and calculated for every stream, either entering or leaving a system, by considering a reasonable process to assess its work potential. Traditionally, the 'useful work potential' of the metabolic heat discarded from the body is assessed in terms of its work potential in a Carnot engine. Since this calculated magnitude is very small, it is deemed as 'not useful for performing work' and described as a very thin stream in Fig. 4(b). The total of the exergy allocated to internal and external work performance and heat generation in Fig. 4(b) is substantially smaller in magnitude when compared with those of energy balance in Fig. 4(a). The observed difference is caused by entropy generation, in association with exergy destruction. Among the major inefficiencies involved in conversion of ATP into mechanical energy (cross-bridge cycling) in skeletal, cardiac and smooth muscle work, we may state inefficiency involved in pumping ions from a low-concentration zone to a high-concentration zone in the Na pumps<sup>(68)</sup>, fraction of the energy, which may be stored in the elastic connections between the myofibrils<sup>(69)</sup>; and the energy which is absorbed during lengthening of the muscle, and conversion into mechanical work during subsequent shortening<sup>(70)</sup>, all of these inefficiencies are among the reasons of entropy generation. Comparison of these figures shows the negative influence of the entropy generation in life processes. Comparison of Fig. 4(a) and (b) shows that the major portion of the exergy provided by the nutrients to the mouse is actually wasted via entropy generation.

### Conclusion

Entropy generation within the context of this study may be regarded as the conversion of a fraction of the energy of a nutrient into unusable form. It was shown in our previous study<sup>(19)</sup> that the genetic make-up of the organisms and the constituents of the diet have a major effect in an entropy generation process. A detailed assessment is presented in this manuscript about how energy of nutrients is allocated to life processes by the pregnant mice. It was shown that the 'internal work' performed by the pregnant mouse to sustain its living processes, including the energy allocated to manufacture the

fetus, was higher than the 'external work' performed. At the beginning and towards the end of pregnancy, internal work performance increased and the external work performance decreased towards the end of pregnancy. The increasing profile of the stored energy in the adipose tissue might be related to this decreasing external work or preliminary preparation for lactation which would start after birth. The other important finding of this thermodynamic assessment is that entropy generation by the pregnant mouse follows a similar profile with the total work performance output at the given conditions.

It is generally believed that approximately 25 % of the energy of the nutrients is converted into internal or external work in human metabolism. In the present study, this proportion was assumed as the first law efficiency for the cellular energy metabolism subsystem of the pregnant mice. The second law efficiency was calculated for the same subsystem and found about 21 %, which is smaller than the first law efficiency. During the energy conversion processes, more exergy than usual may be destroyed to open the way to this observation. The results of this study point the need for new research for minimising the entropy generation by pregnant mice by considering nutrient supply rate as a parameter.

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The authors declare that there are no conflicts of interest.

### Supplementary material

For supplementary material referred to in this article, please visit <https://doi.org/10.1017/S0007114520001646>

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