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Estudi calorimètric del balanç energètic en l'obesitat genètica i nutricional de la rata

Treball que presenta

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per optar al grau de Doctora en Ciències Biològiques.

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Table 1

Composition of the diets offered to Wistar and Zucker rats

component	content in grams per kg (liter for water and milk) water	digestible carbohydrate	lipid	protein	other components
chow pellets	120	587	30	170	93
biscuits	67	655	155	63	60
liver pâté	556	7	291	123	23
bacon	437	0	299	173	91
banana	780	171	1	14	34
tap water	999	0	0	0	1
enriched milk	632	319	19	22	8

The data represent the values used for calculations of food intake. They are the mean of 5 different samples

Table 2

Intake of Wistar and Zucker rats offered a cafeteria diet

strain and diet	period	Wistar control	Wistar cafeteria	Zucker Fa/? control	Zucker Fa/? cafeteria	Zucker fa/fa control	Zucker fa/fa cafeteria	SEM
carbohydrate	30-45	9.13^{a}	9.10^{a}	7.60^{b}	7.47^{b}	12.26^{c}	12.58^{c}	
intake (g/day)	45-60	9.96^{a}	10.92^{d}	$9.91^{a,d}$	$9\,85^{a,d}$	16.95^{e}	13.80^{c}	0.32
lipid intake	30-45	0.47^{a}	2.15^{b}	0.39^{c}	2.23^{b}	0.63^{d}	3.47^{e}	
(g/day)	45-60	0.49^{a}	2.12^{b}	0.51^{a}	2.41^{b}	0.87^{f}	4.04^{g}	0.14
protein intake	30-45	2.65^{a}	$2.32^{b,d}$	$2.20^{b,d}$	2.08^{b}	3.55^{c}	$2.99^{a,e}$	
(g/day)	45-60	2.82^{a}	$2.23^{b,d}$	$2.87^{a,e}$	2.35^{d}	4.91^{f}	$3.25^{c,e}$	0.09

All data are the mean of 6-7 different animals. Means —in the same row— not bearing the same superscript are significantly different ($P<0.05$). The SEM data refer to the complete 30-60 day period.

Statistical significance of differences (ANOVA):

parameters compared	carbohydrate df	P	lipid df	P	protein df	P
STRAIN	2,62	0 0000	2,62	0 0000	2,62	0 0000
DIET	1,62	0 3349	1,62	0 0000	1,62	0 0000
TIME	1,62	0 0000	1,62	0 0000	1,62	0 0000
Wistar – diet	1,62	0 2880	1,62	0 0000	1,62	0 0000
Wistar – time	1,62	0 0008	1,62	0 3892	1,62	0 5742
Zucker Fa/? – diet	1,62	0 7277	1,62	0 0000	1,62	0 0001
Zucker Fa/? – time	1,62	0 0000	1,62	0 0000	1,62	0 0000
Zucker fa/fa – diet	1,62	0 0195	1,62	0 0000	1,62	0 0000
Zucker fa/fa – time	1,62	0 0000	1,62	0 0000	1,62	0 0000

Table 3

Body composition of Wistar and Zucker rats offered a cafeteria diet

age days	strain	diet	weight g	carbohydrate g	lipid g	protein g	water g	energy MJ
30	Wistar	control	92.5[a]	0.15[a]	5.6[a]	11.6[a]	68.6[a]	0.498[a]
	Zucker Fa/?	control	70.3[b]	0.12[b]	4.2[b]	9.0[b]	51.4[b]	0.382[b]
	Zucker fa/fa	control	83.3[a]	0.16[a]	14.2[c,d]	10.8[a]	51.6[b]	0.820[c]
45	Wistar	control	152.1[c]	0.24[c]	11.3[c]	21.0[c]	108.5[c]	0.948[d]
		cafeteria	176.5[d]	0.27[d]	15.6[d]	25.6[d]	122.5[d]	1.230[e]
	Zucker Fa/?	control	122.5[e]	0.22[c,e]	7.3[e]	16.5[e]	86.7[e]	0.682[c]
		cafeteria	146.7[c]	0.19[e]	17.3[f]	18.3[e]	97.2[f]	1.118[f]
	Zucker fa/fa	control	173.7[d]	0.34[f]	37.3[g,i]	23.7[d]	99.0[f]	2.044[g]
		cafeteria	223.6[f]	0.19[e]	65.3[h]	33.0[f]	108.3[c]	3.369[h]
60	Wistar	control	198.8[g]	0.28[d]	18.9[f]	31.9[f]	133.5[g]	1.508[i]
		cafeteria	248.2[h]	0.32[f]	40.8[g]	39.0[g]	150.0[h]	2.547[j]
	Zucker Fa/?	control	165.9[d]	0.28[d]	15.1[d]	26.2[d]	110.2[c]	1.225[e]
		cafeteria	209.1[g]	0.36[f]	32.3[i]	30.1[f]	125.6[d]	2.037[g]
	Zucker fa/fa	control	279.3[i]	0.55[g]	79.4[j]	37.1[g]	145.8[h]	4.031[h]
		cafeteria	350.4[j]	0.69[h]	116.9[k]	45.9[h]	168 7[i]	5.727[k]
		SEM	*7.41*	*0.01*	*2.84*	*1.08*	*3.43*	*0.13*

All data are the mean of 6-7 different animals. Means —for each column— not bearing the same superscript are significantly different ($P<0.05$).

Statistical significance of differences (ANOVA):

parameters compared	weight df	P	carbohydrate df	P	lipid df	P	protein df	water P	df	energy P	df	P
STRAIN	2,93	0 0000	2,93	0 0000	2,92	0 0000	2,93	0 0000	2,93	0 0000	2,93	0 0000
DIET	1,93	0 0000	1,93	0 0268	1,92	0 0000	1,93	0 0000	1,93	0 0000	1,93	0 0000
TIME	2,93	0 0000	2,93	0 0000	2,92	0 0000	2,93	0 0000	2,93	0 0000	2,93	0 0000
Wistar – diet	1,93	0 0000	1,93	0 0001	1,92	0 0000	1,93	0 0000	1,93	0 0003	1,93	0 0000
Wistar – time	2,93	0 0000	2,93	0 0000	2,92	0 0000	2,93	0 0000	2,93	0 0000	2,93	0 0000
Zucker Fa/?–diet	1,93	0 0000	1,93	0 0062	1,92	0 0000	1,93	0 0017	1,93	0 0003	1,93	0 0000
Zucker Fa/?–time	2,93	0 0000	2,93	0 0000	2,92	0 0000	2,93	0 0000	2,93	0 0000	2,93	0 0000
Zucker fa/fa–diet	1,93	0 0000	1,93	0 0059	1,92	0 0000	1,93	0 0000	1,93	0 0000	1,93	0 0000
Zucker fa/fa–time	2,93	0 0000	2,93	0 0000	2,92	0 0000	2,93	0 0000	2,93	0 0000	2,93	0 0000

Table 4

Deposition of carbohydrate, lipid, protein and water in the body of Wistar and Zucker rats offered a cafeteria diet

strain and diet	period	Wistar control	Wistar cafeteria	Zucker Fa/? control	Zucker Fa/? cafeteria	Zucker fa/fa control	Zucker fa/fa cafeteria	SEM
weight increase	30-45	65.4[a]	84.0[b,c]	56.2[a]	76.4[b]	92.9[c]	140.2[d]	
(g)	30-60	112.1[a]	155.7[b]	99.6[a]	138.8[b]	198.4[c]	267.0[d]	3.5
carbohydrate stored	30-45	0.10[a]	0.12[b]	0.11[a,b]	0.07[c]	0.18[d]	0.05[e]	
(g)	30-60	0.13[a]	0.17[b]	0.17[b]	0.24[c]	0.40[d]	0.54[e]	0.01
lipid stored	30-45	6.1[a]	10.1[b]	3.3[c]	13.0[d]	23.6[e]	51.1[f]	
(g)	30-60	13.7[a]	35.3[b]	11.1[c]	29.1[d]	65.7[e]	102.7[f]	1.96
protein stored	30-45	10.1[a]	13.9[b]	7.6[c]	9.4[a]	13.1[b]	22.1[d]	
(g)	30-60	21.0[a]	27.4[b]	17.4[c]	21.1[a]	26.6[b]	35.0[d]	0.43
water stored	30-45	44.2[a,d]	53.8[b,e]	38 2[a]	45.5[a,d]	48.9[b,d]	56.7[e]	
(g)	30-60	69 2[a,b]	81.4[a]	61.7[b]	74.2[a,b]	95.7[c]	117.1[d]	1.59
stored lipid/stored protein	30-45	*0.60*	*0.73*	*0.43*	*1.38*	*1.80*	*2.31*	
ratio (g/g)	30-60	*0.65*	*1.29*	*0.64*	*1.38*	*2.47*	*2.93*	

All data are the mean of 6-7 different animals. Means —in the same row— not bearing the same superscript are significantly different ($P<0.05$). The SEM data refer to the complete 30-60 day period.

Statistical significance of differences (ANOVA):

parameters compared	weight df	P	carbohydrate df	P	lipid df	P	protein df	P	water df	P
STRAIN	2,62	0 0000	2,62	0 0000	2,92	0 0000	2,62	0 0000	2,62	0 0000
DIET	1,62	0 0000	1,62	0 0127	1,92	0 0000	1,62	0 0000	1,62	0 0000
TIME	1,62	0 0000	1,62	0 0108	1,92	0 0000	1,62	0 8863	1,62	0 0000
Wistar – diet	1,62	0 0000	1,62	0 0013	1,92	0 0000	1,62	0 0000	1,62	0 0270
Wistar – time	1,62	0 0000	1,62	0 0000	1,92	0 0000	1,62	0 7255	1,62	0 0000
Zucker Fa/?–diet	1,62	0 0000	1,62	0 0004	1,92	0 0000	1,62	0 0000	1,62	0 0118
Zucker Fa/?–time	1,62	0 0000	1,62	0 2023	1,92	0 0000	1,62	0 0000	1,62	0 0000
Zucker fa/fa–diet	1,62	0 0000	1,62	0 0117	1,92	0 0000	1,62	0 0000	1,62	0 0000
Zucker fa/fa–time	1,62	0 7779	1,62	0 0000	1,92	0 0000	1,62	0 0000	1,62	0 0000

Table 5

Energy balance of Wistar and Zucker rats offered a cafeteria diet

strain and diet.		Wistar	Wistar	Zucker Fa/?	Zucker Fa/?	Zucker fa/fa	Zucker fa/fa	
	penod	control	cafetena	control	cafetena	control	cafetena	SEM
energy ingested (MJ)	30-45	3.41[a]	4.24[b]	2.84[c]	3.80[a]	4.58[b]	6.08[d]	
	30-60	7.13[a]	8.87[b]	6.53[c]	8.35[b]	10.97[d]	12.87[e]	0.14
energy stored (MJ)	30-45	0.48[a]	0.73[b]	0.31[c]	0.74[b]	1.25[d]	2.55[e]	
% of energy ingested		14 1	17 0	10 9	19 5	27 3	41 9	
	30-60	1.04[a]	2.05[b]	0.85[c]	1.66[d]	3.24[e]	4.91[f]	0 09
% of energy ingested		14 6	23 0	13 0	19 9	29 7	38 1	
energy available (ingested – stored) (MJ)	30-45	*2.93*	*3.56*	*2.52*	*3.06*	*3.32*	*3.53*	
	30-60	*6.09*	*6.82*	*5.68*	*6.70*	*7.67*	*7.97*	

All data are the mean of 6-7 different animals. See the text for the method of calculation of composition. Means —in the same row— not bearing the same superscript are significantly different ($P<0.05$).

Statistical significance of differences (ANOVA):

parameters	energy ingested		energy stored	
compared	df	P	df	P
STRAIN	2,62	0 0000	2,62	0 0000
DIET	1,62	0 0000	1,62	0 0000
TIME	1,62	0 0000	1,62	0 0000
Wistar – diet	1,62	0 0000	1,62	0 0000
Wistar – time	1,62	0 0040	1,62	0 0000
Zucker Fa/? – diet	1,62	0 0000	1,62	0 0000
Zucker Fa/? – time	1,62	0 0000	1,62	0 0000
Zucker fa/fa – diet	1,62	0 0000	1,62	0 0000
Zucker fa/fa – time	1,62	0 0000	1,62	0 0000

Legend of Figure

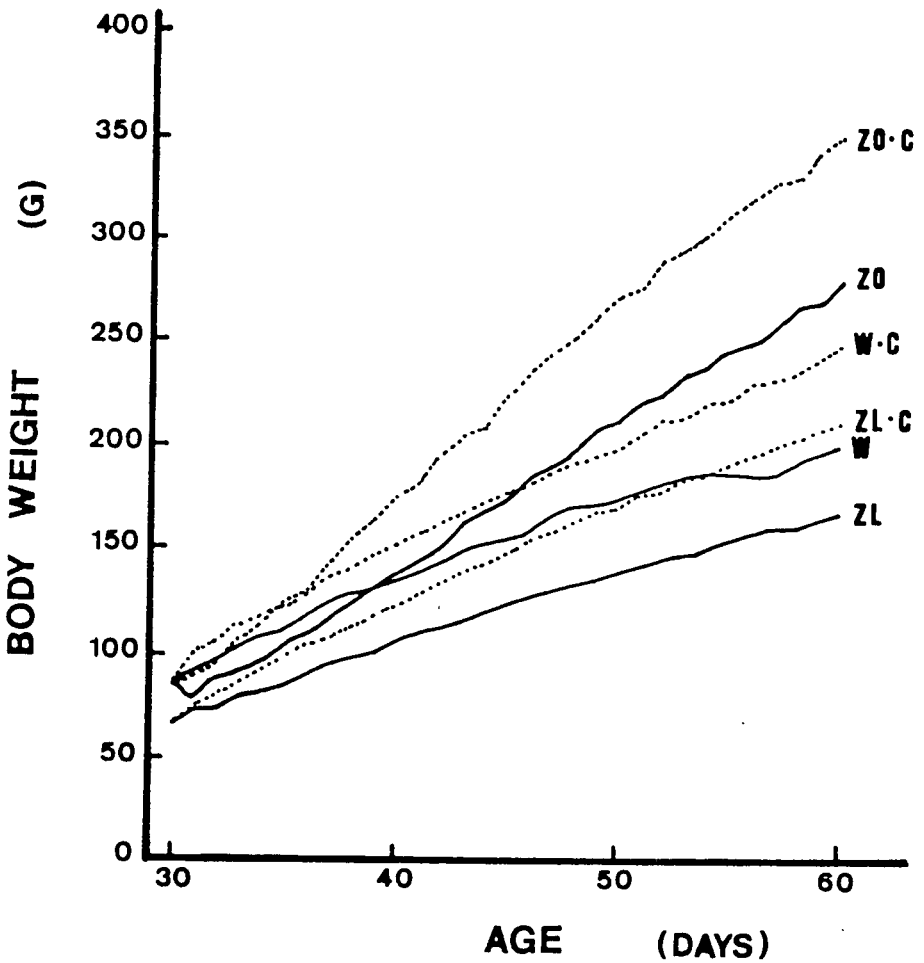
Figure 1

Body weight of the Wistar and Zucker rat groups used in the experiment from days 30 to 60

The graphs represent the mean weight for each group (N=5-7) determined daily. Solid lines correspond to control (pellet) diet, and dot lines to cafeteria-feeding. **ZO • C** Zucker obese cafeteria-fed; **ZO** Zucker obese control-fed; **W • C** Wistar cafeteria-fed; **ZL • C** Zucker lean cafeteria-fed; **W** Wistar control-fed; **ZL** Zucker lean control-fed.

Statistical significance of the differences (ANOVA calculated from values taken at 5-day intervals):

parameters compared	body weight df	P
STRAIN	2,209	0.0000
DIET	1,209	0.0000
TIME	6,209	0.0000
Wistar – diet	1,209	0.0000
Zucker Fa/?–diet	1,209	0.0000
Zucker fa/fa–diet	1,209	0.0000


400
350
300
250
200
150
100
50
0
BODY WEIGHT (G)
30
40
50
60
AGE (DAYS)
ZO·C
ZO
W·C
ZL·C
W
ZL

COMENTARI GENERAL: BLOC I

En aquest primer bloc de treballs hem pogut confirmar la baixa capacitat per generar calor que presenten les rates obeses, en concret en el model d'obesitat genètica que mostren les rates de la soca Zucker (fa/fa); en contraposició a una més alta producció de calor en l'obesitat induïda per la dieta de cafeteria —si més no en les rates Wistar— malgrat que aquesta dieta conté una proporció significativa de lípids i que fomenta una deposició energètica més gran (sobre tot de lípids). Cal remarcar que tot i que les rates obeses ja ingereixen gairebé el doble de lípids que les rates primes quan són alimentades amb la dieta control, la més gran disponibilitat d'aquest substrat en la dieta de cafeteria fa que aquests animals obesos n'arribin a ingerir unes vuit vegades més respecte les rates primes en la dieta control. Això es veu reflectit en la deposició, però no guarda aquesta mateixa proporcionalitat, sinó que en la combinació d'ambdós tipus d'obesitat la deposició és el doble (primer període), o només una mica més (segon període) que en l'obesitat genètica sola, suggerint que les rates obeses ja estan dipositant unes enormes quantitats de lípids i que la dieta de cafeteria hi té poc a afegir.

Quant a l'efecte d'una altra situació de l'estat nutricional dels animals, com és la privació d'aliments, respecte de la producció de calor, hem observat que si bé aquesta té un efecte marcat de disminució de la despesa energètica en les rates primes, no té aquests efectes en les rates fa/fa, indicant que la producció de calor en les rates obeses ja es troba en uns nivells prou baixos, de manera que ni la manca d'aliments pot fer que aquesta disminueixi més.

Finalment, l'efecte de l'administració de sacarosa sobre la producció de calor o sobre la deposició ha estat sempre motiu de controvèrsia. En aquest cas els nostres experiments no fan més que reafirmar uns efectes variables segons la soca, l'edat, etc. En aquest sentit hem pogut veure que si bé en un primer treball trobàvem un efecte potenciador de la termogènesi induïda per la sacarosa de la dieta en les rates Wistar, en un segon treball no s'ha repetit aquesta característica de la sacarosa, i en cap cas ha promogut una despesa de calor significativament més alta que la dieta de cafeteria. Pel que respecta a l'efecte contraposat que comunment s'atribueix també a la sacarosa (augment de pes i de la deposició de lípids), novament ens trobem amb resultats variables, però en cap cas promou un augment de pes més gran que la dieta control o de cafeteria. Pel que fa a la deposició de lípids, hem vist que aquesta pot ser més gran (rates Wistar), igual (rates obeses), o menor (rates Zucker primes) que en els altres dos grups.

BLOC II

Establiment de les condicions de treball en estudis de calorimetria indirecta

treball acceptat per publicar a ARCHIVES INTERNATIONALES DE PHYSIOLOGIE, DE BIOCHIMIE ET DE BIOPHYSIQUE

Analysis of ultradian heat production and aortic core temperature rhythms in the rat.

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SUMMARY

The rhythms of aortic core temperature and overall heat production in Wistar rats was analyzed by using long series of recordings of temperature obtained from implanted thermocouple probes and heat release values from a chamber calorimeter. There was a very high degree of repetitiveness in the presentation of actual heat rhythms, with high cross-correlation values ascertained with paired periodograms. No differences were observed between heat production between male and female adult rats. The cross-correlation for temperature gave similar figures. The cross-correlation study between heat production and aortic core temperature in the same animals was significant and showed a displacement of about 30 minutes between heat release and aortic core temperature. The analysis of heat production showed a strong predominance of rhythms with periods of 24 hours (frequencies <11.6 μHz) or more; other rhythms detected (of roughly the same relative importance) had periods of 8 or 2.2 hours (35 or 126 μHz, respectively). The analysis of aortic core temperature showed a smaller quantitative contribution of the 8 or 2.2 hours (35 or 126 μHz) rhythms, with other harmonic rhythms interspersed (5.1 and 4.0 hours, i.e. 54 and 69 μHz). The proportion of 'noise' or cycles lower than 30 minutes (<550 μHz) was higher in internal temperature than in the actual release of heat. The results are in agreement with the existence of a basic period of about 130 minutes (126 μHz) of warming/cooling of the blood, with a number of other harmonic rhythms superimposed upon the basic circadian rhythm.

INTRODUCTION

Activity and performance are often subjected to and driven by biological rhythms (BÜTTNER & WOLLNIK, 1984). These rhythms modulate the movement, feeding and reproductive behavior in accordance with the daily light (HOFFMAN, 1981) or temperature changes (HELDMAIER *et al.*, 1989) or season temperature variation (GWINNER, 1981) or feeding patterns (DAAN & SLOPSEMA, 1978). Most studies on cyclicity of oscillation of physical or metabolic parameters have been performed on animals subjected to profound changes during these cycles (ARMSTRONG *et al.*, 1986), e.g. hibernating

small mammals (POHL, 1987). However, the pervasive influence of environmental-driven rhythms is well extended even for species no longer subjected to the vagaries of their surrounding physical context. Humans show well defined daily temperature (LOVETT-DOUST, 1979), metabolite (HILDEBRANDT, 1988) and hormone availability (BRANDENBERGER *et al.*, 1987) rhythms. The aortic temperature has been taken as representative of the core temperature since it complies with the definition given by the IUPS (1987) for core temperature and can be considered equal to the temperature of the blood entering the core organs and tissues.

Most studies on temperature cycle variations on rodents are centered in their seasonal adjustment to the conditions of the environment (HOOGENBOOM *et al.*, 1984), dealing with periods longer than the day. The existence of shorter period rhythms of temperature in the rat has recently been observed (CLOSA *et al.*, 1991). The periodicity of these variations is acquired very early during postnatal development (KITTRELL & SATINOFF, 1986) and is directly related with the maintenance of body (core) temperature. There is a basic c. 2-hour heating / cooling period in the core temperature of the laboratory rat (CLOSA *et al.*, 1991). The first stage of the cycle corresponds to a period of increased thermogenesis and higher cardiac output, whilst the second is consistent with lower tissue blood flow and decreased adaptive thermogenesis. Exposure to cold increases the frequency of these heating / cooling episodes (CLOSA *et al.*, 1992).

Since the light/dark cycles deeply influence the rhythms of activity in the laboratory rat (BÜTTNER & WOLLNIK, 1984), alterations of the duration or cyclicity of the illumination phases affects the daily feeding and temperature rhythms (TAKAHASHI *et al.*, 1977), showing the superimposition of other cyclic patterns upon the two basic rhythms defined: a 24-hour period related with light / dark (and indirectly with feeding), and a c. 2-hour period related to the maintenance of core temperature, and very probably depending on the external temperature.

The aim of this study is to analyze the relative and quantitative importance of these rhythms, as well as the possible existence of other ultradian rhythms affecting temperature and heat output, and also the relationship between aortic core temperature and heat production in the laboratory rat.

MATERIALS AND METHODS

Wistar rats (*Rattus norvegicus*) , weighing 200-220 g were used. The animals were housed under standard conditions of light (on from 08.00 to 20.00), temperature (21-22 °C), humidity (70-80 % RH) and ventilation. The animals were kept in collective polypropylene-bottomed cages with wood shavings as bedding material. The rats were fed *ad libitum* with standard rat chow pellets (type A04 from Panlab, Barcelona) and tap water.

The core blood temperature was determined by placing small K-type thermocouple probes (Kane May, UK) upon the lower aorta under ether anaesthesia (CLOSA *et al.*, 1991). The wires passed through a Harvard jacket (Harvard, USA) and were connected to a data storing device (Data-Logger, Kane May, UK), which recorded the aortic core temperature at short (6 min.) fixed intervals. The rats were left to recover for a minimum of 3 days after the implant of the probe. Food consumption and basic temperature rhythms had returned to normal, pre-implantation levels within two days (POHL, 1987). It was thus considered safe to use these animals in the experiments.

Aortic core temperature variations over long periods were measured with the rat kept in an individual cage under the housing conditions stated; the recordings were taken for at least two days after placing the rat in the cage, where it then remained undisturbed. Only the following

days' recordings were analyzed.

The overall heat production was measured, using the same animals, with food and water available, in a multichambered direct circulation calorimeter (Sautin, Castellón, Spain) based on an earlier model developed in our laboratory (DOMÈNECH *et al.*, 1988). The instrument gave a digitalized output of heat production in short fixed intervals. The rats were used to handling and being placed in the chambers before the actual measurements took place. The calorimeter responses were checked with known electric resistances, used for the calculation of the instrument constants; the mean delay in the thermic response to a surge of heat was estimated to be less than six minutes. All measurements were performed at 22 °C.

The consistency of the data analysis was checked using heat production and blood temperature data. Two series of 5 male and 5 female rats were studied in parallel for periods of more than 10 hours. The cross-correlations between different series of data (heat-heat and heat-temperature as well as temperature-temperature) were performed using the Statgraphs program.

Preliminary analysis of rhythms in the actual recordings of aortic core temperature and heat output for a continuous period of 48 hours was performed on the corresponding periodograms using the Statgraphs program. From each periodogram, the frequencies explaining 80 % of the corresponding series were selected. A more detailed analysis was carried out according to the following procedure:

1) Among the set of selected frequencies, those showing the higher amplitudes were fitted, by means of linear regression, to the equation:

$$y_i = A_1 \cdot \sin w_i \cdot t_i + B_1 \cdot \cos w_i \cdot t_i + C \qquad (1)$$

where y_i stands for the temperature or heat output at time t_i, and A_1, B_1 and C denote constants of the system.

2) That frequency producing the lowest sum of squares was selected. The value of this frequency w_1 was refined by determining the sum of squares of frequencies above and below the initial value. The increments tested, positive or negative, were equivalent to an interval of 10 minutes. The value producing the lowest sum of squares was definitively assigned to w_1.

3) The remaining frequencies of the initial set were fitted, as indicated above, to the equation:

$$y_i = C + \sum_{j=1}^{2} A_j \cdot \sin w_j \cdot t_i + B_j \cdot \cos w_j + t_i \qquad (2)$$

4) As in step 2, the frequency producing the highest decrement of the sum of squares was selected, and the statistical significance of this frequency w_2 was determined by an F-test. The refinement described in step 2 was carried out in order to determine the final value of w_2.

5) Steps 3 and 4 were repeated introducing more terms in the parentheses of equation (2) until no further significant improvement was achieved.

The Statgraphs program was implemented on a 286-based Tandon desk top microcomputer. Other calculations were run on a VAX 6310 computer.

RESULTS

Figure 1 shows the results of the cross-correlation of series of data obtained from pairs of male, mixed gender and female rats, as an example of the level of correlation observed between the daily heat output patterns of different animals measured under the conditions described. There were no significant differences

between male and female rats as to the pattern of heat production for a period of 24 hours. The very high coefficients of correlation observed in the center of the graph (there was hardly any deviation with respect to time on the presentation of the basic patterns) shows a high degree of synchronism in the pattern of heat output for male and female rats. There were no differences as to ultradian rhythms between male and female rats. Figure 2 shows a periodogram comparing the cross-correlation between the aortic core temperature recordings of two representative rats. There was also a high degree of correlation for this parameter between series of data from different animals, implying a standard uniform rhythm pattern for all animals tested.

Figure 3 presents the actual 24-hour pattern of heat production of a representative rat. The analysis of the superimposed rhythms gave the basic periods presented in table 1, which are also represented in figure 3 as a composite of the rhythms analyzed. The experimental heat production graphs were decomposed into strong circadian and longer periods, accounting for most of the variations observed, and two distinct shorter rhythms, with similar incidence in the overall pattern: 35 and 126 μHz (periods of 8 and 2.2 hours). These rhythms accounted for about 76 % of the observed pattern, the rest consisting of noise and rhythms or variations of very short period (less than 0.5 mHz, i.e. having periods of less than half hour).

Figure 4 is analogous to figure 3, but here the pattern analyzed is the aortic temperature of a representative rat (the same presented in figure 3). Quantitative analyses of the cycles in this pattern are also presented in the same figure, as well as in table 2. The proportion of the graph that fell under the category of 'noise' or very short rhythms (less than 0.5 mHz) was higher than in the analysis of heat production rhythms, although, the pattern was similar in the presence of the circadian and higher rhythms, as well as the 33 and 126 μHz (periods of 8 and 2.2 hours) rhythms. Two other minor (probably harmonic) frequencies were detected: 54 and 69 μHz (periods of 5.1 and 4 hours), both contributing much less to the analyzed pattern than in the case of heat production graphs.

Figure 5 shows the result of a comparison between the 24-hour pattern of heat production of a rat and the variations of its aortic temperature. The degree of cross-correlation between the two series of data was relatively high. There was slight dephasing between the two sets of data, of about half hour in the case studied: i.e. there is a 30-minute delay in the appearance of a similar pattern of heat output in comparison with that shown by the aortic core temperature. The analysis of pairs of series of data from the animals studied showed comparable patterns, with a delayed correlation between heat production and aortic core temperature.

DISCUSSION

The analysis of series of individual recordings of either heat production or aortic core temperature showed the existence of already known rhythms (i.e. circadian) together with other hitherto unreported periodic variations of the parameters studied. The data thus acquired and analyzed by means of standard methods gave three main series of rhythms: those with periods of 24 hours or longer (i.e. infradian and circadian), those with periods between 24 and 0.5 hours and the rhythms with cycles lower than half an hour. The latter were not considered because of the coincidence of noise and random effects upon both parameters of the common activities of the animal (movement, other muscle activity, 'noise' of the recording instruments, etc.). The objective of this work is to determine the existence and quantitative importance of the ultradian rhythms, since the methodology used precluded a detailed

study of rhythms with periods longer than 24 hours.

The patterns observed between animals under comparable conditions were remarkably uniform. However, the amplitude of these patterns and their baselines, but not their frequencies, were somewhat more variable. It was assumed that these differences were mainly due to individual or idiosyncratic factors. The very high similarity between the heat output rhythms of female and male rats kept under comparable circumstances suggests a low influence of the oestral cycle; we did not determine the situation of our animals in this cycle, but since the sample was large and the measurements repeated, it is worth noting that no significant deviations of the patterns were observed. Male and female rats had a similar heat production pattern, which was also very similar for the whole series of animals, with little individual variation. This may be due in part to the high uniformity of the conditions under which the animals were studied as well as the rapid response and sensitivity of the calorimeter used.

The existence of a 24-hour cycle rhythm in several rat physiological parameters is well known (KAMINSKY & KOSENSNO, 1987), and is a direct correlate of the light period changes, as well as of the feeding patterns associated with them (BRUCKDORFER *et al.*, 1974; Peret *et al.*, 1976). The existence of short ultradian rhythms has also been described in the rat, related to metabolic parameters (STUPFEL & PAVELY, 1990), physical activity (BÜTTNER & WOLLNIK, 1984) or feeding patterns (STUPFEL *et al.*, 1989). The incidence of ultradian cycles in association with hemodynamic and thermogenic stimulation changes has recently been found (CLOSA *et al.*, 1991; 1992). This short-time pattern, of about 2 hours (126 μHz) is related to the sympathetic stimulation of brown adipose tissue thermogenesis (CLOSA, 1990). This rhythm appears in the external heat output pattern, where its magnitude is clear. However, the degree of influence of this pattern upon the aortic temperature is slightly lower than those of the higher harmonic (69 μHz, period of 4 hours) and other ultradian rhythms, that affect the aortic core (and thence the body) temperature more deeply.

The nature of the 54 and 69 μHz (periods of 5.1 and 4.0 hours) aortic core temperature rhythms could only be hinted at either from a humoral or neural pattern that would rhythmically affect the setting of the hypothalamic thermostat. This thermostat is set by other factors (light, energy levels) (MONSON *et al.*, 1983), but we do not know of any basic variable change that could account for this newly found pattern at present.

The influence of the 69 and 55 μHz (periods of 5.1 and 4 hours) patterns upon heat production, however, was undetectable, and thus it is very probable that they will not have a direct bearing on thermogenic regulation, but only on temperature levels. Since the span of variation of the rat aortic temperature under standard conditions is rather narrow (KITTRELL & SATINOFF, 1986; CLOSA *et al.*, 1991), the effects of increased aortic blood temperature within a physiological range would be very small when applied to the defined equations of radiative energy loss (WERNER & BUSE, 1988). Thus no significant effects were observed in heat output. However, the 126 μHz (period of 2.2 hours) rhythm is not only related to changes in aortic core blood and organ temperature, but it also implies an increase in cardiac output and tissue blood flow (including the skin) (CLOSA, 1990). It can be assumed, then, that these changes are the main factor in the modulation of heat loss rather than the direct changes in temperature associated with them. The hemodynamic changes associated with adrenergic stimulation (FOSTER & FRYDMAN, 1978) would then be the essential part of the loss of energy to the environment.

The rat would appear to be able to store a significant fraction of its energy output in the form of heat kept in the body, which results in

increased temperature. This heat is later lost with a comparable, but not coincident, pattern. The actual accumulation of heat that would represent a 0.8 °C increase of temperature of a 200 g rat (assuming a specific heat value comparable to that of water) would be in the range of 0.67 kJ; this accumulation for a period of 30 minutes (i.e. the delay time observed between aortic temperature and heat patterns) would represent c. 0.37 W, i.e. about 20 % of the mean heat output of a rat (RAFECAS *et al.*, 1989). The variations in temperature could then represent the actual storage or loss of heat in the animal in addition to the operation of its thermogenic system. The pattern of release would in part be independent of the accretion of heat, despite being regulated by the same thermostat (BRÜCK & ZEISBERGER, 1987). The dephasing observed when comparing the aortic temperature and heat release patterns can be explained by this heat cumulative behavior of the rat, since the delay observed in the response of the calorimeter could not, by itself, account for the whole span of the differences (less than 6 versus about 30 minutes).

In conclusion, the daily rhythms of heat release in the rat were correlated, but not fully coincident, with the changes observed in the aortic core temperature pattern. The differences were due to the deep influence of 54 and 69 μHz (periods of 5.1 and 4 hours) rhythms superimposed on the basic 126 μHz (period of 2.2 hours) and circadian rhythms, as well as to the existence of a physical heat accumulation rhythm (that raises the body temperature) dephased with the actual loss of this heat, directly related to skin and mucosal circulation rather than to temperature changes. The combined actual patterns observed were a composite of the relative incidence of the individual rhythms and their expressive power.

ACKNOWLEDGEMENTS

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TABLE 1

Analysis of the heat production rhythms observed in the undisturbed Wistar rat.

period hours		approximate frequency μHz	approximate contribution (%) to actual pattern	contribution (%) to 'noiseless' pattern
≥24	≥11.6		57	75
8	35		9	12
2.2	126		10	13
<0.5		<550	24	0

Analysis of the heat production rhythms of a representative rat studied for 48 hours. The contribution to the actual experimental pattern takes into account the 'noise', included in the <550 μHz group. The 'noiseless' pattern is the synthetic curve presented in figure 3, which takes into account the rhythms described and their relative weight.

TABLE 2

Analysis of the core temperature rhythms observed in the undisturbed Wistar rat.

period hours		approximate frequency μHz	approximate contribution (%) to actual pattern	contribution (%) to 'noiseless' pattern
≥24	≥11.6		28	64
8.4	33		4	8
5.1	54		5	12
4.0	69		5	11
2.2	126		3	6
<0.5		<550	65	0

Analysis of the core temperature rhythms of a representative rat studied for 48 hours. The contribution to the actual experimental pattern takes into account the 'noise', included in the <550 μHz group. The 'noiseless' pattern is the synthetic curve presented in figure 4, which takes into account the rhythms described and their relative weight.

Legends of figures

Figure 1
CROSS-CORRELATION FUNCTION BETWEEN HEAT PRODUCTION PATTERNS OF REPRESENTATIVE RATS.
Top, comparison of two female rats; center, comparison of male and female rats; bottom, comparison of two male rats.

Figure 2
CROSS-CORRELATION FUNCTION BETWEEN CORE TEMPERATURE PATTERNS OF TWO REPRESENTATIVE FEMALE RATS.

Figure 3
HEAT PRODUCTION PATTERN OF A REPRESENTATIVE RAT FOR 48 HOURS (LOWER GRAPH) AND THE COMPOSITE OF THE MAIN RHYTHMS DETECTED CORRESPONDING TO THE SAME ANIMAL (UPPER GRAPH).
The bar at the top represents the dark/light periods.

Figure 4
CORE TEMPERATURE PATTERN OF A REPRESENTATIVE RAT (THE SAME AS IN FIGURE 3) FOR 48 HOURS (LOWER GRAPH) AND THE COMPOSITE OF THE MAIN RHYTHMS DETECTED CORRESPONDING TO THE SAME ANIMAL (UPPER GRAPH).
The bar at the top represents the dark/light periods.

Figure 5
CROSS-CORRELATION FUNCTION BETWEEN HEAT PRODUCTION AND CORE TEMPERATURE PATTERNS OF THE SAME REPRESENTATIVE RAT.

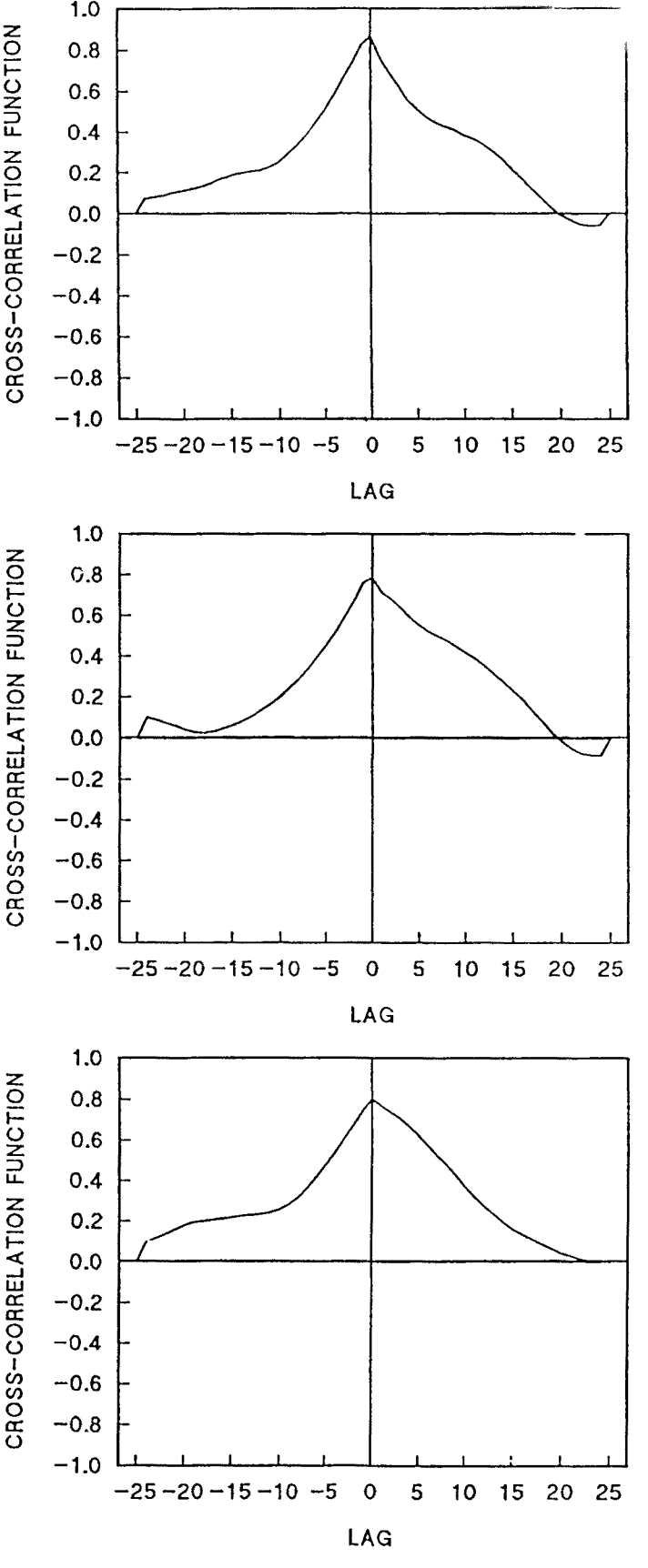
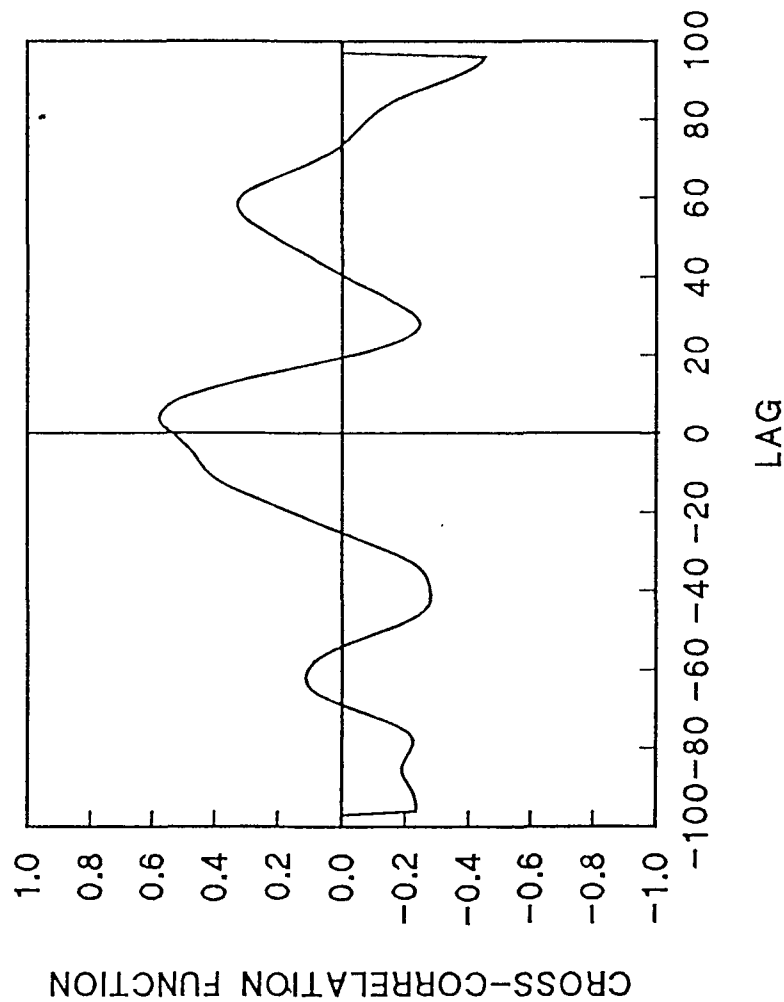

CROSS-CORRELATION FUNCTION
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-25 -20 -15 -10 -5 0 5 10 15 20 25
LAG
CROSS-CORRELATION FUNCTION
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-25 -20 -15 -10 -5 0 5 10 15 20 25
LAG
CROSS-CORRELATION FUNCTION
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-25 -20 -15 -10 -5 0 5 10 15 20 25
LAG

Figura 1


CROSS-CORRELATION FUNCTION
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0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-100 -80 -60 -40 -20 0 20 40 60 80 100
LAG

Figura 2

Figura 3

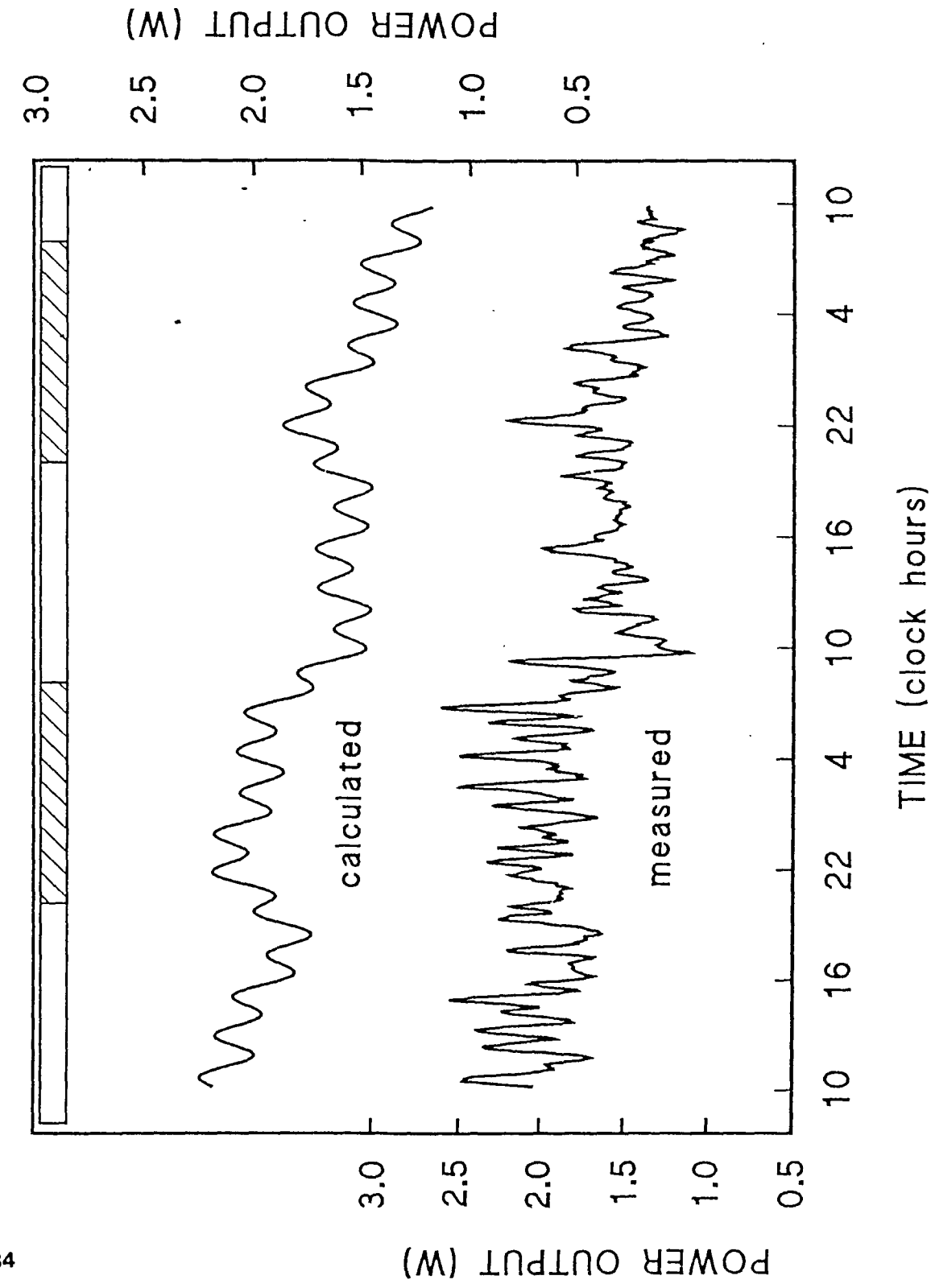
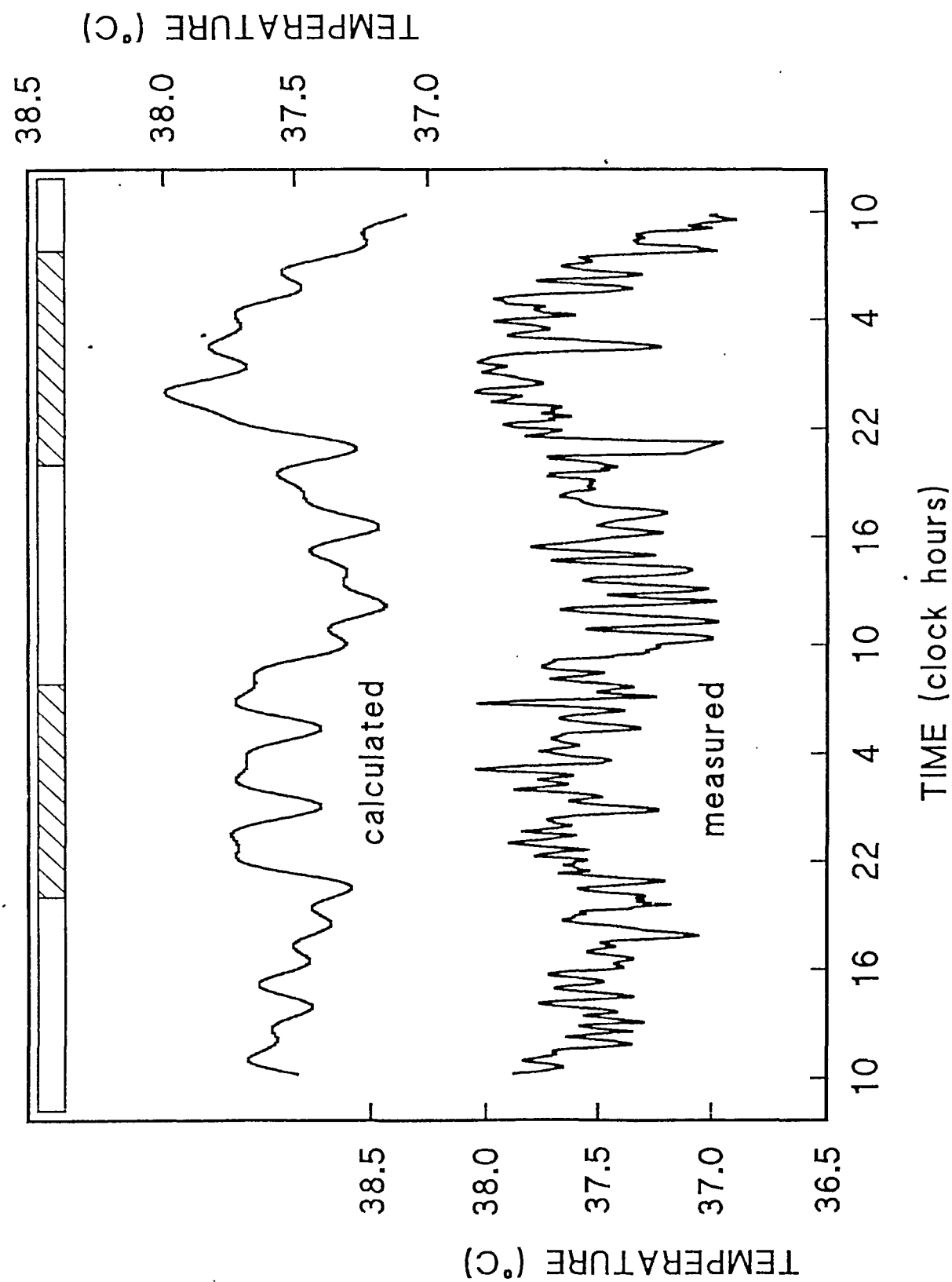

POWER OUTPUT (W)
3.0
2.5
2.0
1.5
1.0
0.5
calculated
measured
10
16
22
4
10
TIME (clock hours)

Figura 4


TEMPERATURE (°C)
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38.0
37.5
37.0
calculated
measured
10
16
22
4
10
16
22
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10
TIME (clock hours)
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38.0
37.5
37.0
36.5
TEMPERATURE (°C)

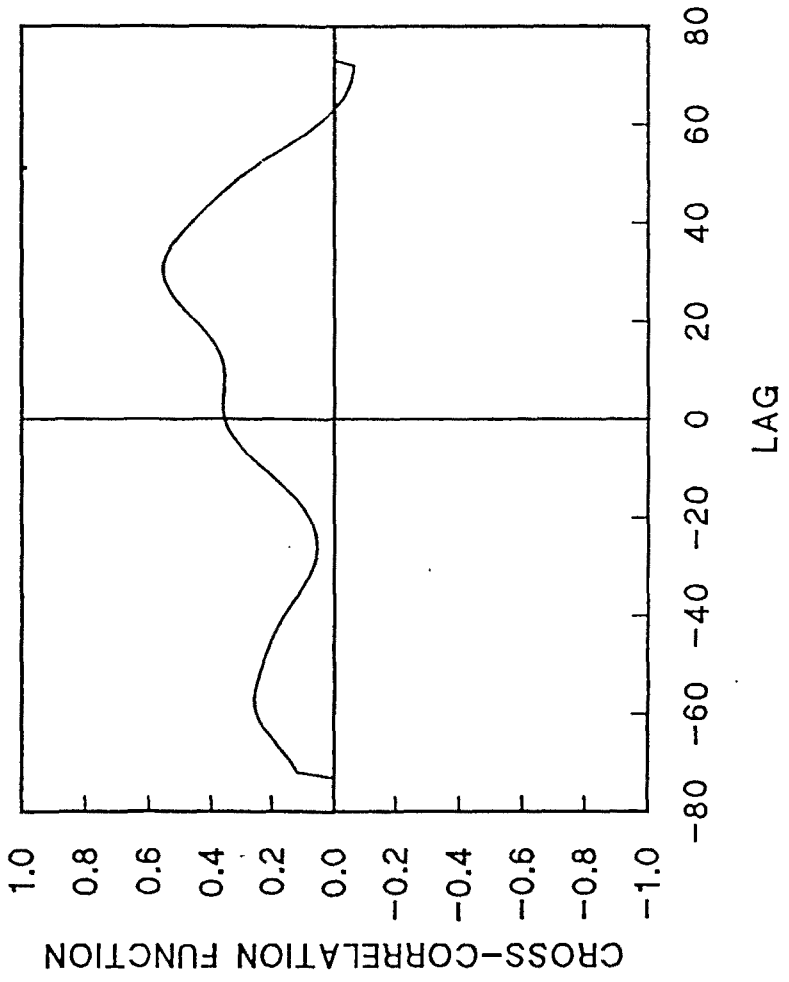
CROSS-CORRELATION FUNCTION
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-80
-60
-40
-20
0
20
40
60
80
LAG

Figura 5

treball enviat per publicar a COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY

Water balance in Zucker obese rats

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ABSTRACT

The water balance in Wistar, Zucker obese and Zucker lean rats, aged 60 days, was measured by determining the amount of water they drank, that contained in the solid food eaten, the water lost through urine and droppings, the net water accrued (estimated from the composition of the body and the daily increase in body weight), the measurement of the water vapour lost and the calculation of metabolic water production by means of the measurement of oxygen consumption, carbon dioxide production and protein oxidation in a 24-hour period.

1. Despite widely different body weights, all three groups of animals accrued a similar proportion of their daily water budget (4.3-4.9 %, i.e. 1.2-1.4 % of the total rat water mass).

2. Wistar and Zucker obese rats had a similar daily water budget despite very different body weights, lean Zucker rats had lower water budgets.

3. Obese and lean Zucker rats produced a more concentrated, and excreted much less urine (the highest urea concentration was found in for obese rats) than Wistar rats.

4. The water lost in the droppings was in the same range as that in urine for obese rats, slightly less for lean Zucker rats and much less in Wistar rats. Obese rats produced a higher amount of stool with respect to the amount of food eaten than the lean animals studied.

5. The contribution of metabolic water to the daily water budget was a 23.6 % for Zucker obese, 22.5 % for Zucker lean and 15.9 % for Wistar rats.

INTRODUCTION

Wild animals often have a tight water budget, since they usually feed upon dry materials and the availability of water is often limited (Schmidt-Nielsen, 1975). As for many rodents, the quantitative importance of metabolic water becomes highly relevant in the rat (Schmidt-Nielsen, 1964). The standard laboratory rat has no such constrictions, since their housing conditions usually include free access to water (Weisbroth *et al.* 1977). Drinking water consumption, however, varies considerably in different stocks and experimental settings (Baker *et al.* 1979). On the other hand, urine production and osmolality or catabolite concentration also show ample variations (Schmidt-Nielsen, 1972). Since it has been suggested that obese Zucker rats (fa/fa) may present alterations in urine production (Fiske *et al.* 1986), perhaps related to their genetic obesity, we have centred this study on their water balance, including a group of lean littermates (Fa/?) as control of the eventual effects of genetic obesity on water budget management.

The group of Wistar rats was included as additional control of the possible differences due to strain, and derived from the diverging process of domestication that affected the rat.

In studies of water balance, the measurement of food, tissue or excreta water content poses no problems except those derived from sampling and volume or weight measurement. However, the estimation of the water balance requires both the measurement of metabolic water production —on the input side— and the losses of water as vapour due to evaporation from lungs and mucosas (Lloyd *et al.* 1979). The estimation of metabolic water production has been often carried out by measuring oxygen consumption for a given time, then calculating the water produced with equations comparable to those used for energy estimations (Blaxter, 1989).

The estimation of water lost as vapour is more complicated, since the water must be trapped before its estimation, which involves the inclusion of the animal within airtight chamber in which the inflow and outflow of air must be accurately monitored; this is further complicated by the emission of water vapour from the drinking outlets, urine and droppings, which interferes with the breath and perspiration water. We have addressed both problems at once, by determining with utmost care the metabolic water production as well as the water vapour released by on-line measurement in an open system using an infrared gas analyzer, and then computing the measurements with time and the known flow of air through the chamber. The 'noise' created by urine, droppings and drinking water evaporation was estimated and subtracted.

MATERIALS AND METHODS

Female Wistar rats (of Iffa-Credo, France, stock) and Zucker rats —lean (Fa/?) and obese (fa/fa)— (of Charles River, USA, stock) were born and reared at the Animal Service of the University of Barcelona. The rats were weaned on day 21 after birth and then fed on standard pellet formulation (type A04 from Panlab, Barcelona). The rats had free access to water and food pellets, they were kept in individual plastic cages (with solid bottoms and wood shavings as bedding material) up to day 60. They were kept in a temperature (21-22 °C), humidity (70–85 %) and light (on from 08:00 to 20:00) controlled environment. Bedding material and soiled cages were changed daily. The animals were maintained in metabolic cages for days 45 to 59, in order to measure (and recover for analysis) all excreta, as well as to determine the amount of food consumed. The rats were weighed daily. On day 59-60 the rats were used for indirect calorimetry estimation of metabolic water and measurement of the water vapour released. Then they were killed and their water content measured.

In each set of rats, three groups of animals were studied: a) Wistar rats (as reference), b) obese Zucker (*fa/fa*) rats (as main subject —obese— of our study) and c) lean Zucker (*Fa/?*) rats (as controls of the latter). All groups were of 6 animals unless otherwise stated; several different series of rats for each experimental group were used. In all cases, the animals were randomly selected, when possible they were from different litters, and no statistically significant differences in body weight for matching age and strain were found between these groups.

This study is circumscribed to a single day, 60, in order to limit its scope to an age at which maturity has been achieved and growth rates are diminishing but have not ceased altogether, i.e. young adults. On days 45 and 60, parallel groups of rats were killed by decapitation and their water body content was measured by differential weighing —before and after 24 hours at 110°C— of samples from the finely minced whole rat cleaned of intestine and stomach contents. The differences in weight

from day to day were correlated with the known water content of the rat on days 45 and 60, and the net gain corresponding to day 60 was extrapolated. The daily amount of drinking water and food consumed were also measured, as was the water content of their excreta. The daily urinary nitrogen excretion was measured by determining its urea content (Fawcett and Scott, 1960), and total N with an elemental N analyzer (Carlo Erba NA-1500); the nitrogen-gap data necessary for the calculation of protein oxidation were taken from a previous study (Esteve *et al.* 1992a, 1992b).

In order to determine experimentally the amount of water lost through evaporation and transpiration, and to calculate the production of metabolic water with time, the measurement of oxygen consumption, carbon dioxide production and water vapour released were performed with the 59-60 day rat in a glass chamber (1.50 dm^3) receiving a flow of synthetic air (nominally 21 % O_2 79 % N_2, with no CO_2 nor H_2O) from a compressed gas cylinder, at a final pressure in the range of 0.1 MPa. The chamber was immersed in a thermostat-controlled water bath, kept at 25 °C (i.e. 298 K). The chamber contained a small glass water-drinking bottle (weighed before and after maintaining the rat in the chamber) and had a cellulose compress placed under a wide wire mesh for absorption of excreta.

Air inflow was measured and kept at 11 $ml \cdot s^{-1}$ for all lean animals and 22 $ml \cdot s^{-1}$ for obese Zucker rats. The gas efflux was also measured and its water, carbon dioxide and oxygen content were measured with two infrared gas-analysis systems (one for water and the other for CO_2), serially connected to a paramagnetic oxygen detector (all from Leeds & Northrup, England). The analogic signals of these instruments were digitalized with an RTI-800 interface card (Comelta, Barcelona) and then processed and stored in a desktop computer. Before —and after— every 24-hour measurement of gas exchange, the system was calibrated with standard gas mixtures.

The flow of nitrogen through the chamber was considered unaltered by the presence of the rat in the chamber (Ferrannini, 1988); the moles of oxygen, carbon dioxide and water vapour were calculated at fixed intervals (5 minutes) by taking the mean of 10 measurements of each parameter within that period. The results were stored in the computer. The water vapour lost was estimated directly, since all water present in the efflux air came from the rat, as the input air was completely dry. The eventual interference of vapour coming from urine and droppings was eliminated by measuring the water vapour yield of blank runs —2 hours each— of the system (with no rat) in which the bedding compress was humidified with an amount of 0.9 % NaCl equivalent to half the daily urine output of the rat (i.e. 8 ml for Wistar, 5 for Zucker obese and 3.5 for Zucker lean). The mean water vapour readings for this time were subtracted from the values measured to obtain the net amount of water vapour released by the rats.

The 24-hour oxygen consumption and carbon dioxide production at the given intervals, as well as the mean daily nitrogen excretion and nitrogen gap size (Esteve *et al.* 1992a, 1992b) were used for the estimation of protein, carbohydrate and lipid oxidized by the rat in 24 hours (*unpublished data*). From these data, the amount of metabolic water produced was derived with standard calculations (McLean and Tobin, 1987). The data are means of four consecutive intervals (i.e. data are given for every 20 minutes) and of 5-6 different animals. All data were presented adjusted to the local European winter time (+1 hour GMT).

RESULTS

Table 1 presents the basic experimental data obtained from 60-day rats. The size of obese rats was 68 % higher than that of lean Zucker rats, which were also 20 % smaller than

Wistar rats. The percentage water content of obese rats was lower (because of their very high fat content) than that of both lean groups; the water mass of obese Zucker rats was in a similar range to that of Wistars, and higher than that of Zucker lean animals. Again, Zucker obese rats ate more food than the lean Wistar and Zucker rats, thus ingesting much more energy. As a consequence of higher food intake, obese Zucker rats generated a higher mass of faecal pellets per day than the other two groups. However, their percentage of faecal water was practically identical to that of lean Zucker rats and in the same range as those of Wistar rats.

Urinary N excretion closely followed the proportions of food ingested for all three groups, with highest values for Zucker obese rats. The urea concentration in the urine released by lean Zucker rats practically doubled that of Wistar rats, and that of obese Zucker rats was more than three times the Wistar concentration.

Figure 1 shows the measured rates of water vapour lost throughout the 60th day of the three groups of rats, together with the calculated rates of production of metabolic water. Wistar rats showed a pattern of water emission directly related with the illumination (feeding) cycle, with a maximum towards the end of the dark period. The production of metabolic water followed roughly the same pattern, with higher synthesis during the dark period. Zucker obese rats showed a higher dispersion of data, as well as a higher degree of short-term oscillations with time. Here, the pattern of water emission presented a trough at the mid–dark period, with maximal values well within the lighted interval. The production of metabolic water also presented wide short term variations and followed the water emission pattern most of the time, but remained low at the end of darkness and the beginning of the light cycle. Lean Zucker rats presented a flatter pattern, with maximal variations overall of about 20 %, roughly pointing to minimal water emission during the light cycle. The metabolic water production pattern did not present long-term cyclic variations.

The water balances of Wistar and Zucker rats are presented in absolute terms on Table 2, and in percentage values in Figure 2. The water intake of Wistar and Zucker obese rats was similar, that of lean Zucker rats being about 24 % lower; however, the quantitative importance of metabolic water was higher (23.6 and 22.5 %) for Zucker rats than for Wistars (15.9 %).

Wistar rats excreted a much larger amount of urine than Zucker rats, showing —conversely— lower losses in their droppings. When expressed as percent of the water budget, the Wistar rat vapour losses were in a similar range to the losses through urine. Zucker rats showed a similar pattern, with most of the water losses being accounted for as water vapour. Obese Zucker rats lost a comparable amount of water in droppings and urine.

The water accrued was similar for all three groups, despite gross differences in their weight increase from day 59 to day 60 (3.1 ± 0.3 g, 7.0 ± 0.2 g and 2.9 ± 0.2 g, respectively for Wistar and obese and lean Zucker rats), since a large proportion of the weight gained by obese rats consisted of fat. The water retained by lean and obese rats was a fairly similar percentage of total water input.

When the water balance data are expressed as a percentage of the total water mass of the rat, we obtain the indexes presented in Table 3. The proportion of daily metabolic water generated versus total water was higher for obese than for both lean rat groups; however, these differences disappeared when expressed as the ratio of metabolic water versus energy ingested (27.8 $\mu l \cdot kJ^{-1}$, 23.2 $\mu l \cdot kJ^{-1}$ and 28.5 $\mu l \cdot kJ^{-1}$, respectively for Wistar, obese and lean Zucker rats). The figures for water accrued, evaporated water and daily water turnover were similar: the t½ for the rat water mass were about 41 h, 39 h and 50 h for —respectively—

Wistar, obese and lean Zucker rats. Urine water as a percentage of total water was lowest for lean Zucker rats, and highest for Wistars, the latter more than doubling that of the former; these differences were even magnified when the urine water was related to the energy intake: 75.3 $\mu l \cdot kJ^{-1}$, 24,2 $\mu l \cdot kJ^{-1}$ and 30,1 $\mu l \cdot kJ^{-1}$ respectively for Wistar, obese and lean Zucker rats.

DISCUSSION

The —redundant— direct assessment of all parameters involved in the estimation of the rat hydric balance is in itself a way to check the accuracy and adequacy of the measurements. As observed in Table 2, the deviation for the mean values between calculated and actually determined water retention were minimal for Wistar and Zucker obese, and lower than 6 % of the daily water budget for the lean Zucker rats, for which the difference between the two data is more patent. This overall homogeneity or coherence of the experimental and derived sets of data gives more credibility to the balances, in comparison with the common practice of measuring most of the data and deriving the rest from the available data.

The direct measurement of evaporation–transpiration water losses represents an improvement for the measurement of oxygen and carbon dioxide changes and is also an improvement on the systems based on the trapping of water (Atwater and Benedict, 1905), since the gas measurements allow on line water emission estimation and do not pose the problems derived from partial water retention, backpressures and high deadweight of the water traps (Levine and Morgan, 1990). This same arrangement enabled us to estimate metabolic water production at very short intervals.

There are significant differences between Wistar and lean Zucker rats as to water budget management. The main differences can be found in the larger proportion of urine water lost by Wistars and a somewhat higher evaporation-transpiration in Zucker rats. The overall movement of water represents a faster turnover for Wistar rats. Since all animals had all free access to drinking water (i.e. had an adequate —*ad libitum*— supply of water), we can presume that they did not suffer from thirst. The urinary concentration of urea of lean Zucker rats was about twice that of Wistars, thus it can be safely assumed that the main factor involved in the establishment of the urine volume in lean Zucker (and even more so in obese Zucker) rats is the need to eliminate nitrogen catabolites, essentially urea (Passmore and Eastwood, 1986), as occurs in the rat and many other species.

The possibility exists, however, that urea did not constitute the main contributor to the osmolality of the urine of these rats, since mineral components contribute in a high proportion to this osmolality (Passmore and Eastwood, 1986), and this is a limiting factor for the production of urine in mammals (Schmidt-Nielsen, 1975). In a parallel study, we determined the levels of alkaline and other metals in urine, as well as phosphate and other anions (unpublished results). Their combined concentration was a mean 542 $mEq \cdot l^{-1}$, 1120 $mEq \cdot l^{-1}$ and 990 $mEq \cdot l^{-1}$, respectively, for Wistar, obese and lean Zucker rats. When these values are added to the urine amino acid concentrations —15-16 mM for all three groups (*unpublished results*)— and to urea, we have an approximate idea of the osmolality of the urine of the three groups of rats: 1.0 $Eq \cdot l^{-1}$ for Wistar and 2.6 $Eq \cdot l^{-1}$ and 1.8 $Eq \cdot l^{-1}$ for obese and lean Zucker rats. Then, the factor limiting urine production in , Zucker rats as compared with Wistars is neither their mineral (essentially potassium and sodium chloride) content nor its osmolality; the differences between groups are in the same range as those indicated for urea.

The losses of water with the droppings constitute a significant share of the daily water budget of obese —and to a lesser extent of

lean— Zucker rats water. However, the water content of the faecal pellets of all three groups of rats was similar, but the amount of unused food in the stool, i.e. amount of faecal pellets produced with respect to the quantity of food eaten (g of solids in the droppings / g solids ingested) shows some differences between the groups studied: 0.163 for Wistar, 0.205 for obese Zucker and 0.157 for lean Zucker rats. There are no differences in this respect in either group of non-obese rats, suggesting that this relative digestive loss of efficiency may be related to this form of obesity. Thus, obese rats seem to extract a lower proportion of nutrients from their food (Esteve, 1992), which affects —albeit slightly— the management of their water allowance by increasing their faecal losses.

It has been found that obese Zucker rats, in addition to obesity and a defective thermogenic apparatus (Goldbole *et al.* 1978) show altered renal function (Fiske *et al.* 1986). It should nevertheless be pointed out that their ability to excrete highly concentrated urine is fully maintained. The altered pattern of urine production and catabolite concentration found in obese rats when compared with their lean counterparts can be tentatively related to their altered adrenal steroid production pattern (Bray, 1990), known to affect their circulating levels of corticosterone (Saito and Bray, 1983), with induced alterations in circadian rhythms (Luke *et al.* 1991), but which may also affect their mineralocorticoid component.

Despite these obesity-related aspects, there are significant differences in urine production and its share in daily water budget management between lean Zucker rats (of Sherman and Merck M ancestry) and Wistar rats. In both cases, however, metabolic water contributes in a similar proportion to the water input when compared with the total water mass of the rat, and also when compared with their energy intake. The tighter control of urinary losses and lower amount of water drunk suggest that Zucker rats are more adapted to dry conditions, i.e., their behaviour conforms to current hypotheses to the effect that the rat is not excessively dependent on drinking water in the management of their water balances (Schmidt-Nielsen and Schmidt-Nielsen, 1950). It would, however, be unwise to generalize, since our results suggest that rats of different stock may exhibit varying water-management strategies.

ACKNOWLEDGEMENTS

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TABLE 1

Weight and balance data of 60-day Wistar, obese and lean Zucker rats

	Wistar	Zucker obese	Zucker lean
Weight (g)	198.8 ± 4.2	279.3 ± 8.4 *#	165.9 ± 3.9 *
Rat water content (%)	67.15 ± 0.38	45.05 ± 0.53 *#	66.40 ± 0.38
Rat water mass (g)	133.5 ± 2.8	125.8 ± 2.8 #	110.2 ± 2.6 *
Food consumed (g/day)	16.68 ± 0.59	29.65 ± 0.46 *#	17.89 ± 0.69
Ingested energy (kJ/day)	223 ± 7	396 ± 5 *#	239 ± 7
Droppings water content (%)	56.5 ± 1.2	59.4 ± 2.0	59.9 ± 1.8
Urinary urea (mM)	449 ± 25	1460 ± 60 *#	825 ± 50 *
Urinary N (as urea, mg/day)	453 ± 55	843 ± 34 *#	366 ± 45

The data are the mean ± SEM of 6 different animals. All data have been referred (calculated) for rats on day 60.
Statistical significance of the differences between groups (Student's *t* test):
Differences versus Wistar rat values: * = $P < 0.05$
Differences between obese and lean Zucker rats: # = $P < 0.05$.

TABLE 2

Water balances of 60-day old Wistar and lean and obese Zucker rats

	Wistar	Zucker obese	Zucker lean
INPUT			
Water drunk	30.7 ± 3.6	26.1 ± 1.2	21.1 ± 1.4 *
Water in solid food	2.0 ± 0.1	3.6 ± 0.1 *#	2.2 ± 0.1
Metabolic water [1]	6.2	9.2	6.8
total	**38.9**	**38.9**	**30.1**
OUTPUT			
Urine water	16.8 ± 3.2	9.6 ± 0.4 *	7.2 ± 1.3
Water in the droppings	3.1 ± 0.2	7.8 ± 0.9 *#	4.0 ± 0.2 *
Water vapour lost [4]	17.4 ± 2.1	19.6 ± 3.1	19.0 ± 2.6
total	**37.3**	**37.0**	**30.2**
WATER NET BALANCE			
Measured water balance [2]	**+1.7**	**+1.8**	**+1.6**
Calculated water balance [3]	+1.6	+1.9	−0.1

All values are expressed in grams per day. They represent the mean ± SEM of 4-6 different animals.
Statistical significance of the differences between groups (Student's *t* test):
Differences versus Wistar rat values: * = $P < 0.05$
Differences between obese and lean Zucker rats: # = $P < 0.05$.
[1] Mean values measured in another comparable set of animals from 24-hour oxygen consumption, carbon dioxide production (Figure 1) and nitrogen balance data.
[2] Mean values of net water accumulation calculated from the net mean weight increase and the rat composition changes measured in another series of animals from days 45 to 60.
[3] Arithmetic difference between mean total water intake and water losses.
[4] Cumulative evaporative losses (transpiration + evaporation from respiratory mucosas etc.), measured in another set of rats (those used for the estimation of metabolic water production, Figure 1).

TABLE 3

Water balance data of 60-day Wistar, obese and lean Zucker rats related to their body water mass

	Wistar	Zucker obese	Zucker lean
% OF WATER MASS			
metabolic water	4.7	7.3	5.4
water accrued	1.2	1.4	1.2
water turnover	29.2	30.9	23.9
urine water	12.6	7.7	5.7
evaporated water	13.0	15.6	15.1

All data are expressed as percent of the whole rat water mass (calculated from Tables 1 and 2).

LEGENDS TO FIGURES

Figure 1

Water vapour emission and metabolic water production of 60-day Wistar, lean and obese Zucker rats during a period of one day

The values shown in the dark graph are the 20-minute means (4 mean measurements of 5 minutes each) of 6 rats water vapour emission. The upper black graph of each drawing represents the water vapour losses, the accompanying dot graphs show the upper and lower ranges of the mean ± SEM. The lower graph represents the calculated metabolic water production. The time scale is set at GMT +1 hours. The bar at the top shows the periods of light and darkness.

Figure 2

Water balances of 60-day Wistar, obese and lean Zucker rats, expressed as percentages of the daily water budget

The data presented are mean values calculated from those of Tables 1 and 2. In all cases, 100 % represents the mean total day-60 water input.

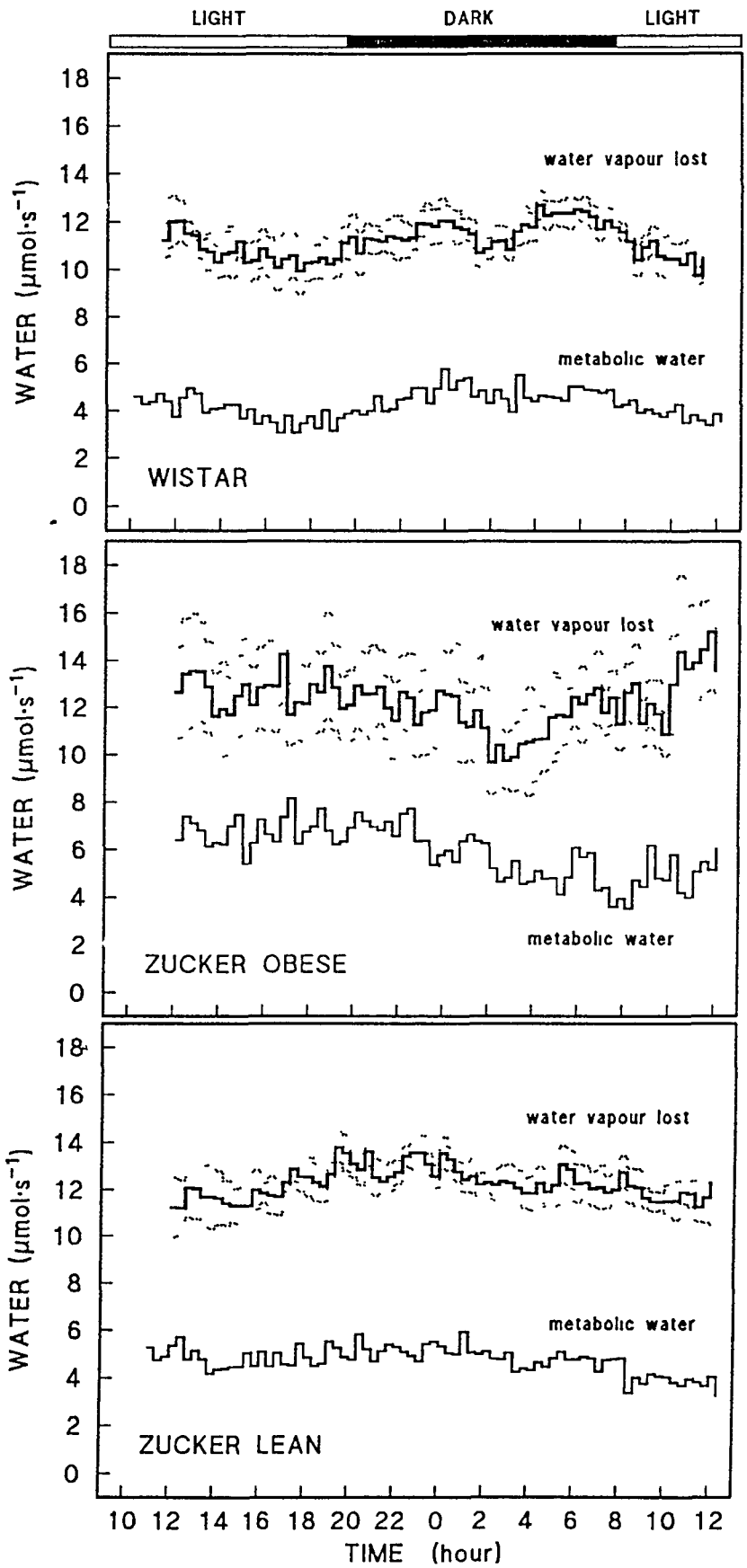
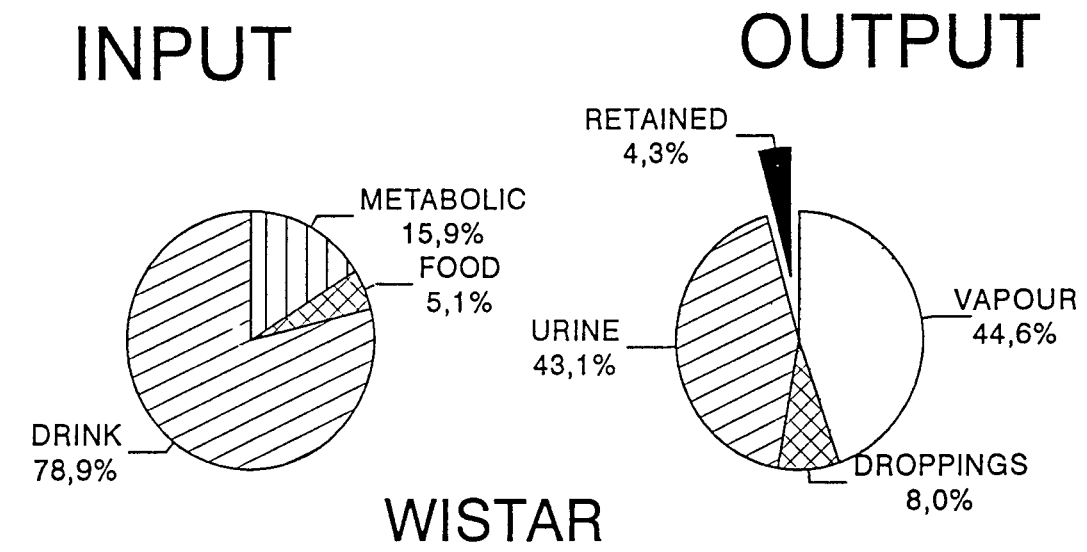
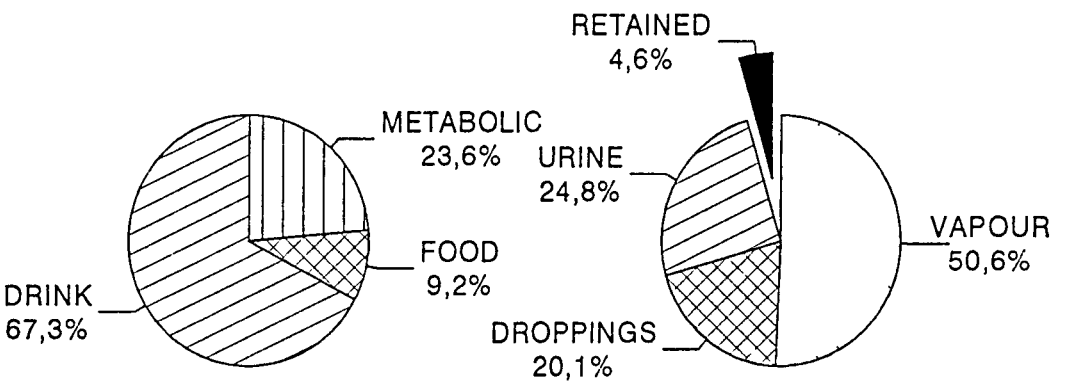

LIGHT
DARK
LIGHT
WATER (µmol·s⁻¹)
water vapour lost
metabolic water
WISTAR
ZUCKER OBESE
ZUCKER LEAN
TIME (hour)
10 12 14 16 18 20 22 0 2 4 6 8 10 12

Figura 1


INPUT
OUTPUT
METABOLIC
15,9%
FOOD
5,1%
DRINK
78,9%
RETAINED
4,3%
VAPOUR
44,6%
URINE
43,1%
DROPPINGS
8,0%
WISTAR


METABOLIC
23,6%
FOOD
9,2%
DRINK
67,3%
RETAINED
4,6%
URINE
24,8%
VAPOUR
50,6%
DROPPINGS
20,1%
ZUCKER OBESE

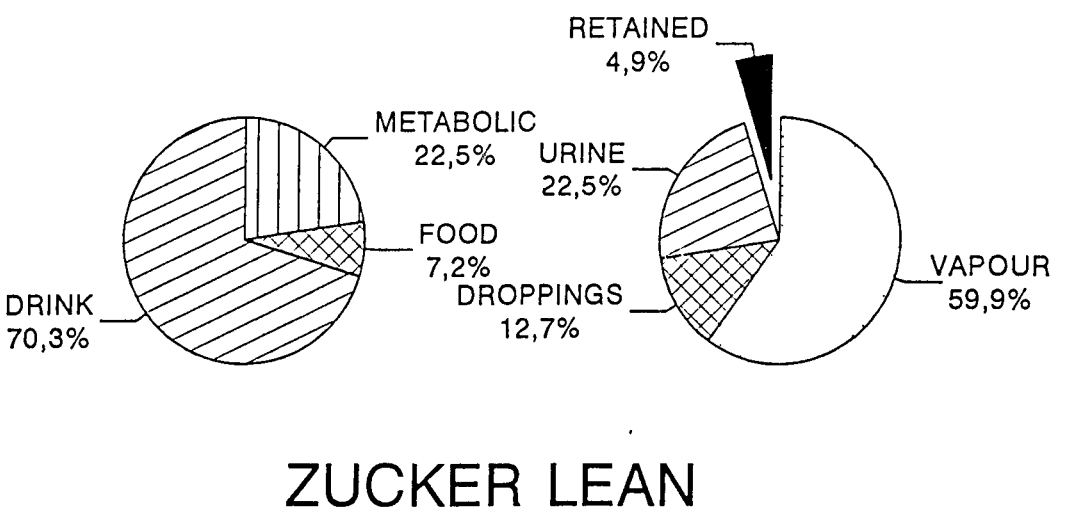

METABOLIC
22,5%
FOOD
7,2%
DRINK
70,3%
RETAINED
4,9%
URINE
22,5%
VAPOUR
59,9%
DROPPINGS
12,7%
ZUCKER LEAN

Figura 2

treball enviat per publicar a JOURNAL OF APPLIED PHYSIOLOGY

Indirect calorimetry estimation of carbohydrate and lipid consumption in obese rats

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ABSTRACT

The energy consumption of series of 60-day rats of Wistar, lean and obese Zucker stock have been studied by means of direct and indirect calorimetry, and by establishing their energy balance through measurement of food intake and retention. Calorimetric studies encompassed a 24-hour period, with gas and heat output measurements every 2 or 5 minutes respectively for direct and indirect calorimetry. The analysis of fat composition (diet, whole rat, and synthesized and oxidized fat) showed only small variations that had only a limited effect on the overall energy equation parameters. A gap in the nitrogen balance, which represents a urinary N excretion lower than the actual protein oxidized, resulted in serious deviations in the estimation of carbohydrate and lipid oxidized when using the equations currently available for indirect calorimetry. Analysis of the amino acid composition of diet and rat protein as well as of the portion actually oxidized, and correcting for the nitrogen gap allowed the establishment of a set of equations that gave a much better coincidence of the calculated data with the measured substrate balance. The measured heat output of all rats was lower than the estimated values calculated by means of either indirect calorimetry or direct energy balance measurement; the difference corresponded to the energy lost in water evaporation, was in the range of 1/5th of total energy produced in the three rat stocks. Wistar rats showed a biphasic circadian rhythm of substrate utilization, with alternate lipid synthesis/degradation that reversed that of carbohydrate, concordant with its nocturnal feeding habits. Zucker rats did not show this rhythm; obese rats synthesized huge amounts of fat during most of the light period, consuming fat at the end of the dark period, which suggests more diurnal feeding habits. Lean Zucker rats showed a similar, but much less marked pattern.

INTRODUCTION

Indirect calorimetry has long been an essential tool in studies of overall energy and substrate balances in undisturbed animals (22). The correct application of this technique poses several practical problems (28), which have been partially circumvented by the development of precise methods for gas volume and concentration analysis, improved chambers (3) and

optimized formulas for calculation (15,28). In all cases, the correct estimation of heat and the substrate being currently oxidized needs precise measurements of VO_2 and VCO_2 as well as of protein oxidation (41).

There are a number of formulas for calculation provided the basic data are available, such as the complete and detailed formulations of Ferrannini (15) and those developed by McLean and Tobin (28) from the bomb calorimeter studies of Brouwer (5). These studies —and other carried out along the same lines (27)— represent the current state-of-the-art for indirect calorimetry calculations.

Many studies on obese Zucker rats have shown their amazing ability to convert large amounts of diet carbohydrate (and protein) into stored lipids (30). The obese Zucker rats metabolize large amounts of diet protein (21), and excrete substantial amounts of nitrogen in their urine (12); however, recent studies have found that other rat strains (13) and mammals (7) show a sizeable nitrogen "gap" (12), i.e., a significant proportion of the nitrogen absorbed is not found in the urine and is reputedly lost as nitrogen gas. This is a generalized problem (9) and it has been postulated that N_2 emissions can affect the basic gas measurements used in indirect calorimetry (6). Since obese Zucker rats show hyperphagia, and accumulate only a fraction of the nitrogen ingested (10), it was assumed that the nitrogen gap could significantly affect any indirect calorimetry measurements based essentially upon nitrogen excretion data as an index of protein oxidation.

The present work was devised, then, to check the applicability of the current indirect calorimetry formulas to hyperphagic obese animals. In addition, the possible effect of lipid and protein composition of the rats on the applicability of the indirect calorimetry formulas developed was also determined.

MATERIALS AND METHODS

Animals:

Female Wistar rats (of Iffa-Credo, France, stock) and Zucker rats —lean (Fa/?) and obese (fa/fa)— (of Charles River, USA, stock) were bred at the Animal Service of the University of Barcelona. The rats were weaned on day 21 and fed thereafter on a standard pellet formulation (type A04 form Panlab, Barcelona Spain), containing 59 % metabolizable carbohydrate (essentially starch), 3 % lipid (mainly fish and soybean oil), and 17 % protein (a mixture of fish meal, soy and cereal protein), 6 % minerals and 4 % fibre, with a nominal energy content of 14.6 $kJ \cdot g^{-1}$. The rats had free access to water and food pellets, and were kept in individual plastic cages (with solid bottoms and wood shavings as bedding material) from day 30 to 60. The animals were kept in a temperature (294-295 K), humidity (70–85 % relative humidity) and light (on from 08:00 to 20:00) controlled environment. Bedding material and soiled cages were changed daily. One group of rats was kept in the same surrounding within metabolic plastic cages (Tecniplast, Gazzada Italy), and used for balance studies from days 30 to 60.

In all experiments, three groups of animals were studied: a) Wistar rats (as reference), b) obese Zucker (*fa/fa*) rats (as main subject —obese— of our study) and c) lean Zucker (*Fa/?*) rats (as controls for the obese rats). The presence of rats of different original strains: Wistar and Sherman and Merck M for the Zucker rats (42), was considered adequate as a way to check the applicability of the methods used to rats from different stocks. Several different series of rats for each experimental group were used. All series were of 5-6 animals unless otherwise stated. In all cases, the animals were randomly selected, when possible they were from different litters, and no statistically significant differences in body weight for matching age and strain were detected.

Experimental setup:

This study is circumscribed to a single

day, 60, in order to limit its scope to an age at which maturity has been achieved, and growth rates are diminishing but have not ceased altogether, i.e. young adults.

Three series of animals were studied; the first were killed on day 45, the second were kept in metabolic cages from day 45, and weighed daily from day 55-60; their food consumption was measured and their urine and droppings were recovered and analyzed (days 55 to 59). On day 60 the rats of this second group were used for indirect calorimetry experiments, and then killed. The carcasses of rats of 45 and 60 days were analyzed and their composition was computed together with the increases in body weight of the last four days, so as to obtain an estimate of the composition of the weight accrued. The third series of rats was used solely for the direct calorimetry measurements on day 60.

The computed data on food ingested, nutrients available (i.e. ingested nutrients corrected for the absorptive efficiency), body composition, weight increase and composition of excreta were used to estimate net energy, nitrogen, carbohydrate and lipid balances, all referred to day 60, so as to make the results comparable with the direct and indirect calorimetry measurements.

Gas measurements:

Gas measurements were performed with the rat in a glass chamber (1.50 dm^3) receiving a flow of synthetic air (nominally 21 % O_2 79 % N_2, with no other components) from a compressed gas cylinder, at a final pressure in the range of 0.1 MPa. The chamber was immersed in a thermostat controlled water bath, kept at 298 K. The chamber contained a glass tube with drinking water (weighed before and after the experiment as a measure of the water drunk), and food pellets. The rat was supported in the chamber by a wire mesh, under it, a sanitary absorbing compress was placed to retain the urine and droppings.

Air inflow was measured and kept at 13.8 $ml \cdot s^{-1}$ for all lean animals and 27.6 $ml \cdot s^{-1}$ for obese Zucker rats. The gas efflux was also measured and its water, carbon dioxide and oxygen content were measured with two infrared gas analysis systems (one for water and the other for CO_2) serially connected to a paramagnetic oxygen detector (all from Leeds & Northrup, England). The analogic signals of these instruments were digitalized with an RTI-800 interface card and then processed and stored in a desktop computer.

Before —and after— every gas exchange measurement, the system was calibrated with two standard gas mixtures: $O_2/N_2/CO_2$ and O_2/N_2. After establishing the ranges for oxygen and carbon dioxide, the system was calibrated with water-saturated air, adjusting the scale using a water vapor saturation / temperature table (40).

Gas measure calculations:

The flow of nitrogen across the chamber (456 $\mu mol \cdot s^{-1}$ for lean and 913 $\mu mol \cdot s^{-1}$ for obese animals) was considered unchanged by the presence of the rat in the chamber, irrespective of the volumes of the gas entering and leaving the chamber or their temperatures. From the air inflow and its known composition (measured with the chamber empty), the moles of nitrogen and oxygen entering the system per unit of time were determined. The percentage composition of the effluent gas was used to determine its actual volume by equating the moles of nitrogen at both sides of the chamber (15). From here, the moles of oxygen and carbon dioxide were calculated at fixed intervals (5 minutes) by calculating the mean of 10 measurements of each parameter taken at intervals within that period. The results (moles of oxygen consumed, and moles of carbon dioxide produced, and respiratory quotient) were stored in a diskette.

Energy and substrate calculations:

The ingested nutrient values were computed, taking into account the probable losses of materials in the digestive process; thus, the lipids absorbed were considered to be 95 % of those ingested (25), for carbohydrate the same percentage was applied (29). In the case of protein, the previously determined (*unpublished results*) absorptive efficiency for the three groups of rats was applied to the ingested protein values. The energy equivalence factors used for all calculations were those proposed by Brouwer (5).

The lipid and protein balance analysis, as well as the known content of carbohydrate in the diet ingested allowed the determination of the net amount of protein, lipid and carbohydrate oxidized by the rats on day 60. These data were then compared with those derived from the application of current formulas for estimation of substrate oxidation.

The mean composition (empirical formulas) of the different samples were calculated from the measured lipid fatty acid (32) and protein amino acid composition for the different lipid/protein batches. The lipid formulas are given as triacylglycerols, and those for protein as amino acyl residues (i.e. amino acids minus a molecule of water).

The values given in the Tables for oxygen consumption and carbon dioxide production for lipids and proteins per gram of foodstuff were calculated using the mean molecular weights derived from the mean empirical formulas. These calculations were performed assuming complete oxidation for lipids and complete conversion of all amino acid N either to urea or to N_2.

Since the values for protein oxidation referred to a whole day, it was not possible to determine the instantaneous rates of protein degradation, and thus, in the calculation of lipid and carbohydrate oxidation values for a given time we had to assume (at least as an approximation) that the protein oxidation values were uniform for the 24 hours of the study. In order to minimize the influence of the nitrogen gap in the calculations, it was decided to use the net oxidized protein value in the formulas instead of the smaller value of urinary nitrogen excretion (12).

The O_2 and CO_2 data were combined with the net protein degradation values and applied to the indirect calorimetry formulas to obtain the energy, and carbohydrate and lipid oxidation values given in the tables and figures. In the figures, the data presented are means of four consecutive intervals (i.e. data are given every 20 minutes) and of 5-6 different animals. All data were presented adjusted to local European winter time (GMT+1 hour).

The parameters for protein degradation were adjusted proportionally for the existence of an N gap, i.e., the formula assumes that —for Wistar rats fed on standard diet— about 1/10th of the N oxidized does not appear in the urine (13), and in addition, it draws more oxygen and produces more carbon dioxide than the protein converted into urea.

The parameters of the basic set of indirect calorimetry equations were determined from the actual values found for oxidation of the diet consumed.

Direct calorimetry

In addition to indirect calorimetry performed as indicated above, sets of 4 animals per group aged 60 days were used for the measurement of their heat release by means of direct calorimetry. A differential adiabatic multi-chambered calorimeter, developed in our Laboratory (8) was used. Since the instrument walls were made of copper, the rats were kept in the dark for the duration of the experiment (24-26 hours). The rats had free access to water and food during the experiments. The calorimeter was connected via a converter analogic/digital card with another desktop computer. Heat release measurements were taken at intervals of 15 s, and were computed every 2 minutes and stored in a diskette. In the graph, the means of 10

such 2-minute measurements for 4 animals are presented. Previous work in our Laboratory has shown that rats kept 24 hours in darkness preserve their circadian and ultradian rhythms practically unchanged.

Energy equivalence of evaporative water losses

Since direct calorimetry does not incorporate the heat losses incurred with water evaporation, the energy equivalence of this water evaporation was calculated from the cumulative water vapor measurements performed with the infrared gas analysis system on 60-day rats. The heat of evaporation constant of water at the temperature of the chamber (40) was used for the estimation of this component of the heat output of the rat.

The heat lost in converting water to vapor was determined by using the equation:

$$W = H \times (56969.2 - 44 \times T) / t$$

where W is the power (in W), H the water evaporated (in moles), T the temperature (in K) and t the time (in s). The water vapor lost was estimated directly, as all water present in the efflux air came from the rat, since the input air was completely dry. The possible interference of vapor coming from urine and droppings was eliminated by measuring the water vapor yield of blank runs —2 hours each— of the system (with no rat) in which the bedding compress was humidified with an amount of 0.9 % NaCl equivalent to half the daily urine output of the rat (i.e. 8 ml for Wistar, 5 for Zucker obese and 3.5 for Zucker lean). The mean water vapor readings for this time were used as blanks and subtracted from the real measurements to obtain the net amount of water vapor released by the rats.

RESULTS

Table 1 shows the basic energy and nitrogen balance data of the three groups of rats used. As expected, the 60-day Zucker obese rat was heavier than either lean Zucker or Wistar rats of the same age; the rate of weight increase and storage of protein and —especially— fat were also higher for obese rats, as was their food intake. Their energy production, calculated from available ingested energy data alone, also reflects this trend, since Wistar and lean Zucker rats had an energy output of 85-88 % of their intake but obese Zucker rats lowered this figure to about 69 %. Since obese rats deposited a much lower proportion of protein than of lipids, their N excretion rate was much higher, with a lower N *gap* (12).

In Table 2, the mean lipid composition of Wistar and Zucker rats is presented in the form of empirical formula of their triacylglycerols. For each group, values are given for the total rat lipid, for the fraction accrued and for the fraction oxidized. The size (chain length) of the fatty acids in the triacylglycerols oxidized for all three groups was distinctly smaller than those of the other fractions. In general, obese rats had somewhat smaller triacylglycerols. Nevertheless, the differences between groups and types of lipid were very small, resulting in only modest changes in the oxygen and carbon dioxide values obtained from their theoretical oxidation. The maximal difference between any two values was lower than 10%.

Table 3 presents a similar set of data referred to the mean amino acid residue in the rat protein, as well as in the accrued and oxidized fractions. With protein the variability was much higher, with varying proportions of nitrogen and sulphur. As expected, the complete oxidation of protein ($N \rightarrow N_2$) resulted in higher theoretical oxygen consumption and higher carbon dioxide production than its elimination as urea. The mean O_2 and CO_2 calculated values for the protein oxidized (to urea) by Zucker rats were very close to those of diet protein, but those corresponding to Wistar rats were higher. When the values for total oxidation (to N_2) are compared, the similarities between

the three sets of oxidized protein and the diet were closer, with even less changes in the respiratory quotient.

The three sets of formulas presented in Table 4 were used for the estimation of energy, carbohydrate and lipid consumed shown in Table 5. The deviations for small figures of net lipid synthesis (Wistar and lean Zucker rats) gave the poorest fits. There were significant ($P<0.05$, Student's t test) differences between the measured balance data and those obtained with the commonly used formulas. The mean carbohydrate values were also rather disparate, but the differences were not significant because of a high dispersion of individual data. In all cases, the formulas postulated in this study, drawn from the actual composition of the diet and using protein balance data instead of excreted nitrogen values, resulted in better fits for all three groups of rats.

Figure 1 shows the oxygen consumption values for a whole day for Wistar and Zucker rats. The dispersion of data was much more marked for lean and obese Zucker rats than for Wistars. In all three groups, there was a maximum oxygen consumption at the end of the dark period, and a minimum in the first half of the light period. Figure 2 presents the data for carbon dioxide production. The form of the curves was very similar to that of oxygen consumption for lean Zucker rats, but there was little resemblance for the other two groups.

The respiratory quotients (Figure 3) changed little with time in lean Zucker rats, followed a bimodal pattern (peaks at the first third of both dark and light periods) in Wistar rats and showed deep variations in Zucker obese rats, with a maximum in the light period and a minimum at the end of the dark.

The use of the formulas postulated allowed the determination of the variations in time of lipid and carbohydrate oxidation, as presented in Figure 4. The pattern shown by the respiratory quotient gave rise to two complementary graphs for carbohydrate and lipid oxidation in Wistar rats. Maximal carbohydrate oxidation was found in the mid-dark period, with a minimum just at the end of the light period. This coincided with the maximal lipid oxidation. The net lipid synthesis concurred with the peaks of carbohydrate oxidation in the middle of the dark and light periods. Lean Zucker rats showed much less variation, with only small changes in carbohydrate and lipid oxidation by the last third of the dark phase, and fairly uniform carbohydrate oxidation and lipid synthesis for the rest of the day. A similar —scaled-up— pattern was observed in obese Zucker rats. These animals actively synthesized lipid at the expense of carbohydrate for most of the day (light period), and consumed part of this lipid with very little carbohydrate oxidation during the last half of the dark interval.

In Figure 5, the heat production values of 60-day rats estimated with the indirect calorimetry formula postulated in this study, together with the direct calorimetry data are shown. The heat output data (direct calorimetry) were consistently lower than those obtained from indirect calorimetry calculations. Water vapor release data are presented in Table 6, together with the means of the calorimetric data presented in Figure 5. The mean heat loss from water was similar to the differences between the mean 24-hour values for heat production (indirect calorimetry) and for total measured heat output (direct calorimetry). The amount of heat lost through the estimated water evaporation was in the range of the order of 1/5th of all heat produced (22 % for Wistar, 18 % for Zucker obese and 23 % for Zucker lean rats).

DISCUSSION

The main systems used for the estimation of energy balances of animals are direct (24) and indirect calorimetry (33); the direct measurement of substrate energy balances has been also used (2). Direct calorimetry gives accurate,

direct and readily measurable data (15), but its applicability is usually restricted to small animals, since it requires increasingly complex systems for its application to large organisms, such as humans (24). Another drawback is the need to estimate the amount of heat lost through evapo-transpiration processes, since it cannot be readily measured with calorimeters (37).

On the other hand, indirect calorimetry can be easily scaled-up and applied with advantage to larger animals, since they interchange measurable gas volumes more easily. Indirect calorimetry, however, also has some disadvantages, such as the —slight— variation of oxygen/heat equivalences, depending on the substrates being used (5,26), the hindrances related with net substrate synthesis —essentially fat— (15), and the need to procure data on protein oxidation (41). There are also other methodological aspects that limit the full use of indirect calorimetry, such as the interference in gas volume, composition and flow measurements resulting from the presence of considerable amounts of water in the expired air. In addition, the formulas available for energy calculations have been derived from bomb calorimeter heat production measurements of standard substrates, not always comparable with the materials being oxidized (15).

The direct measurement of substrate balances in living animals also has serious intrinsic drawbacks: difficulty in measuring the residual energy in droppings and urine or estimation of the efficiency of assimilative processes so as to obtain available energy data (38), as well as the need to carefully evaluate the energy equivalence of the materials stored/lost by the animal during a given period of time. In a live animal this is very difficult to measure, since gains/losses of weight may be due to changes in fat or water and/or protein stores (20).

The use of the three approaches on an uniform group of animals from three different stocks (with different fat management, size, genetic background but having in common their application in comparative experiments) has been used in this work as a way to compare the techniques, correct flaws and validate their applicability. The data on substrate–energy balances have been considered the most accurate, since they derive from the measurement of actual tissue and feed composition, and incorporate a number of data taken from animals under similar conditions (14). The substrate–energy balance data show a sizeable nitrogen gap for all groups tested (12,13). This, together with the slightly different amino acid composition of the proteins ingested and oxidized (as well as that constituting the protein pool of the animal) can result in widely changing amounts of oxygen drawn for their oxidation —on a weight basis—, also altering the carbon dioxide generated. Despite being fed the same standard pellet diet, the three groups of rats presented a different pattern of protein deposition/oxidation. This may have a profound effect on the application of the standard equations for calculation of energy production and other substrate (carbohydrate, lipid) oxidation.

The problems with protein oxidation measurement are not limited to this point, however, since the mean 24-hour data calculated from balances or those obtained from their excretion through urine emission could not be compartmentalized in short time-frames: the rats do not urinate at timed intervals, and anyway the urine N is only a —large— fraction of all N excreted, in itself an index of net protein oxidation. Since there is no known method for measuring the variation of protein oxidation in time-frames shorter than those used here, we reverted to the N balance data for a 24-hour period, assuming for the sake of the calculations, as is often assumed (15,28), that the rate of protein oxidation is *uniform* for all the period studied.

The possible influence of fat synthesis/oxidation has been found to be much smaller than expected, since the composition of the rat fat

stores is fairly uniform (19,32), and the management/oxidation of dietary fat results in very similar oxygen consumption/carbon dioxide production calculated on a lipid weight basis. Thus, this factor has not been further explored.

The problem of water evaporation both as a heat–loss factor and as an interference in the measurement of oxygen and carbon dioxide balance has been addressed by simply measuring it. This has been found to be more precise than the simple removal of most water by means of a water trap (1), since these do not often result in completely dried gas; in addition they alter the temperature of the efflux and can induce a slight buildup of pressure within the system because of resistance to the gas flow.

The gas flow was maintained high in order to prevent the incidence in the measurements and calculations of the N_2 potentially generated by the rat (6). The N_2 production calculated for all groups of rats studied was less than 0.6 $\mu l \cdot s^{-1}$ (i.e. a mere 0.06 % of the input gas), thus it was considered that the flow of N_2 entering the chamber was equal to that leaving it. All calculations were done on a molar rather than on a volume basis, since the relationship to substrate utilization was thus more direct and the formulas were simplified by the elimination of all reference to temperatures.

The basic energy substrate to energy conversion factors used are those of Brouwer (5), very similar to other comparable sets of data available in the literature (23). The basic structure of the equations for the measurement of substrate oxidation and heat production was that put forward by Weir (41), and used subsequently in most indirect calorimetry studies (16). They rely on the wide difference in respiratory quotients found when oxidizing carbohydrate or fat and equate these mean theoretical respiratory quotients with the non–protein respiratory quotient, thus estimating the proportions of fat and carbohydrate oxidized (11,15,22). The equation derived from the present work uses the respiratory quotients corresponding to the mean protein and lipid of the standard diet.

The application of two widely used sets of indirect calorimetry equations and our derived equation to the data obtained from the experimental sets of animals gave differently fitting results. The data calculated with the equations we propose here gave better fits than the other, probably because they were derived from data coherent with the experimental measurements. It must be noted that the widest relative deviations from the expected results were observed in the measurements of very small amounts of lipid synthesized. Using the literature cited equations, the values for lipids were overestimated in all cases, as was carbohydrate in most cases. The differences can be attributed to the different approach to protein oxidation: in the Ferrannini (15) and McLean & Tobin (28) equations, the urinary N value is used instead of our protein balance data, the difference between the two entities being essentially due to the underestimation of protein oxidation resulting from the former approach. It can be argued that data on urinary N excretion are more readily available than actual protein balance measurements, although, both sets of data can be easily correlated if the size of the nitrogen gap is known, thus the approach we used can be easily adapted for studies with live and undisturbed animals.

The direct calorimetry data gave sustainedly lower values for heat output than the heat production results found with indirect calorimetry; since the latter fitted fairly well with those calculated from the energy balance data, it can be safely assumed that direct calorimetry values are lower than expected. The evaporative heat loss values obtained from coherent water emission data fit fairly well in the interval between direct and indirect calorimetry data. The proportion of heat lost in the rat through evaporation of water was, however, higher than usually assumed or expected (36). The relative constancy of the proportion of water versus heat produced for the three groups of rats agrees

with the assumption that most of that water came from the lungs and mucosas, in itself related to the concentrations of oxygen, carbon dioxide and water in the expired air, which are maintained fairly constant in the long run (35). These emissions are not related to obesity or leanness, nor to idiosyncratic aspects of the strain investigated, despite wide differences in these same rat stocks as to water balance management (*unpublished results*).

In addition to these methodological aspects, the data also provide some extra insight on the energy balance management of obese and lean rats. Wistar rats show a well known circadian cyclicity (18) on the consumption of oxygen, production of CO_2 and, especially on the respiratory quotient, whereas, lean Zucker rats show almost no oscillations at all, and obese Zucker rats present a single-trough pattern that contrasts with the double crest pattern of Wistar rats. In addition to these cycles, there is a much shorter period pattern observed in Wistar rats (18) that is substituted in all Zucker rats —especially in obese rats— by a very wide band of individual variation and oscillation with time. Despite the buffering of the data presented —resulting from the representation of means of 20 minutes—, the degree of variation of Zucker rats is wider than that of Wistars. The emission of carbon dioxide also shows less uniform data than those of oxygen consumption, a consequence of the pattern of release in periodic bursts of CO_2 (39).

The cyclicity is more apparent when the calculated oxidation/synthesis of carbohydrate and lipid are presented. As expected, obese rats synthesize —and store— huge amounts of fat (31), essentially at the expense of dietary carbohydrate and protein (21). However, at the end of the dark period there was a net consumption of lipid, with very low utilization of carbohydrate. This pattern —but to a much lower setting— was observed in Zucker lean rats. The highest consumption of carbohydrate in obese rats was observed in the light hours, suggesting diurnal habits for these animals. The actual oxidation of carbohydrate coincides with the light hours with a couple of hours of delay —probably the time needed for the food eaten to be available for oxidation. This contrasts with the data on the Wistar rats, a nocturnal animal (34), in which the lowest carbohydrate oxidation coincides with the end of the light period —and the highest lipid oxidation surge. The high lipid turnover and the enormous accumulation of fat in obese Zucker rats are in agreement with their known hyperphagia (4) and lack of functionality of their brown adipose tissue thermogenic system (17), since they derive a high amount of body heat from the conversion of carbohydrate into fat, thus helping to maintain their temperature and thermal homeostasis.

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TABLE 1

Mean daily parameters of 60-day Wistar and Zucker rats.

parameter	Wistar	Zucker obese	Zucker lean
body weight (g)	198.8 ± 4.2	279.3 ± 8.4	165.9 ± 3.9
weight increase ($g \cdot day^{-1}$)	3.11 ± 0.31	7.03 ± 0.18	2.89 ± 0.18
weight increase ($‰ \cdot day^{-1}$)	15.6 ± 1.6	25.2 ± 0.6	17.4 ± 1.1
net protein accrued ($g \cdot day^{-1}$)	0.73 ± 0.05	0.90 ± 0.04	0.65 ± 0.03
net lipid accrued ($g \cdot day^{-1}$)	0.51 ± 0.03	2.80 ± 0.06	0.52 ± 0.02
net protein intake ($g \cdot day^{-1}$)	2.27 ± 0.06	4.03 ± 0.07	2.43 ± 0.06
net lipid intake ($g \cdot day^{-1}$)	0.45 ± 0.01	0.80 ± 0.01	0.49 ± 0.01
carbohydrate intake ($g \cdot day^{-1}$)	9.30 ± 0.23	16.53 ± 0.21	9.98 ± 0.26
total energy intake (W)	2.58 ± 0.08	4.58 ± 0.25	2.77 ± 0.08
energy production (W)	2.19 ± 0.06	3.17 ± 0.02	2.43 ± 0.04
urinary N ($mg\ N \cdot day^{-1}$)	199 ± 10	455 ± 21	239 ± 4
nitrogen *gap* (%)[1]	19.1	9.5	16.2
water vapour released ($ml \cdot day^{-1}$)	17.4 ± 2.1	19.6 ± 3.1	19.0 ± 2.6
O_2 consumption ($mmol \cdot day^{-1}$)	392 ± 20	540 ± 46	428 ± 42
CO_2 release ($mmol \cdot day^{-1}$)	387 ± 12	579 ± 10	422 ± 10
mean respiratory quotient	0.987	1.071	0.984

All data are the mean ± SEM of 4-6 different animals. The results refer to day 60 and have been determined from body weight, composition, balance and consumption data taken at different intervals as indicated under Materials and Methods.

[1] Proportion of total oxidized protein not found as urine nitrogen, expressed as a percentage.

TABLE 2

Lipid composition, and oxygen consumption and carbon dioxide production equivalents for 60-day Wistar and Zucker rats

lipid	Empirical formula $C_RH_SO_6$ R	S	mean MW	O_2 mmol/g	CO_2 mmol/g	RQ
diet ingested	57.6	103.6	890.8	90.38	64.66	0.7154
Wistar rats						
total rat lipid	55.8	102.8	868.4	90.41	64.25	0.7107
accrued	57.0	106.7	886.7	90.98	64.28	0.7065
oxidized	54.7	103.9	883.8	95.76	61.85	0.6459
Zucker obese rats						
total rat lipid	54.6	101.7	852.9	90.31	64.02	0.7089
accrued	53.7	100.2	840.6	90.12	63.88	0.7088
oxidized	52.8	99.3	829.3	90.03	63.71	0.7077
Zucker lean rats						
total rat lipid	55.8	103.7	869.3	90.57	64.19	0.7087
accrued	55.8	104.5	870.1	90.70	64.13	0.7071
oxidized	47.1	109.9	771.5	92.81	61.09	0.6582

The empirical formula data are mean values calculated from the composition of the fats from 6 different animals for each breed (32). Mean molecular weight (MW) or triacylglycerols, the equivalence of mmoles of oxygen needed for the oxidation of 1 gram of the fat, the carbon dioxide produced and the respiratory quotient (RQ) have been calculated from the mean empirical formula.

TABLE 3

Protein composition, and oxygen consumption and carbon dioxide production equivalents for 60-day Wistar and Zucker rats

	mean amino acyl residue *empirical formula* $C_RH_SO_TN_US_V$					mean	O_2	CO_2	
protein	R	S	T	U	V	MW	mmol/g	mmol/g	RQ
diet ingested	4.78	7.45	1.58	1.39	0.067	111.7	34.6	30.3	0.8763
							53.3	*42.8*	*0.8030*
Wistar rats									
total rat protein	4.40	6.95	1.15	1.33	0.064	98.82	47.2	37.8	0.8014
							57.3	*44.5*	*0.7776*
accrued	4.44	7.02	1.46	1.32	0.064	104.16	43.9	36.4	0.8282
							53.4	*42.7*	*0.7993*
oxidized	5.11	7.85	1.83	1.47	0.074	121.4	42.5	36.0	0.8467
							51.6	*42.1*	*0.8152*
Zucker obese rats									
total rat protein	4.44	7.03	1.44	1.34	0.075	104.5	34.3	29.7	0.8659
							53.9	*42.7*	*0.7920*
accrued	4.47	7.04	1.45	1.31	0.073	104.6	34.9	31.9	0.8765
							53.7	*42.8*	*0.7962*
oxidized	4.98	7.68	1.66	1.46	0.034	115.5	34.0	30.5	0.8959
							53.0	*43.1*	*0.8056*
Zucker lean rats									
total rat protein	4.53	7.19	1.45	1.35	0.078	106.2	34.8	30.0	0.8607
							53.9	*42.7*	*0.7920*
accrued	5.03	8.10	1.38	1.45	0.151	115.7	38.2	30.9	0.8109
							57.0	*43.5*	*0.7631*
oxidized	4.79	7.37	1.64	1.40	0.026	111.5	33.7	30.4	0.9030
							52.5	*43.0*	*0.8186*

The empirical formula data are mean values calculated from the composition of the fat samples from 6 different animals for each breed (32). Mean molecular weight (MW) or triacylglycerols, the equivalence of mmoles of oxygen needed for the oxidation of 1 g of the fat, carbon dioxide produced and the respiratory quotient (RQ) have been calculated from the mean empirical formula.

The values for oxygen and carbon dioxide and RQ shown in *italics* correspond to the values calculated assuming a complete conversion of all N in the amino acyl residues to N_2 gas; the data shown in non–italicized type correspond to the assumption that all N in the amino acyl residues is converted to urea.

TABLE 4

Equations used for the estimation of heat production, carbohydrate and lipid oxidized from oxygen consumption, carbon dioxide production and nitrogen balance data.

FERRANNINI (1988)

$L_{(g/min)} = 1.67 \times (VO_2 - VCO_2) - 1.92 \times N$

$\dot{G}_{(g/min)} = 4.55 \times VCO_2 - 3.21 \times VO_2 - 2.87 \times N$

$EPR_{(kcal/min)} = 3.91 \times VO_2 + 1.10 \times VCO_2 - 3.34 \times N$

oxygen flow —VO_2— in l/min

carbon dioxide flow —VCO_2— in l/min

oxidised protein —N— in mg of urine N/min

EPR = energy production ratio

parameters calculated for a temperature of 273 K

MCLEAN & TOBIN (1987), using values given by BROUWER (1965)

$L_{(g)} = 1.719 \times (VO_2 - VCO_2) - 1.963 \times N$

$G_{(g)} = 4.174 \times VCO_2 - 2.968 \times VO_2 - 2.446 \times N$

$H_{(kJ)} = 16.18 \times VO_2 + 5.02 \times VCO_2 - 5.99 \times N$

oxygen consumed —VO_2— in l

carbon dioxide produced —VCO_2— in l

oxidised protein —N— in mg of urine N

parameters calculated for a temperature of 273 K

all measures are referred to a given period of time

Formulas proposed:

$L_{(g)} = 38.8803 \times (MO_2 - MCO_2) - 0.1664 \times P$

$G_{(g)} = 94.87 \times MCO_2 - 67.873 \times MO_2 - 0.528 \times P$

$E_{(kJ)} = 352.1 \times MO_2 + 121.99 \times MCO_2 + 2.51 \times P$

oxygen consumed —MO_2— in moles

carbon dioxide produced —MCO_2— in moles

oxidised protein —P— in g

all measures are referred to a given period of time

TABLE 5

Comparison of the application of equations for the measurement of carbohydrate and lipid consumption from gas interchange and protein catabolism data applied to Wistar and Zucker rats on day 60

parameter	balance data	FERRANNINI (1988)	MCLEAN & TOBIN (1987)	formulas proposed
Wistar				
lipid (g)	−0.06 ± 0.02	−0.19 ± 0.01	−0.20 ± 0.01	−0.06 ± 0.02
carbohydrate (g)	9.30 ± 0.23	10.68 ± 0.50	9.97 ± 0.50	9.29 ± 0.65
heat (kJ)	189.3 ± 5.1	180.7 ± 9.3	184.5 ± 9.6	189.2 ± 9.8
Zucker obese				
lipid (g)	−2.00 ± 0.06	−2.32 ± 0.19	−2.38 ± 0.19	−2.03 ± 0.16
carbohydrate (g)	16.53 ± 0.21	18.83 ± 1.44	14.51 ± 1.45	16.59 ± 1.58
heat (kJ)	273.6 ± 1.3	250.9 ± 21.5	258.0 ± 22.1	268.7 ± 23.0
Zucker lean				
lipid (g)	−0.03 ± 0.03	−0.21 ± 0.01	−0.22 ± 0.01	−0.04 ± 0.01
carbohydrate (g)	9.98 ± 0.26	11.49 ± 1.00	10.80 ± 0.99	10.00 ± 1.14
heat (kJ)	210.0 ± 3.4	196.8 ± 19.4	201.1 ± 19.8	206.7 ± 20.4

The data are the mean ± SEM of 4-6 different animals, and are referred to a period of 24 hours. The means of the data used for the calculations are presented in Table 1.

TABLE 6

Mean daily heat production values for 60-day Wistar and Zucker rats

parameter	Wistar	Zucker obese	Zucker lean
—calculated energy data obtained from substrate balances:			
Net energy ingested	2.58	4.58	2.77
Heat theoretically produced	2.19	3.17	2.43
—calculated indirect calorimetry data:			
Heat production	2.19	3.11	2.34
—measured direct calorimetry data:			
Heat released	1.75	2.44	1.87
—calculated water-energy relationships:			
Heat loss from water evaporation	0.49	0.56	0.54

All data are expressed in W, and represent the mean (24-hour) energy production/output of a 60-day rat of the indicated breed.

LEGENDS OF FIGURES

Figure 1

Oxygen inflow of Wistar, lean and obese Zucker rats over one day

The values shown in the dark graph are the 20-minute means (4 mean measurements of 5 minutes) of 5 (Wistar), 4 (Zucker obese) or 6 (Zucker lean) rats oxygen consumption. The dot lines represent the maximal and minimal values for the corresponding periods.

The time scale is set at GMT +1 hours. The bar at the top shows the periods of light and darkness.

Figure 2

Carbon dioxide outflow of Wistar, lean and obese Zucker rats over one day

The values shown in the dark graph are the 20-minute means (4 mean measurements of 5 minutes) of 5 (Wistar), 4 (Zucker obese) or 6 (Zucker lean) rats carbon dioxide release. The dot lines represent the maximal and minimal values for the corresponding periods.

The time scale is set at GMT +1 hours. The bar at the top shows the periods of light and darkness.

Figure 3

Respiratory quotient of Wistar, lean and obese Zucker rats over one day

The values shown in the graph are the means of the mean data presented in Figures 1 and 2. They are mean values for 20-minute intervals.

The time scale is set at GMT +1 hours. The bar at the top shows the periods of light and darkness.

Figure 4

Carbohydrate and lipid oxidized by Wistar, lean and obese Zucker rats over one day

The carbohydrate values are shown as a dot line and refer to the left ordinate axis. The lipid values are presented as a solid line and refer to the right ordinate axis. All data are the mean calculated values for 20-minute intervals, taken from the data of Figures 1 and 2.

The time scale is set at GMT +1 hours. The bar at the top shows the periods of light and darkness.

Figure 5

Energy consumed by Wistar, lean and obese Zucker rats over one day

The solid line corresponds to the substrate oxidation power determined through indirect calorimetry, the data are the means for 20-minute intervals calculated from the data in Figures 1 and 2. The dot line corresponds to the measured heat output of another group of rats, determined through direct calorimetry; the data are the means for 20-minute intervals of the measurements done every 2 minutes on 5 (Wistar and lean Zucker) or 4 (Zucker obese) rats. The 24-hour means for all these graphs are presented in Table 6.

The time scale is set at GMT +1 hours. The bar at the top shows the periods of light and darkness.

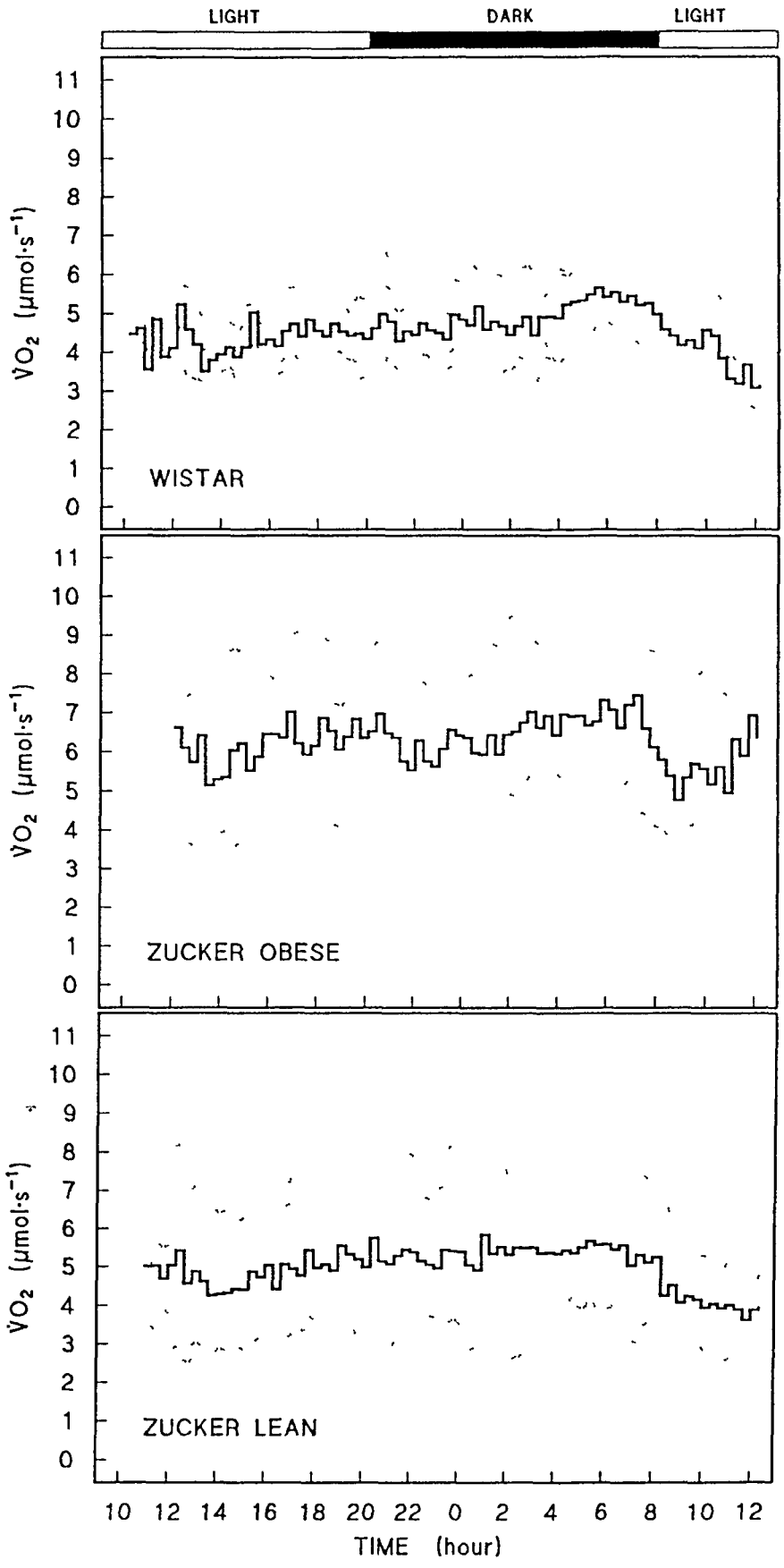

LIGHT
DARK
LIGHT
VO_2 ($\mu mol \cdot s^{-1}$)
WISTAR
ZUCKER OBESE
ZUCKER LEAN
10 12 14 16 18 20 22 0 2 4 6 8 10 12
TIME (hour)

Figura 1

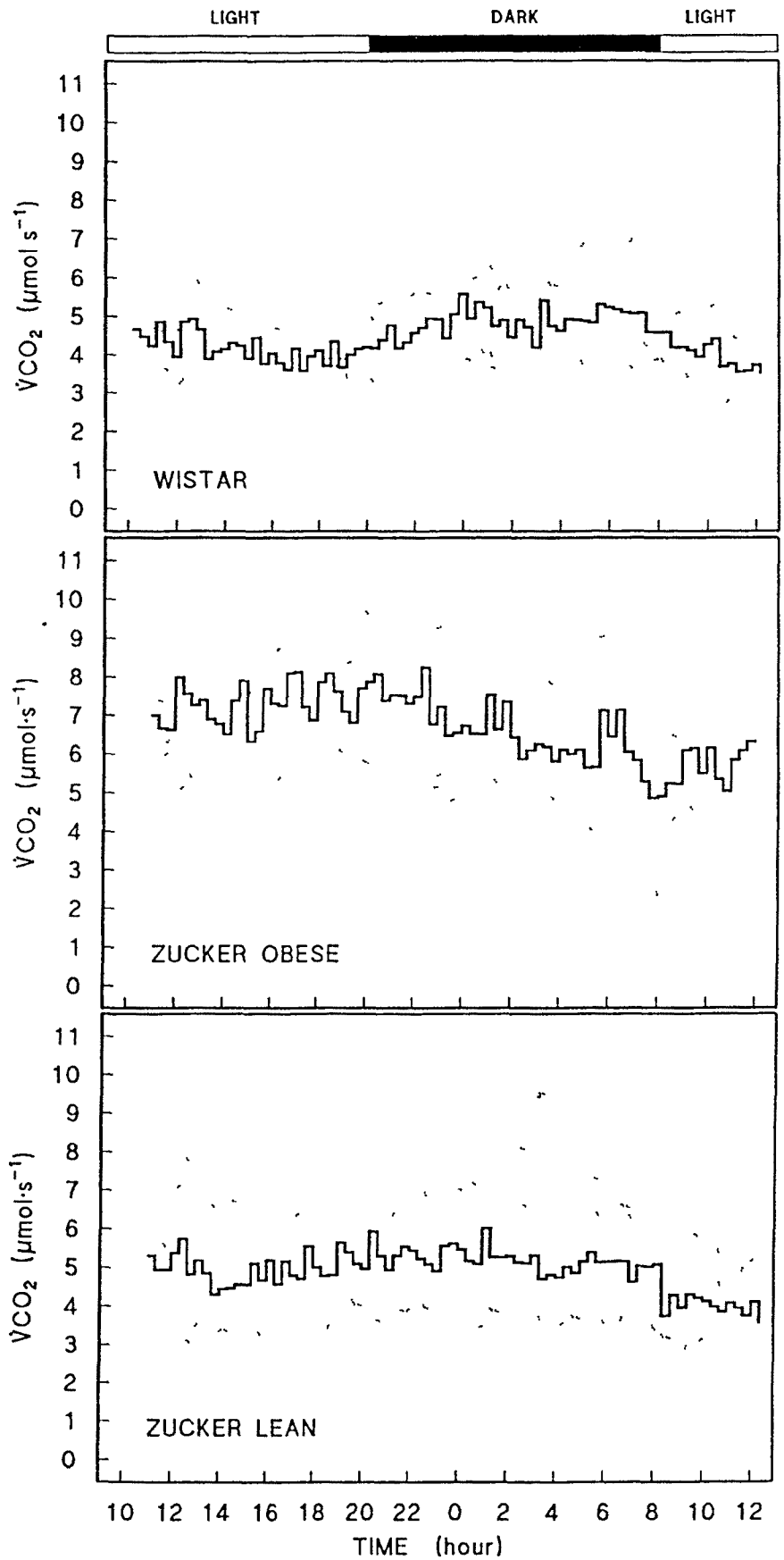
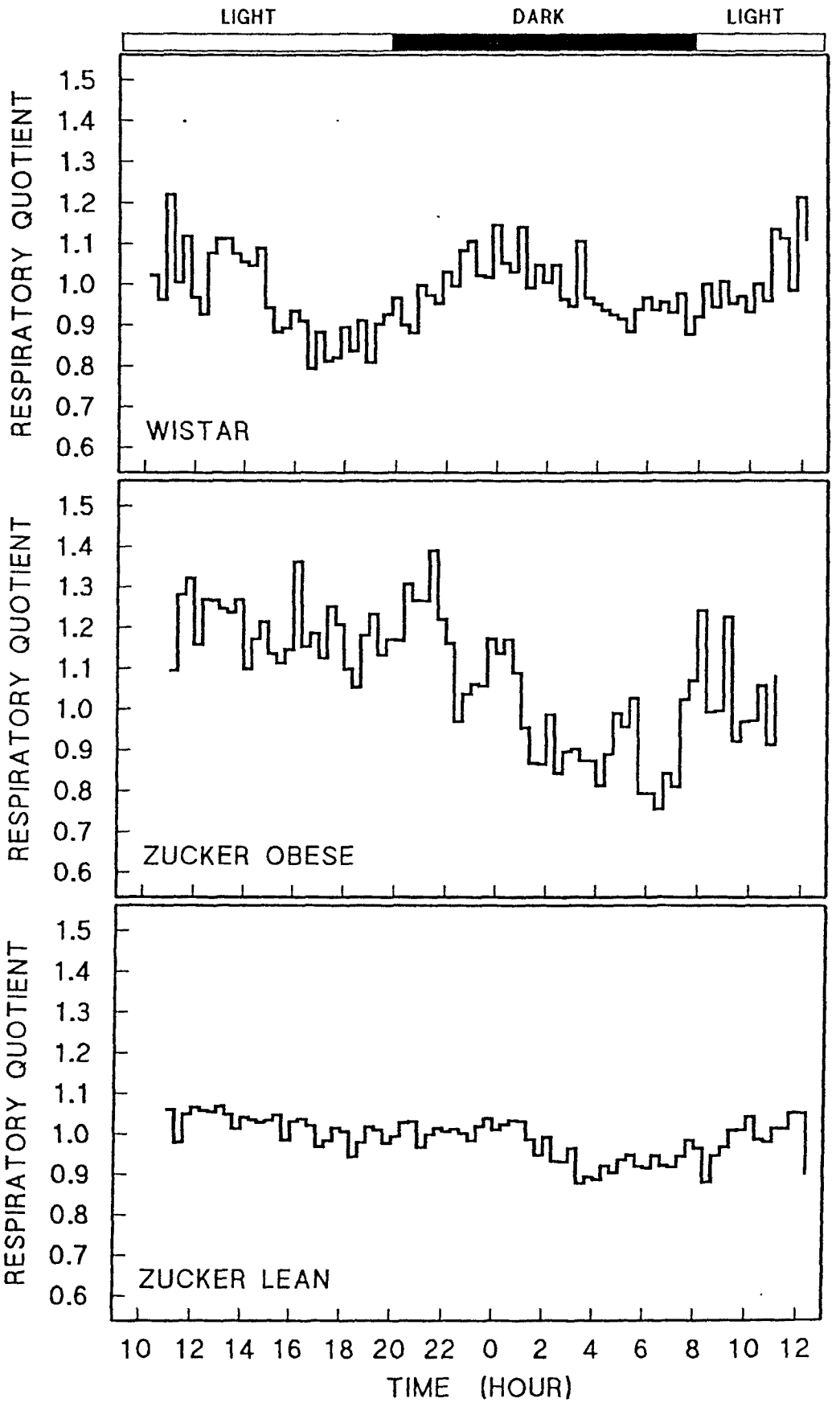

LIGHT
DARK
LIGHT
$\dot{V}CO_2$ (μmol s^{-1})
WISTAR
ZUCKER OBESE
ZUCKER LEAN
10 12 14 16 18 20 22 0 2 4 6 8 10 12
TIME (hour)

Figura 2


LIGHT
DARK
LIGHT
RESPIRATORY QUOTIENT
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1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
WISTAR
ZUCKER OBESE
ZUCKER LEAN
10 12 14 16 18 20 22 0 2 4 6 8 10 12
TIME (HOUR)

Figura 3

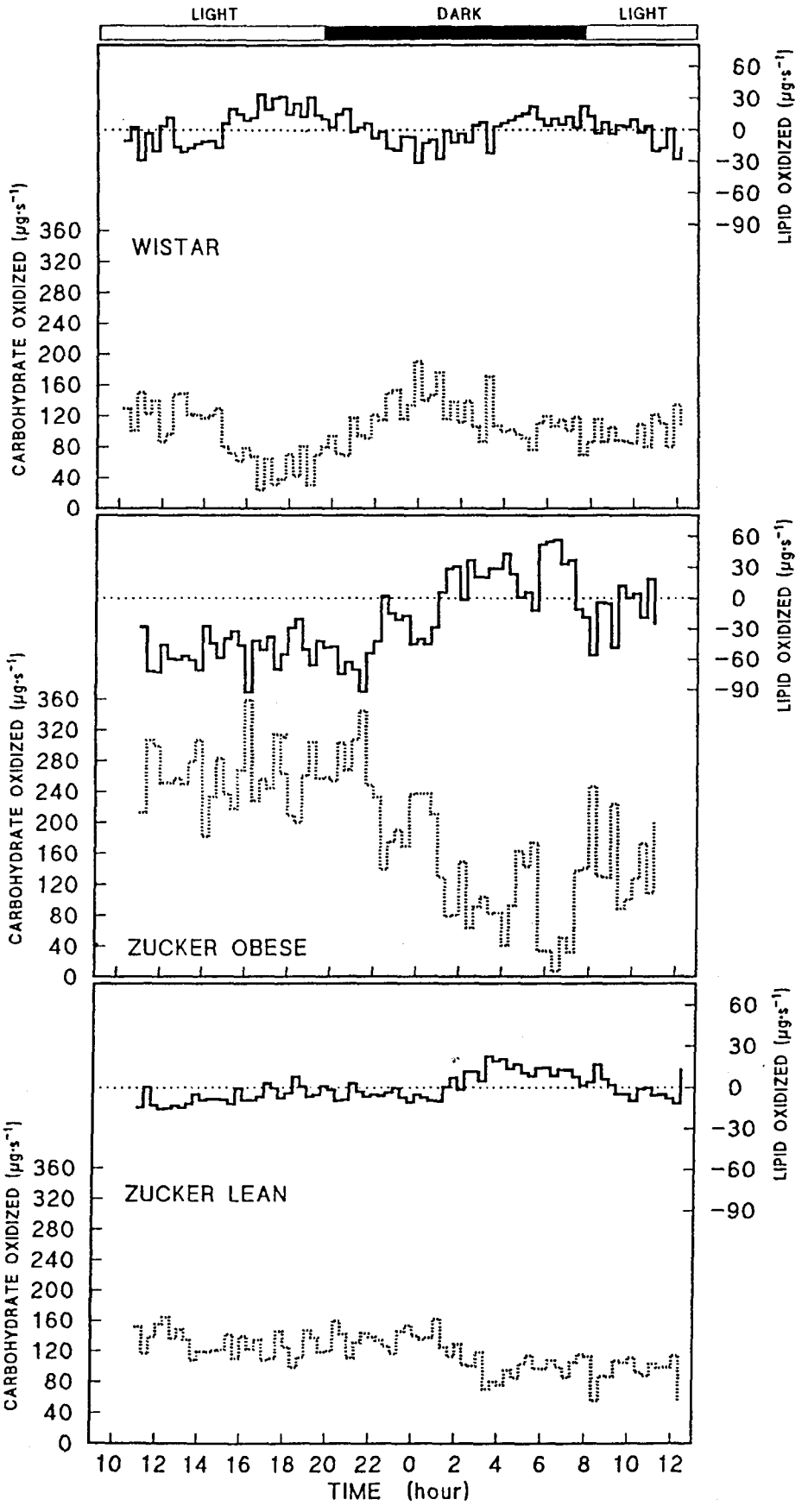
LIGHT
DARK
LIGHT
WISTAR
ZUCKER OBESE
ZUCKER LEAN
CARBOHYDRATE OXIDIZED (µg·s⁻¹)
LIPID OXIDIZED (µg·s⁻¹)
0
40
80
120
160
200
240
280
320
360
60
30
0
-30
-60
-90
10 12 14 16 18 20 22 0 2 4 6 8 10 12
TIME (hour)

Figura 4

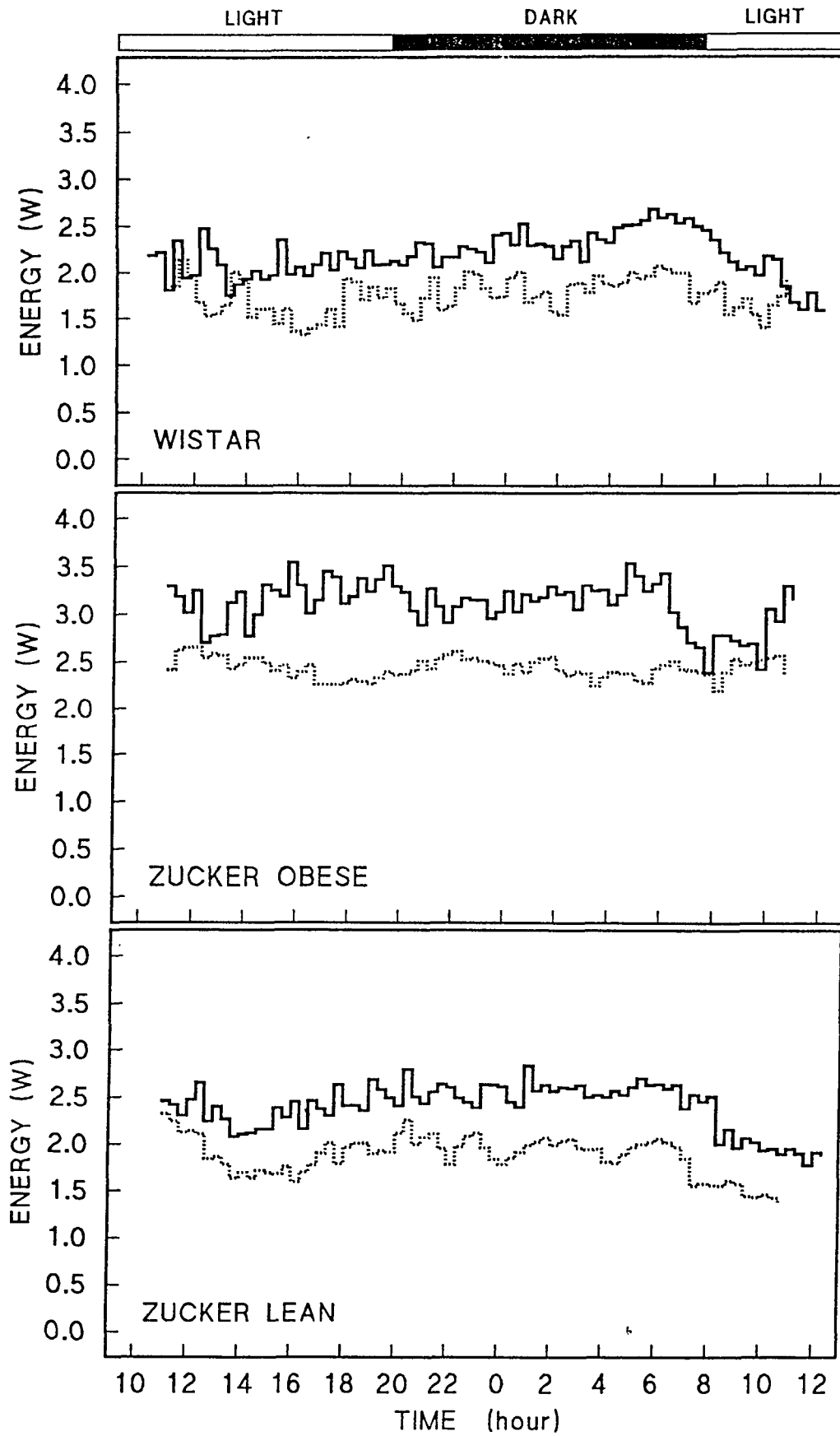
LIGHT
DARK
LIGHT
ENERGY (W)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
WISTAR
ZUCKER OBESE
ZUCKER LEAN
10 12 14 16 18 20 22 0 2 4 6 8 10 12
TIME (hour)

Figura 5

COMENTARI GENERAL: BLOC II

L'existència de canvis en la temperatura nuclear ens va fer plantejar si aquests es podien reflectir en canvis de les pèrdues globals de calor de l'animal. Això ens va portar realitzar l'anàlisi dels ritmes d'expressió d'ambdós paràmetres. De les dades presentades s'en dedueix que els ritmes de producció de calor estan correlacionats, però no són totalment coincidents amb els canvis en la temperatura central. Les diferències es deuen a la gran influència dels ritmes de quatre i cinc hores en la temperatura central, que no es manifesten en la despesa global de calor. Això podria deures a l'existència de ritmes d'acumulació de calor, posats de manifest per l'elevació de la temperatura corporal, que estan parcialment desfasats amb respecte a les pèrdues de calor, però que podrien estar més relacionades amb la circulació cap a la pell i mucoses que no pas amb els canvis de temperatura.

La utilització del sistema d'anàlisi de gasos ens va permetre mesurar l'aigua evapo-transpirada per l'animal, i completar d'aquesta manera les mesures de calorimetria directa, comprovant que els valors persistentment més baixos de la calorimetria directa en comparació a la indirecte, corresponen —tal com era d'esperar— a que en la calorimetria indirecte es mesura la producció de calor, mentre que en la directa es mesuren les pèrdues de calor sensible, entre les que hom no inclou les pèrdues per evapo-transpiració, que han de ser mesurades independentment. Això també ens va permetre disposar d'un conjunt homogeni totes les dades per poder realitzar un estudi complet del balanç d'aigua. En aquest estudi hem pogut posar de manifest l'existència de diferències en la utilització de l'aigua entre les rates Wistar i les Zucker. Les principals diferències estan en la gran quantitat d'aigua que perden les rates de la soca Wistar per l'orina, i que impliquen una concentració d'urea en orina que a les rates Zucker és el doble de les Wistar. Segons això, l'urea podria ser el principal determinant del volum d'orina en les rates Zucker. Però al calcular l'osmolalitat aproximada de l'orina tenint en compte a més de l'urea, els minerals i altres components excretats en ella, trobem que les diferències són del mateix ordre de les trobades per l'urea, el que fa concloure que les diferències no són degudes a alteracions pròpiament renals sinó que molt probablement es deuen a un ajustament diferent del control neuro-endocrí de l'excreció d'aigua i sals. Un altre punt a destacar és la pèrdua més gran d'aigua amb les femtes de les rates obeses, doncs malgrat que la proporció era similar per tots els grups, les rates obeses eliminaven un volum més gran de femtes, de manera que la quantitat de matèria fecal sòlida respecte de la quantitat ingerida era més gran per les rates obeses que pels dos grups de rates primes, fet que podria indicar una menor capacitat d'extracció de nutrients dels aliments, és a dir, una menor eficiència del procés de digestió relacionada amb aquest tipus d'obesitat.

Així mateix les mesures amb el sistema d'anàlisi de gasos van ser corregides per les pèrdues de nitrogen que no es detecten per l'orina, segons dades recollides en el treball paral·lel abans esmentat. Les equacions utilitzades van ser contrastades amb les dades del balanç realitzat amb animals comparables, arribant a la conclusió que les que més s'acosten a aquests valors són les derivades a partir de la composició dels lípids i proteïnes de la dieta, donant millors aproximacions que altres equacions amplament utilitzades. També es va poder observar que el fet que les rates

obeses sintetitzin quantitats importants de lípids a partir de la utilització de glúcids no té un efecte apreciable. Aquests animals obesos només oxiden lípids en les hores de foscor, indicant uns hàbits més diürns que no els típics de les rates Wistar que són animals típicament nocturns. També en aquest cas això es pot comprovar en el fet que en les hores de llum oxiden menys glúcids i més lípids que no pas en les de foscor.

BLOC III

Dinàmica dels greixos en l'animal obès intacte

treball enviat per publicar a MOLECULAR AND CELLULAR BIOCHEMISTRY

Fatty acid utilization by young Wistar rats fed a cafeteria diet.

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ABSTRACT

The content and accretion of fatty acids in 30, 45 and 60–day old Wistar rats fed either reference chow or a cafeteria diet has been studied, together with their actual fatty acid intake during that period. Diet had a small overall effect on the pattern of deposition of fatty acids, but the deposition of fat was much higher in cafeteria rats. The fat-rich cafeteria diet allowed the direct incorporation of most fatty acids into lipid storage, whilst chow-feeding activated lipogenesis and the deposition of a shorter chain and more saturated type of fatty acids. During the second month of the rat's life, the elongation pathway as well as Δ^9–desaturase became functional, thus helping to shape the pattern of fatty acids actually accrued. The 60-day rats showed a relative impairment in the operation of Δ^5–desaturase, since their lipids had a higher C20:4/C20:3 ratio than those of the diet ingested. Cafeteria-diet feeding minimized this effect since the large supply of dietary polyunsaturated fatty acids made the operation of the elongation-desatuarse pathways practically unnecessary.

INTRODUCTION

It is widely accepted that most of the fat stored by rodents during post-weaning growth as well as by the adult is the product of active lipogenesis (1) taking place mainly in the liver and adipose tissue (2). The standard diet given to experimental rats contains usually small amounts of fats (3), usually rich in polyunsaturated fatty acids, and thence the accretion of fat is mainly driven by the relative abundance of carbohydrate. This is especially magnified in cases of genetic obesity (4).

The "cafeteria" diets (5) were introduced in the 70's as a way to generate hyperphagic conditions in the rat similar to those experienced by humans consuming highly palatable choice diets. The diets actually selected by the rats have a common high lipidic content (6), with relatively unchanged —and proportionally lower— protein and carbohydrate (7). Constant exposure to these diets from weaning results in

high growth rates (8) and important fat accretion (9), with enormous increases in energy intake (7,10) and heat production (11) through increased thermogenesis.

This research has been designed as a balance study, in which the actual intake of most individual fatty acids is measured and compared with the composition of the fat newly deposited in young rats fed either the cafeteria or a standard chow reference diet. The differences between the two sets of figures can provide information on the processes of handling fatty acids both from dietary and lipogenic origin.

MATERIALS AND METHODS

Animals and diets:

Female Wistar rats (bred at the University of Barcelona Animal Service from Charles River France stock) were used 30 days after birth (weaned on day 22). The rats were housed individually, either in polypropylene-bottomed cages with wood shavings as absorbent material, or in polycarbonate metabolic cages (Tecniplast Gazzada, Italy) which allowed for the daily estimation of the consumption of individual food items. The rats were maintained in a light (on from 08.00 to 20.00), humidity (70-80 % relative humidity) and temperature (21-22 °C) controlled environment.

Control rats were fed a reference pellet diet (type A04 from Panlab, Barcelona) and tap water *ad libitum*. All studies involving the measurement of food intake were carried out with the rats kept in metabolic cages. The rats given the cafeteria diet were presented daily with a fresh offering of biscuits, liver pâté, bacon, banana, chow pellets (as indicated above), tap water and whole milk complemented with 333 g/l sucrose plus 10 g/l of a mineral and vitamin supplement (Gevral, Cyanamid Ibérica) (7). All the materials were previously weighed and presented in excess (c. 20-30 % higher than the expected consumption). Twenty-four hours later, the remaining debris was isolated, identified and weighed. The drying of food leftovers was corrected by determining the amount of water lost in one day from known weight food samples left in an otherwise empty cage. This diet is a simplified version of an earlier cafeteria diet developed and studied by us (7,8), scaled down by selecting only the items actually consumed in significant portions. The animals were weighed every day at the same hour (11.00 to 12.00).

Experimental setup:

For each dietary group (reference– and cafeteria–fed rats) three sets (5-7 rats each) of animals were studied, being killed at 30 (0 days of dietary treatment), 45 (15 days of treatment) or 60 (30 days of treatment) days after birth. The 60–day rats were used for daily individual diet intake analysis (metabolic cages) throughout the 30 days of the study. The other rats (30–, and 45–days old) were kept individually in ordinary cages, where they were fed the same diets; their intake was not measured.

On days 30, 45 or 60, the allotted groups of rats were weighed and immediately killed by decapitation. Their corpses were again weighed (the difference being the net loss of blood and fluids) and then dissected. The content of their intestine was carefully removed and weighed. The weights recorded and used for calculations were the empty body weights. The remaining carcass was then minced and ground with a blender, sampled and stored at –23 °C until processing.

Analytical procedures:

The ground carcasses were sampled following a previously tested sampling protocol (12). The samples were homogenized with a Politron homogenizer; the resulting smooth paste was then used for analysis. The constituents of the diets given to the animals were also ground and homogenized, and then subjected to the same analyses. Blood samples were also analyzed and used to correct the data obtained

for the different groups, accounting for the blood lost during the killing and dissecting process.

Total lipids were extracted from the samples (in duplicate) homogenizing the samples with a chloroform: methanol mixture (13). The lipids were saponified under reflux for 90 minutes with 12 g/l NaOH in ethanol and the methyl ester derivatives of all fatty acids were obtained (14) and analyzed in a Perkin-Elmer Sigma 3B gas chromatograph using a 30m x 0.75 mm i.d. Supelcowax 10 (Supelco Inc, USA) minicolumn, and a flame ionization analyzer. Helium was used as mobile phase. Standards for the extraction and derivatization procedures were run in parallel. Internal standards of C13 were introduced in all samples prior to methylation. Standards of the fatty acids listed in table 1 (from Sigma, USA) were also run with each batch of samples, which were chromatographed in duplicate.

Calculations and statistics:

The analyses of body composition of the series of rats killed on days 30 and 45 were used for the calculation of the composition of the rats kept in metabolic cages (killed at 60 days after one month of daily measurement of intake and body weight) on days 30 and 45. Since their weights were very close ($P > 0.95$, Student's *t* test) and the feeding scheme was the same, it was assumed that the percentage composition of the three series of rats was identical for matching age and diet.

The composition of the lipid samples, the proportion of lipid and the mass of the rat paste allowed the estimation of the amount of each fatty acid present in each of the three age-groups studied, and allowed the quantitative estimation of the differences between the fatty acids stored, always related to the actual empty body weight of the animal referred to the age of comparison.

The known amount of each food item consumed daily by each rat and its fatty acid composition (Table 1) were used to establish the amount of each fatty acid ingested by each rat for each day. The tabulated data for each diet component were combined in order to determine the gross intake of each fatty acid during each of the 15-day periods studied. Statistical comparisons between the groups were made with a standard two–way ANOVA program, as well as with Student's *t* test.

RESULTS

Table 1 presents the fatty acid composition of the lipids from the foods given to the experimental animals. The proportion of lipid content and the weights of the rats used are seen in table 2. The percentage of fat changed little from 30 to 45 days, increasing more up to 60 days in reference diet-fed rats. In cafeteria-fed rats, the increase was much more marked for both stretches of time, with lipids constituting a larger proportion of body weight than in the rats fed the reference diet.

The fatty acid compositions of the carcasses of 30–, 45– and 60–day rats are presented in Figure 1 for the two dietary groups. With increasing age, the amount of C22 and C24 saturated fatty acids increased in some cases (C24) by an order of magnitude. There was a comparable increase in the presence of odd carbon fatty acids as well as in mono–unsaturated fatty acids. The increases in linoleic acid (C18:2) were more directly related to the diet than to age, since in reference–diet rats there was an actual decrease in the amount of this fatty acid present in the carcass, whereas in cafeteria-fed rats the increase observed followed the pace of other unsaturated fatty acids. With age —and body size— the increase in poly–unsaturated fatty acids was also significant, and perhaps more related to diet than to age, despite the general tendency to increase observed for most of these fatty acids. The cafeteria diet provoked a significant overall increase in practically all fatty acids compared with the

reference diet-fed rats. The chow-fed 45-day rats, however had higher amounts of some fatty acids (C17, C14:1, C20:2 and C20:3) than their cafeteria-fed counterparts, despite their smaller size.

The data on fatty acid composition of reference diet– and cafeteria–fed rats during the 30-day period studied are presented in Figure 2. The fatty acid composition of all groups was remarkably similar, with a net predominance of C16, C18:1 and, to a lesser extent, C18:2, C16:1 and C24, all other fatty acids being present in proportions well below 5 %. When comparing the 30–day rats with older reference diet– and cafeteria–fed animals, the most prominent changes were the decreases in the proportions of C18 and C18:2, and the increase in C18:1. Cafeteria–fed rats showed a more marked tendency to increase the accumulation of more unsaturated C18's, with lower losses in linoleic (C18:2) acid; in addition, in cafeteria–fed rats the relative weight of C17 and C24 were lower than in the reference–diet animals.

Figure 3 presents the amounts of fatty acid actually ingested in the whole 30-day period compared with the amounts accrued during the same period, both for reference diet– and cafeteria–fed rats. In general, reference diet–fed controls accumulated more fatty acids than those present in the food eaten, with the significant exceptions of C18:2 and C18:3, especially abundant (42 % of all fatty acids) in the brand of chow pellets used. On the other hand, cafeteria–fed rats showed consistently lower deposition figures than those of ingestion, with the only exceptions of C14:1 (practically identical, as was C24) and C16:1, distinctly accrued in larger amounts than ingested. Figure 4 shows the net differences in fatty acid ingested and accrued. In reference diet–fed rats, the net synthesis of fatty acids was maximal for C16, C16:1 and C18:1, with highest degradation proportions in C18:2 and C18:3. Cafeteria-fed rats degraded mainly C16, C18, C18:1 and C18:2, but most fatty acids were also degraded in larger proportions than ingested. The only fatty acids showing changes not significant were C24 and C14:1. A net synthesis of C16:1 was also observed.

The mean values for chain length and unsaturation for the fatty acids present in the different groups of rats studied, as well as in their intake and differentially accrued lipids are shown in Table 3. The mean chain length changed little in cafeteria–fed rats, with a little more alterations in reference diet–fed rats, the maximal values being observed on day 45. The degree of unsaturation again changed less from 30 to 60 days in the rats fed a cafeteria diet than in reference diet–fed controls, which showed a trend towards more saturated fatty acids. During the 30-45 day period the fatty acids present in the diet consumed by control rats had longer chains and were much more unsaturated than the fatty acids deposited. Cafeteria–fed rats showed a practically identical mean profile for the fatty acids eaten and accrued. In the 45–60 day period, the situation was similar for cafeteria–fed rats, but now the reference diet–fed had a mean accrued fatty acid chain length very close to that of the intake, whilst the number of double bonds was yet smaller than those of intake, and distinctly lower than those of cafeteria-fed rats.

The ratios C20:4/C20:3 and C20:3/C18:2 for ingested lipids and those of 30– and 60–day rats are presented in table 4. The C20:4/C20:3 ratio decreased in both dietary groups from day 30 to day 60. This effect of age was more marked in the group fed the cafeteria diet. The C20:4/C20:3 ratios were higher for 30-day (both dietary groups) and 60-day reference-fed rat lipids than those of the diet ingested. The rat lipid C20:3/C18:2 ratios were higher than those of the diet ingested in all cases. From day 30 to 60, the rat lipid C20:3/C18:2 ratio increased four-fold in reference-fed rats, increasing only by 20 % in cafeteria diet-fed rats.

DISCUSSION

Rats offered a cafeteria diet with a composition akin to that used here show a remarkably uniform daily intake of fat, carbohydrate and protein (7). The different batches of reference diet used maintained a uniform fatty acid composition (15). The ingestion of diets rich in energy and fat, such as the cafeteria diets (6), increased the accumulation of fat in experimental animals (7,9). The accumulation is effected mainly in accordance with the specific fatty acid profiles that characterize the species, rather than following the patterns of the fat ingested (16). This is possible because of the ability to select, degrade, elongate, and, up to a certain point, desaturate the newly arrived fatty acids (17). The main point is, however, the extent to which lipogenesis must compensate for the shortcomings of the diet in fatty acid availability with respect to fat storage. In humans, it has been shown that even a small amount of dietary fat actually inhibits lipogenesis (18), thus facilitating the direct and selective incorporation of dietary fatty acids into reserve fats. It is thus very probable that the cafeteria diet would actually prevent a significant *de novo* synthesis of fatty acids. The practical identity between the mean chain length and double bonds of the fatty acids ingested and those deposited supports this idea.

The data obtained show high uniformity in the fatty acid composition of the rats despite changing diet, in agreement with previous reports (19) that showed a relative uniformity in the fatty acid composition of tissues. This is achieved by different means in reference diet– and in cafeteria–fed rats. In the former, there is active and significant synthesis of fatty acids. The major subject of synthesis appears to be the most abundant saturated fatty acid, palmitic acid (C16). The elongation pathway for saturated fatty acids seems to become functional shortly after birth (20), in order to provide the fatty acids needed for membrane structures, especially in the brain (21). The elongation process is further matured during the second month of life of the rat (days 30-60), as shown by the increases in C22 and C24 from 30 to 45 and then to 60 days. A similar appreciation can be made on the ability to desaturate at C-9 to produce C16:1 and C18:1. However, in that case —Δ^9–desaturase— the system is already functionality at 30 days, since all 45-day rats show greatly increased levels of these monounsaturated fatty acids. In cafeteria-fed rats, these fatty–acid modifying pathways also seem fully functional, as observed in the net synthesis of palmitoleic (C16:1) acid.

In spite of the ability of the rat metabolism to adapt the ingested fatty acid pattern to match the accretion needs, the diet has an appreciable effect —albeit small— upon the composition of the fat accrued (16,22,23). Reference diet-fed rats, which consume a diet with scant amounts of most fatty acids, actually oxidize most of the 18C polyunsaturated fatty acids eaten, and must, therefore, synthesize all of the other fatty acids needed. The resulting fat is much less unsaturated than that already present in the 30-day old rat (with a sizeable part of it —the fat accumulated up to day 21— coming from the dam's milk), and than that in the chow pellets, as well as than that actually accrued by cafeteria-fed rats. In addition, the youngest group has some difficulty in producing longer fatty acids, as shown by the short mean chain value for that period.

Cafeteria-fed rats, in contrast, need to get rid of excess fat. They incorporate fatty acids with a mean chain length and unsaturation very similar to those of 30-day rats and to those of the diet they ate, thus their degradation of diet fatty acids consists simply in trimming down the pattern ingested to that needed. In general, the degradative effort is focused more on saturated and polyunsaturated fatty acids, and cafeteria rats select a higher share of dietary monounsaturated fatty acids for fat accretion.

The essential polyunsaturated fatty acid availability of both cafeteria and reference diets seem to be in large excess —in disagreement with some claims suggesting that the cafeteria diet could be defficient (24)—, since they were degraded to a large extent by the rats in both diet groups. In addition, the actual loss of linoleic acid observed in reference diet–fed rats was produced despite a very large amount of this fatty acid in the diet, which eventually can speed up its metabolic utilization.

The data presented suggest that there is a relative loss of Δ^5–desaturase activity with maturation from 30 to 60 days after birth, as shown by the polyunsaturated fatty acid ratios of table 4. The decrease in the C20:4/C20:3 ratio shown by reference diet-fed rats seems to indicate that these animals maintain —albeit diminished— their Δ^5–desaturase activity, since the ratios were higher than those of the diet ingested. This is further stressed by the increase observed in the C20:3/C18:2 ratio with age, suggesting that the elongation and Δ^6-unsaturation processes actually proceeded unhampered, thus inducing a relative accumulation of C20:3 because of the postulated Δ^5–desaturase inability to use this fatty acid for the synthesis of C20:4, thus the Δ^6–desaturase activity may not be a limiting step in the synthesis of C20:4, as occurs in humans (25). Cafeteria diet-fed rats lost practically all their Δ^5–desaturase capability in the 30-60 day period, since their C20:4/C20:3 ratios were almost the same as those of the diet they ingested. The C20:3/C18:2 ratio, on the other hand, suggested that all the elongation–desaturation pathway ending in the synthesis of arachidonic acid (C20:4) is severely depressed as a whole, because their high dietary provision renders the process unnecessary.

As indicated previously (8) the feeding of cafeteria diet to young rats has an effect upon their growth and fat and protein deposition similar to a continuation of lactation, with much higher rates of protein and fat accretion (12) and lower urea production (8) than controls fed the usual cereal-based rat chow. The data in the present study agree with that view, since the mean of fatty acids deposited was practically identical to that present in the weaned 30-day old rat. The animals weaned —and later fed continuously— on reference diet chow pellets showed a distinct differential fatty acid accretion pattern, with a higher predominance of shorter and more saturated fatty acids than those present in the weaned rat, and even than those present in the diet. Thus, the protein– and fat–rich cafeteria diet can act, in that respect, as a continuation of lactation, preventing the shift towards lipogenesis and protein and amino acid oxidation that typifies weaning in the rat (26,27), and maintaining the high growing potential and efficiency that characterize lactation (8).

In conclusion, the study has shown that diet affects only in a small part the fatty acid composition of —growing— young Wistar rats. Diet actually affects fat deposition quantitatively rather than qualitatively. The main difference between a fat-rich and carbohydrate-rich diet may lie in the stimulation/inhibition of net lipogenesis. During the second month of the rat's life, the elongation pathway as well as Δ^9–desaturase become functional, while there is a relative loss of the Δ^5–unsaturation capability, thus helping to shape the type of fatty acids actually accrued.

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TABLE 1

Fatty acid content of the foods consumed by cafeteria-fed rats

Fatty acid	chow pellet	liver pâte	bacon	banana	biscuit	milk
saturated chain						
C10	n.d.	0.23 ± 0.11	0.88 ± 0.50	n.d.	6.40 ± 1.74	0.34 ± 0.01
C12	0.02 ± 0.01	0.47 ± 0.10	0.49 ± 0.14	n.d.	44.2 ± 5.6	0.37 ± 0.00
C14	0.14 ± 0.01	2.69 ± 0.29	3.59 ± 0.47	n.d.	14.9 ± 0.4	1.70 ± 0.12
C15	0.04 ± 0.01	1.39 ± 0.41	1.10 ± 0.62	n.d.	1.13 ± 0.17	0.27 ± 0.04
C16	4.14 ± 0.39	59.7 ± 9.5	72.2 ± 9.2	0.09 ± 0.03	24.7 ± 1.4	5.72 ± 0.03
C17	0.24 ± 0.05	4.60 ± 0.77	4.02 ± 1.21	n.d.	2.52 ± 0.25	0.38 ± 0.07
C18	1.11 ± 0.05	20.7 ± 2.1	26.1 ± 4.3	0.02 ± 0.01	14.2 ± 0.9	2.42 ± 0.21
C20	0.35 ± 0.09	2.76 ± 0.56	3.04 ± 0.68	0.01 ± 0.01	1.62 ± 0.28	0.14 ± 0.06
C22	0.79 ± 0.20	5.07 ± 0.05	4.76 ± 0.87	0.02 ± 0.01	2.54 ± 0.11	0.27 ± 0.12
C24	1.31 ± 0.03	7.03 ± 1.60	6.08 ± 0.62	0.07 ± 0.04	4.57 ± 0.47	0.65 ± 0.35
monounsaturated						
$C14:1^{9}$	0.04 ± 0.01	0.72 ± 0.24	0.61 ± 0.35	n.d.	0.80 ± 0.12	0.17 ± 0.01
$C16:1^{9}$	0.14 ± 0.01	5.00 ± 0.45	6.43 ± 0.87	0.01 ± 0.01	1.80 ± 0.09	0.25 ± 0.00
$C18:1^{9}$	4.66 ± 0.64	96.4 ± 12.7	117.1 ± 16.1	0.05 ± 0.03	28.3 ± 2.2	4.77 ± 0.09
$C20:1^{11}$	0.32 ± 0.03	3.54 ± 0.49	3.09 ± 0.81	0.01 ± 0.01	1.53 ± 0.14	0.21 ± 0.04
$C22:1^{13}$	0.23 ± 0.06	2.56 ± 0.41	2.69 ± 0.51	0.01 ± 0.01	1.03 ± 0.15	0.11 ± 0.09
polyunsaturated						
$C18:2^{9,12}$	11.1 ± 1.0	20.9 ± 2.3	32.5 ± 4.3	0.05 ± 0.02	8.85 ± 0.74	0.47 ± 0.01
$C18:3^{9,12,15}$	0.84 ± 0.07	1.96 ± 0.23	2.50 ± 0.26	0.04 ± 0.01	1.15 ± 0.17	0.20 ± 0.01
$C20:2^{11,14}$	0.18 ± 0.01	2.92 ± 0.13	3.91 ± 0.59	0.01 ± 0.01	1.31 ± 0.11	0.10 ± 0.08
$C20:3^{8,11,14}$	0.52 ± 0.24	2.46 ± 0.38	2.51 ± 0.45	0.02 ± 0.02	1.10 ± 0.14	0.14 ± 0.06
$C20:4^{5,8,11,14}$	0.39 ± 0.15	2.02 ± 0.33	2.49 ± 0.41	0.01 ± 0.01	0.78 ± 0.07	0.08 ± 0.04

All data are the mean ± SEM of 5 duplicate different samples for each food item. The data are expressed in mg of the fatty acid per gram of fresh food (per ml in the case of milk).
n.d. = not detected.

TABLE 2

Weights and lipid composition of young rats fed reference and cafeteria diets

age (days)	diet	weight (g)	percentage of lipid
30		90 ± 2	6.3 ± 0.3
45	reference	152 ± 4 ▲	7.8 ± 0.5 ▲
	cafeteria	177 ± 2 *	9.3 ± 0.2 *
60	reference	199 ± 4 •#	10.0 ± 0.8 •#
	cafeteria	248 ± 5 *•#	17.3 ± 1.6 *•#

The data are the mean ± SEM weight and lipid content (as a percentage of body weight). N = 12-20 rats for weight and 6 for lipid composition.
Statistical significance of the differences between means (Student's *t* test):
cafeteria versus reference diet: * = $P < 0.05$
45– versus 30–day: ▲ = $P < 0.05$
60– versus 45-day: • = $P < 0.05$
60– versus 30–day: # = $P < 0.05$

TABLE 3

Mean length and unsaturation values for the component, ingested and deposed fatty acids in young rats fed reference and cafeteria diets

		COMPOSITION		INTAKE		DEPOSITION	
period and age group	diet	mean chain length	mean number of double bonds	mean chain length	mean number of double bonds	mean chain length	mean number of double bonds
30–day	*(reference)*[1]	*17.3*	*0.74*				
30-45	reference			18.2	1.26	17.4	0.56
	cafeteria			17.4	0.73	17.5	0.71
45–day	*reference*	*18.3*	*0.67*				
	cafeteria	*17.5*	*0.72*				
45–60	reference			18.2	1.26	18.0	0.55
	cafeteria			17.7	0.70	16.9	0.76
60–day	*reference*	*17.6*	*0.63*				
	cafeteria	*17.2*	*0.76*				

The figures presented were calculated from the data of Figures 1 and 2
Food ingested —and its fatty acid composition— was estimated from the differences in amount of food offered and that left over.

[1] Reference diet and dam's milk, since these animals remained with their dams up to day 21 and then were fed only the reference diet

TABLE 4

Ratios of polyunsaturated fatty acids present in the lipids of rats fed reference and cafeteria diets, as well as in the diet they ingested

age and diet group		C20:4/C20:3 ratio	C20:3/C18:2 ratio
30 days	(reference)[1]	1.87	0.102
60 days	reference	1.35	0.480
	cafeteria	0.89	0.124
30-60 day ingested diet	reference	0.75	0.047
	cafeteria	0.84	0.083

The figures presented were calculated from the data of Figures 1 and 2

[1] Reference diet and dam's milk, since these animals remained with their dams up to day 21 and then were fed only the reference diet

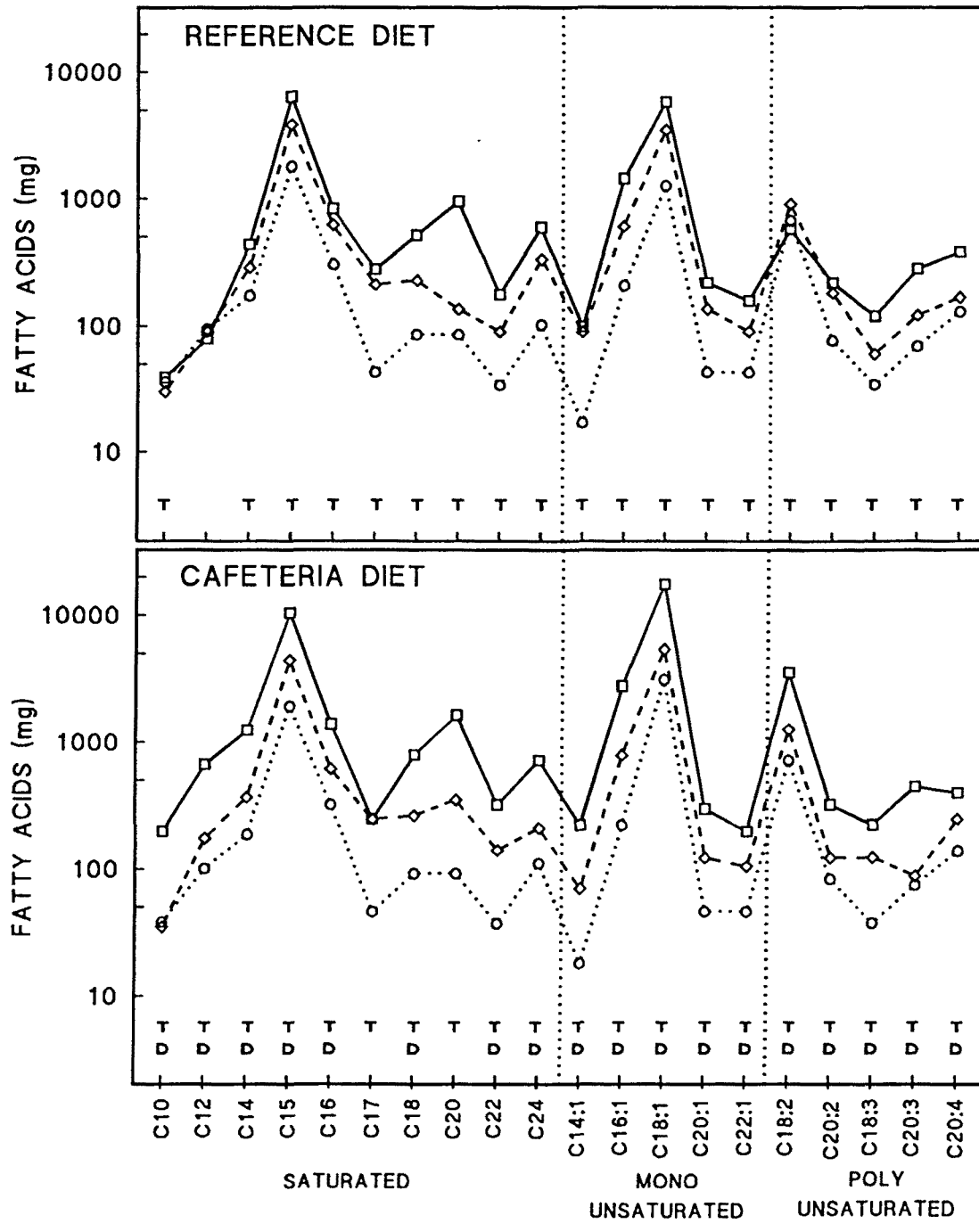

FIGURE 1: Total fatty acid content in the body of Wistar rats fed reference or cafeteria diets from 30 to 60 days after birth.

Upper panel, reference diet, lower panel, cafeteria diet. Dotted line and open circles: 30-day old rats; Dashed line and diamonds: 45-day old rats; Solid line and squares: 60-day old rats. Each symbol represents the mean of 6 different animals. The scale for fatty acids is logarithmic.

Statistical significance of the differences between groups (two–way ANOVA):

A "T" in a given fatty acid column means that there is a significant ($P<0.05$) effect of time, a "D" means a significant ($P<0.05$) effect of dietary treatment.

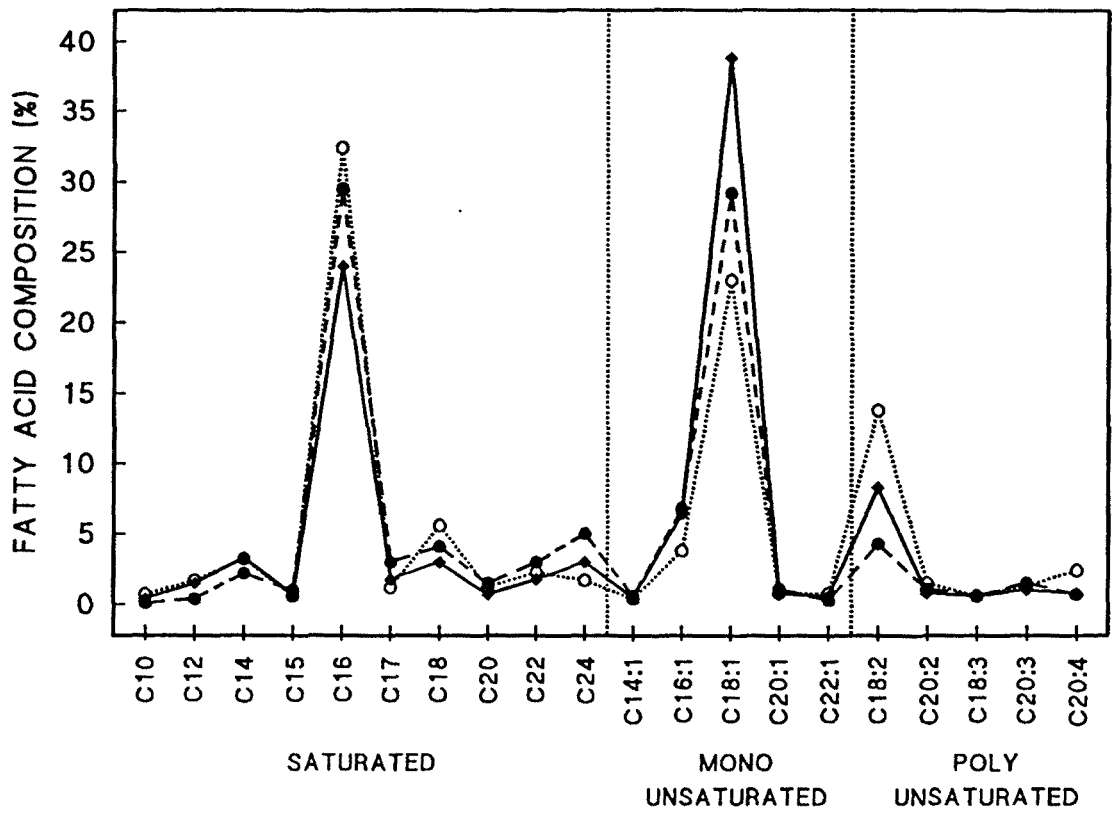

FIGURE 2: Percentage fatty acid composition of Wistar rats fed reference or cafeteria diets from 30 to 60 days after birth.

Percentage of each fatty acid weight with respect to the total fatty acid content in the whole rat lipids. Dotted line and open circles: 30-day old rats; Dashed line and black circles: 60-day old rats fed reference diet; Solid line and black diamonds: 60-day old rats fed cafeteria diet.

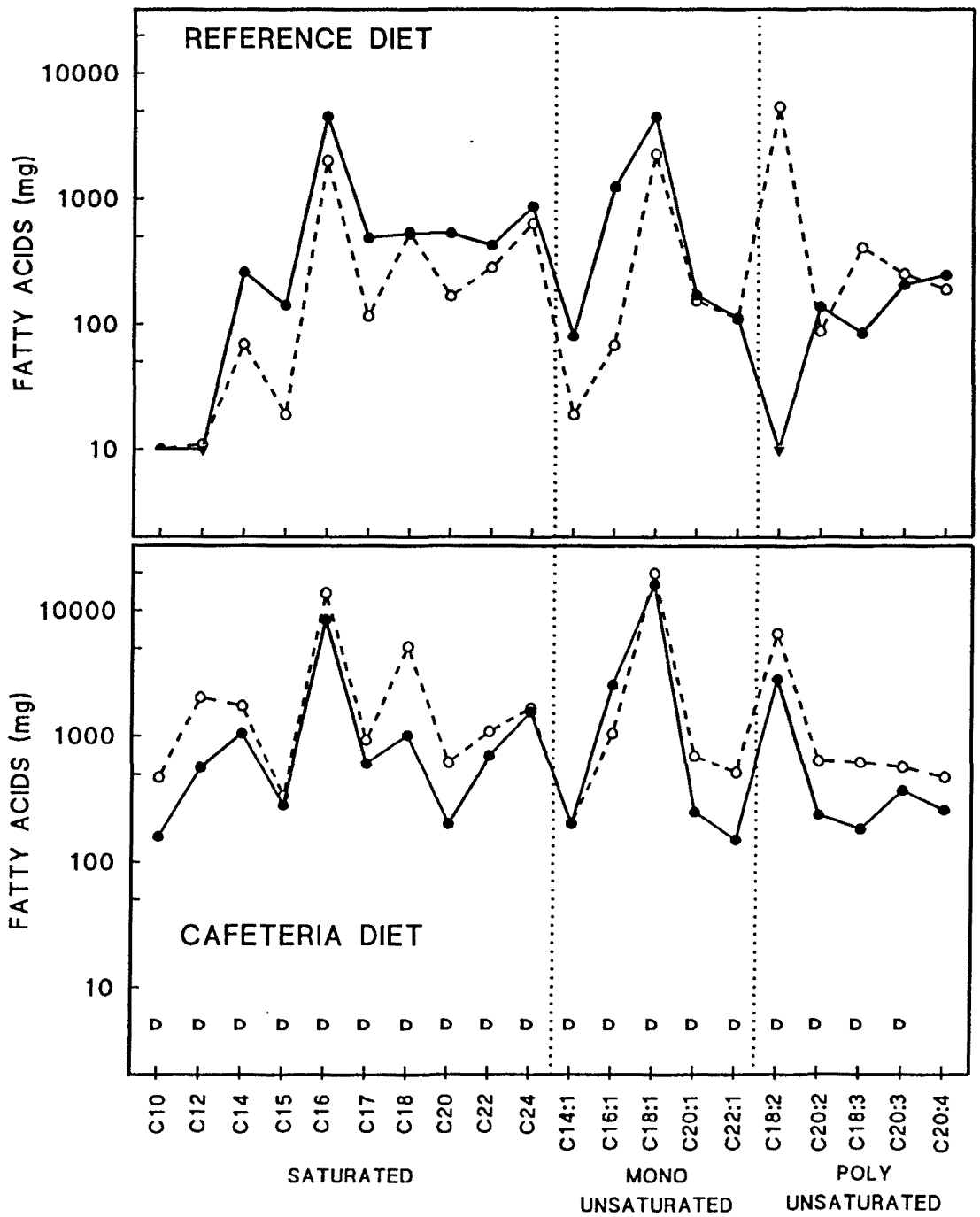

FIGURE 3: Differences between the amount of each fatty acid ingested with the diet and that accumulated in the tissues of Wistar rats fed reference or cafeteria diets from 30 to 60 days after birth.

Upper panel, reference diet, lower panel, cafeteria diet. In both cases, the solid line and black circles represent the amount of fatty acid accrued during the 30 days of dietary treatment. The dashed line and open circles are the amount of each fatty acid actually ingested during the same period of time. Upper semicircles: data lower than 10 mg; Triangles: negative values. *Statistical significance of the differences between groups (Student's t test): A "D" means a significant ($P<0.05$) difference between reference and cafeteria diets in the amount of fatty acid deposited. In addition, all fatty acids showed a significant difference between reference and cafeteria diets in the amount of fatty acid ingested.*

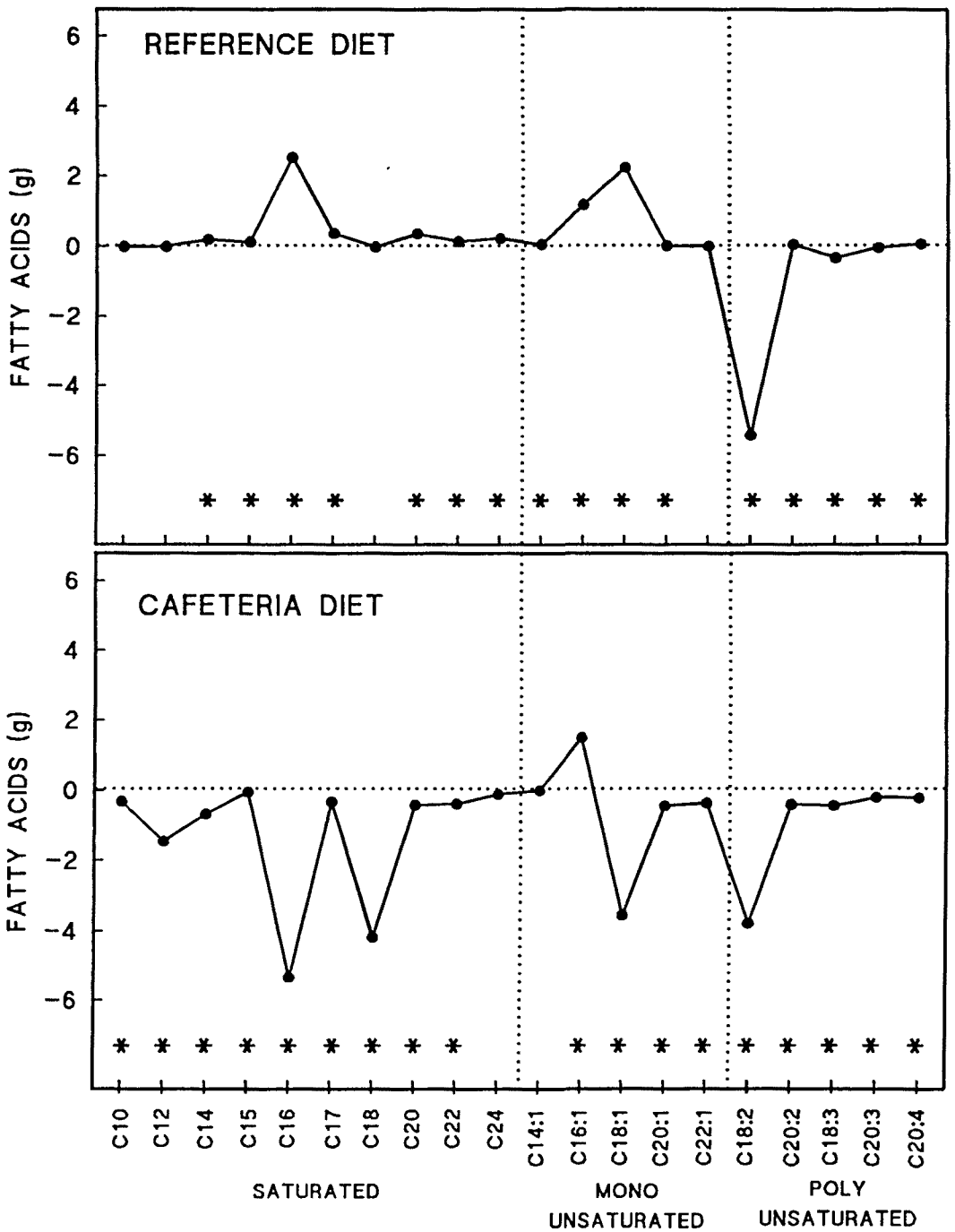

FIGURE 4: Net differences between the fatty acids ingested and those accrued by Wistar rats fed a reference or cafeteria diet from days 30 to 60

The data have been calculated from those in figure 3. THey show the net differences between the amopunt of each fatty acid ingested and that actually accrued in the rat lipid stores. The data are presented in a linear scale. Positive values indicate net synthesis, negative values net oxidation.

Statistical significance of the differences between groups (Student's t test):

An asterisk "" in a given fatty acid column means that there is a significant difference ($P<0.05$) between the ingested and accrued fatty acid.*

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Deposition of dietary fatty acids in young Zucker rats fed a cafeteria diet.

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SUMMARY

The content and accretion of fatty acids in 30, 45 and 60–day old Zucker lean Fa/? and obese fa/fa rats fed either reference chow or a cafeteria diet has been studied, together with their actual fatty acid intake during each period. Diet had little overall effect on the pattern of deposition of fatty acids, but quantitatively the deposition of fat was much higher in cafeteria–fed rats. The fat–rich cafeteria diet allowed the direct incorporation of most fatty acids into the rat lipids, whilst chow–feeding activated lipogenesis and the deposition of a shorter chain and more saturated pattern of fatty acids. Genetic obesity induced a significant expansion of net lipogenesis when compared with lean controls. Cafeteria–fed obese rats accrued a high proportion of fatty acids, which was close to that ingested, but nevertheless showed a net *de novo* synthesis of fatty acids. It is postulated that the combined effects of genetic obesity and a fat–rich diet result in high rates of fat accretion with limited net lipogenesis. Lean Zucker rats show a progressive impairment of their Δ^5–desaturase system, a situation also observed in obese rats fed a reference diet. In Zucker obese rats, cafeteria feeding resulted in the alteration of the conversion of C18:2 into C20:3. The cafeteria diet fully compensated for these drawbacks by supplying very high amounts of polyunsaturated fatty acids.

INTRODUCTION

The genetically obese Zucker fa/fa rat is widely used as a model of obesity (1). This strain is characterized by hyperphagia and a high ability to store fat (2), as a consequence of impaired ability for adaptive thermogenesis (3). The Zucker fa/fa rat is, thus, unable to compensate for any excess energy ingested, by means of diet induced thermogenesis, and has a poor response to cold exposure (4).

The "cafeteria" diets were introduced in the 1970's as a way to generate hyperphagic conditions in the rat similar to those experienced by humans eating highly palatable choice diets (5), with the purpose of using an animal model of obesity which is closer to the situation in humans than genetic obesity. The diets actually selected by the rats have a common high lipidic content (6), with relatively unchanged —and proportionally lower— protein and carbohydrate (7). The constant exposure to these diets from weaning results in high growth rates (8) and substantial fat accretion (9), with high increases in energy intake (7) and increased thermogenesis (10).

The effects of cafeteria diet–induced hyperphagia and genetic obesity are not fully additive (11,12), but nevertheless fa/fa rats accumulate huge amounts of fat irrespective of the fat content on the diet offered to them.

The research presented here has been designed from the model of a balance study, in which the actual intake of the most representative and abundant individual fatty acids is measured and compared with the composition of the fat newly deposited in growing young rats —lean Fa/? controls and obese fa/fa rats— fed either the cafeteria or a standard chow reference diet. The differences between the two sets of figures provide information on the predominant processes of handling fatty acids of both dietary and lipogenic origin.

MATERIALS AND METHODS

Animals and diets

The animals used in this study were female Zucker Fa/? and fa/fa rats (bred at the University of Barcelona Animal Service from heterozygous parents [Fa/fa] of Harlan–Olac UK stock). Experiments began 30 days after their birth (weaned on day 22). The rats were housed individually, either in polypropylene–bottomed cages with wood shavings as absorbent material, or in polycarbonate metabolic cages (Tecniplast Gazzada, Italy) which allowed the daily estimation of the consumption of individual food items. The cages were maintained in a light (on from 08.00 to 20.00), humidity (70–80 % relative humidity) and temperature (21–22 °C) controlled environment.

Control rats were fed a commercial reference pellet diet (type A04 from Panlab, Barcelona, containing 170 g/kg protein, 587 g/kg digestible carbohydrate, and 30 g/kg lipids) and tap water *ad libitum*. All studies involving the measurement of food intake were carried out with the rats kept in metabolic cages. The rats given the cafeteria diet were presented daily with a fresh offering of biscuits spread with liver pâté, bacon, banana, chow pellets (as indicated above), tap water and whole milk complemented with 333 g/l sucrose plus 10 g/l of a mineral and vitamin supplement (Gevral, Cyanamid Ibérica) (12). All the materials were previously weighed and presented in excess (c. 20–30 % higher than expected consumption). Twenty-four hours later, the remaining debris was isolated, identified and weighed. The drying of food leftovers was corrected for by determining the amount of water lost in one day from known weight food samples left in a cage with no rats. This diet is a simplified version of an earlier cafeteria diet developed and studied by us (7), scaled down by selecting only the items actually consumed in significant portions. The animals were weighed every day at the same hour (11.00 to 12.00).

Experimental setup

For each breed (lean Fa/? or obese fa/fa) and dietary group (reference– and cafeteria–fed rats) three sets (5–7 rats each) of animals were studied, being killed at 30 (0 days of dietary treatment), 45 (15 days of treatment) or 60 (30 days of treatment) days after birth. The 60–day group rats were used for daily individual diet intake analysis (metabolic cages) for the whole 30 days of the study. The other rats (45–day, and 30 day old rats) were kept individually in ordinary cages, where they were fed the same diets; their intake was not measured.

On days 30, 45 or 60, the allotted groups of rats were weighed and immediately killed by decapitation. Their corpses were again weighed (the difference being the net loss of blood and fluids) and then dissected. The content of their intestine was carefully removed and weighed. The weights recorded and used for calculations were the empty body weights. The remaining carcass was then minced and ground with a blender, sampled and stored frozen at –23 °C until processing.

Analytical procedures

The ground carcasses were sampled following a previously tested sampling protocol (12). The samples were homogenized with a Politron homogenizer; the resulting smooth paste was then used for analysis. The constituents of the diets given to the animals were also ground and homogenized, and then subjected to the same analytical procedures. Blood samples were also analyzed and used to correct the data obtained for the different groups accounting for the blood lost during the killing and dissecting process.

Total lipids were extracted from the samples (in duplicate) homogenizing the samples with chloroform : methanol (13). The lipids were saponified under reflux for 90 minutes with 12 g/l NaOH in ethanol, and the methyl ester derivatives of all fatty acids were obtained (14) and analyzed in a Perkin–Elmer Sigma 3B gas chromatograph using a 30 m x 0.75 mm i.d. Supelcowax 10 (Supelco Inc, USA) minicolumn, and a flame ionization analyzer. Helium was used as carrier phase. Standards for the extraction and derivatization procedures were run in parallel. Internal standards of C13 were introduced in all samples prior to methylation. Standards of the fatty acids listed in Table 1 (from Sigma, USA) were also run with each batch of samples, which were chromatographed in duplicate.

Calculations and statistics

The analyses of body composition of the series of rats killed on days 30 and 45 were used for the calculation of the composition of the rats kept in metabolic cages (killed at day 60, after one month of daily measurement of intake and body weight) on days 30 and 45. Since their weights were very close ($P > 0.95$, Student's *t* test) and the feeding scheme was the same, it was assumed that —for each breed— the percentage composition of the three series of rats was identical for matching age and diet.

The composition of the lipid samples, the proportion of lipid and the mass of the rat paste were used to estimate the amount of each fatty acid present in each of the three age–groups studied, and allowed the quantitative estimation of the differences between the fatty acids stored, always related to the actual empty body weight of the animal referred to the age of comparison.

The known amount of each food item consumed daily by each rat and its fatty acid composition (Table 1) were used to establish the amount of each fatty acid ingested by each rat for each day. The tabulated data for each diet component were combined in order to determine the gross intake of each fatty acid during each of the 15–day periods studied. Statistical comparisons between the groups were performed with a standard two– or three–way ANOVA program, as well as with Student's *t* test.

RESULTS

Table 1 shows the fatty acid composition of the food items presented in the cafeteria diet. In Table 2, the weights and percentages of lipids found in the different groups of rats are listed. Obese rats started at 30 days with a 20 % higher weight than their lean counterparts; after one month, their weight was almost double than of the lean rats. Cafeteria feeding actually increased body weight and fat content in all groups of rats, up to more than one third of body weight being made up of lipid in the 60–day cafeteria–fed fa/fa rats.

Figure 1 depicts the individual fatty acid composition of the four diet–breed groups, measured at 30, 45 and 60 days. In all groups the overall fatty acid deposition showed a generalized increase with age, maximal for older obese rats feeding on the cafeteria diet. Lean Fa/? rats showed the lowest global amount of fatty acids when fed the reference diet. The rats accrued most fatty acids in a fairly uniform way, except for slight changes in the amount of

C18:2, whose stores were practically the same at 30 as at 60 days, except in obese rats fed the cafeteria diet. The levels of the shorter chain saturated fatty acids actually decreased from 30 up to 60 days. The levels of C20:2 and C20:3 showed little change from 30 to 45 days. Obese Zucker rats fed the reference diet also showed a decrease of C10 from 30 to 60 days. In addition, there was practically no increase in odd–carbon fatty acids, or in 14:1, from 45 to 60 days, with an actual decrease in C22:1 and unchanged C18:2 levels from 45 to 60 days. The 45–day levels of C20 were also higher than those found on day 60.

The administration of a cafeteria diet to lean rats resulted in a similar pattern to that described for obese rats fed the reference diet. The amount of long (C20 to C24) saturated fatty acids actually decreased from 45 to 60 days, with small changes in C15, C17, C14:1 and C22:1 levels. In cafeteria–fed lean rats, polyunsaturated fatty acids showed a significant accumulation of linoleic (C18:2) acid from 30 to 45 days, which was later reversed up to day 60. All other polyunsaturated fatty acids actually decreased —or were unchanged, as is the case with C20:3— from day 45 to day 60, despite consistent increase in the 30–45 day period.

Obese rats fed the cafeteria diet showed a much more streamlined pattern in relation to time. For all fatty acids, 45–day values were higher than those at day 30, and those at day 60 were much higher. The overall pattern of accrual was very close to that shown by 45–day lean rats fed the cafeteria diet, but the differences between the age groups were essentially quantitative rather than qualitative, as can be seen in Figure 2, which shows the percentage of each fatty acid in the rat lipids.

The quantitative importance of C16 for lean rats was maintained in spite of age and diet. However, the other main fatty acid, C18:1, increased its share with age, the lipid–rich cafeteria diet further increasing its proportion. Lean rats also showed an age–dependent loss of importance of C18:2 and other polyunsaturated fatty acids. Cafeteria feeding, in this respect resulted in a lower proportion of polyunsaturated fatty acids and long–chain saturated fatty acids, in contrast with their higher share in 60–day rats fed the reference diet.

Obese rats showed a very similar overall pattern of fatty acid distribution. However, the extent of change induced by diet or breed upon the basic lean rat–reference diet pattern was small. The proportion of C18:1 changed much less than in lean rats with age and cafeteria diet, but the percentage of C16 decreased with age (the cafeteria diet actually maintained the 30–day proportion of C16). The effect of obesity on polyunsaturated fatty acid proportions was not marked when compared with the lean rats fatty acid profile. The proportion of these fatty acids actually decreased with age. Here, the cafeteria diet resulted in a higher percentage than those found in either group fed the reference diet.

The fatty acids ingested and those accrued during the 30–day period studied in the four diet–breed groups of rats are presented in Table 3. Figure 4 shows the net differences between fatty acid ingestion and accretion. Lean rats fed the reference diet had higher accretion values than the amounts of most fatty acids present in the diet. There was a net synthesis of C16 and C18:1, as well as small amounts of C16:1. Other fatty acids accumulated in much smaller amounts, although their quantitative contribution was fairly small. Linoleic acid (C18:2) was oxidized in a large proportion, with little of it actually incorporated into the lipids of the rat. This same pattern of lower deposition–than intake —but on a much smaller scale— was observed for C18:3, C20:3 and C20:4. In fact, the only polyunsaturated fatty acid that was actually synthesized in net amount, albeit small, was C20:2. Obese rats fed the reference diet presented a very similar pattern to that, but here the differences between intake and accretion were —in general—

much larger; the lowest differences between the two sets of data could be found for saturated fatty acids, C20 or higher, C22:1 and C20:3. Obese rats show a very similar pattern of net lipogenesis / fatty acid oxidation than their lean counterparts fed the same reference diet. The fa/fa rats synthesize *de novo* large amounts of C16 and C18:1, with important net production of C14, C18 and, especially, C16:1. The tendency to use the excess dietary C18:2 and other polyunsaturated fatty acids was also maintained.

Lean rats fed the cafeteria diet showed a completely reversed pattern of intake / deposition, since here all fatty acids —except C16:1— were ingested in larger amounts than those actually accrued. The differences were more marked for C16, C18, C18:1 and C18:2, but were also important for most saturated and all polyunsaturated fatty acids. Linoleic acid, a very important diet component, was quantitatively oxidized, since the amount found in a 30–day rat was higher than that of a 60–day.

The obese rats fed a cafeteria diet showed an extremely close intake pattern to that of the fatty acids actually accrued. Although the differences were statistically significant for most fatty acids, the differences between patterns were very small given the sheer size of the amounts involved, e.g., a rat in this group contains c. 50 g of oleic acid, about 15 % of its body weight!. The obese rats fed the cafeteria diet accrued less saturated fatty acids —except for C16— than those ingested: essentially C18:1 and C16:1. Obese rats oxidized large amounts of C18 and the shortest saturated fatty acids: C10, C12, C14. This group of rats, however, oxidized only small amounts of their dietary polyunsaturated fatty acids.

From the data presented in Table 3 (and in Figure 4), the mean net synthesis of fatty acids for the 30–day period can be estimated as 0.21 g (lean, reference diet), –37.6 g —net degradation— (lean, cafeteria diet), 46.8 g (obese, reference diet) and 7.11 g (obese, cafeteria diet).

Table 4 shows the mean fatty acid group composition, as well as mean chain length and number of double bonds in the diet ingested and the portion of lipid accrued in the 30–day period studied. The reference diet contained a higher mean number of fatty acid chain double bonds than the cafeteria diet and the rat lipids. During the 30–45 day period, the degree of unsaturation of the fatty acids accrued was similar in all groups and close to that of the rats' lipids presented in Table 5. However, in the 45–60 day period, the fatty acids accrued were much more saturated in all groups —except for cafeteria–fed obese rats, where the reverse is true— than either the fatty acids from the diet or those of the rats' lipids. The proportion of saturated fatty acids in the accrued fat increased with age —except in cafeteria-fed obese rats—, at the expense of unsaturated fatty acids, especially polyunsaturated.

Table 5 presents the mean fatty acid composition of the lipids from rats at different ages, fed either the reference or cafeteria diet. There was a remarkable uniformity in the mean length of the fatty acids actually present in the rats' lipids, all about 17.3 carbons per molecule. The degree of unsaturation changed a little more, being generally higher in all cafeteria–fed groups, and lowest in genetically obese rats. Lean rats fed the reference diet consistently decreased the mean unsaturation of their fatty acids with age, essentially at the expense of the polyunsaturated fatty acid fraction. The cafeteria diet maintained this tendency in lean rats, with an increased predominance of the monounsaturated fatty acids proportion. Obese rats fed the reference diet showed less marked changes in fatty acid composition with age, with only slight decreases in the saturated and polyunsaturated fractions and increases in monounsaturated fatty acids, with overall maintenance of the mean unsaturation value. Cafeteria–diet feeding helped maintain a higher degree of unsaturation in the lipids of obese rats, with lower saturated

and increased monounsaturated fatty acids.

Table 6 shows the ratios between the amounts of C20:4 and C20:3 found in the 30– and 60–day carcasses as well as in the diet ingested by the two breeds of rats, and also the C20:3/C18:2 ratio. The C20:4/C20:3 ratio decreased with age in all groups except for obese rats fed the cafeteria diet, in which actually it increased. The C20:3/C18:2 ratio increased with age in lean rats regardless of the diet they ingested. Obese rats fed the reference diet increased this ratio slightly less, but those fed the cafeteria diet showed very low values.

DISCUSSION

In agreement with previous studies (7), the amount of fat ingested by the rats fed a cafeteria diet was remarkably uniform, despite changes in the age / size of the rats. The fatty acid intake pattern was also very similar in lean and obese rats, as observed in Table 3 for both breeds of rats. This remarkable uniformity supports the view that feeding on a cafeteria diet practically equals the ingestion of a fat–rich diet with consistent and uniform fatty acid composition (15). This is further stressed by the very small differences observed in the mean chain length and unsaturation of the fatty acids ingested for the age and breed groups fed the cafeteria diet.

In spite of the differences between the fatty acid composition of the diet and that of the rats' lipids, the proportions of each fatty acid incorporated into the rat lipids was very similar for all diet and breed groups. The predominance of C16, C18:1 and C18:2 is in agreement with the fatty acid composition of the adipose tissue of rats (16). This uniform incorporation was accomplished from very different lipid content in the diet (a mean 7.9 % —lean— or 9.5 % —obese rats— in the cafeteria diet selected versus a 3.0 % in the reference diet), and was maintained even when the amount of fat actually deposited in one month ranged from a mere 11.7 g for lean rats fed the reference diet to a mean 106 g for obese rats fed the cafeteria diet. The uniformity in fatty acid composition is achieved by different means; in obese rats —especially those fed the reference diet— there is significant active net synthesis of fatty acids.

The prime subject of net fatty acid synthesis appears to be the most abundant monounsaturated fatty acid, oleic acid (C18:1), followed by palmitic acid (C16). Lean rats synthesize palmitic and oleic acids when fed the reference diet, using-up most of the (excess) polyunsaturated dietary fatty acids at the same time; when fed the cafeteria diet, the rats incorporate dietary amino acids massively, adapting their degradation / synthesis to fit uniform fatty acid patterns.

In spite of the ability of the rat metabolism to match the ingested fatty acid pattern to that needed for accretion, the diet has an appreciable effect —albeit small— upon the composition of the fat accrued. The reference diet contains small amounts of most fatty acids; however, the rats fed with it actually oxidize most of the polyunsaturated fatty acids eaten, and must, therefore, synthesize a large proportion of the fatty acids they need. The resulting fat is more saturated than the fat already present in the 30–day old rat (with a sizeable part stemming from the dams' milk), or that found in the chow pellets.

The elongation pathway for saturated fatty acids seems to become fully functional during the second month of life (days 30–60), since the increases in C22 and C24 rose considerably from day 30 to 45, although the pace is somewhat slower thereafter.

The similarity between the mean size and unsaturation of fatty acids found in the cafeteria diet and those accrued from days 30 to 45 supports the notion that a fat–rich diet —like the cafeteria diet— results in growth and accretion closely resembling that observed

during lactation (8). This pattern changes in the 45 to 60–day period, probably as maturation proceeds. The interpretation just advanced, however, could not explain the differences in the mean fatty acid unsaturation of rats fed the reference diet, which is more marked for the older rats. This can be partially explained by a decrease in the Δ^5–desaturase activity: the decrease observed with age of the C20:4/C20:3 ratio in Zucker lean rats clearly points to the existence of such a loss of activity with increasing age. This is further stressed by the concomitant increases in the C20:3/C18:2 ratio: i.e. there is a relative accumulation of C20:3 that cannot be converted into C20:4 because of a deficiency in the Δ^5–desaturase system. In addition, these combined ratios suggest that the elongation and Δ^6–desaturase systems are fully functional, since this pathways becomes active shortly after birth (17), providing the polyunsaturated fatty acids needed for brain and other tissue development (18). This altered Δ^5–desaturase and unchanged elongation and Δ^6–desaturase activity was also observed, with varying degrees of intensity, in lean rats fed either diet, as well as in obese rats fed the reference diet. On the other hand, the obese rats fed the cafeteria diet showed a C20:4/C20:3 ratio that was practically unchanged by age and very close to the values of the cafeteria diet actually eaten, together with a much decreased C20:3/C18:2 ratio, suggesting a deep alteration of the pathway between C18:2 and C20:3. This can be partially explained by the limiting–rate role of Δ^6–desaturase, as has been described for humans (19). The failure of the pathway is partly and efficiently counteracted by the wide availability of these polyunsaturated fatty acids in the diet.

The complete pathway from C18:2 to C20:4 is thus altered in both lean and obese Zucker rats in the transition from post–lactation to maturity (i.e. from 30 to 60 days of age). Obesity or cafeteria diet feeding resulted in a deeper alteration of the Δ^5–desaturase system. The combination of obesity and the cafeteria diet affected the pathway even earlier. In general, the cafeteria diet renders the whole pathway practically inoperative, because the high dietary availability of the polyunsaturated fatty acids amply covers the rat requirements, thus the emphasis is placed on their oxidation for energy rather than their bio–transformation.

In the rat, lipogenesis from carbohydrate and other available fuels helps to compensate for the shortcomings of the diet as to fatty acid availability with respect to energy (fat) storage (20). In humans, it has been shown that even a small presence of dietary fat actually inhibits lipogenesis (21), thus facilitating the direct and selective incorporation of dietary fatty acids into reserve fats. The presence of a 10 % lipid in the diet prevents lipogenesis from glucose in the rat (22,23).

It can safely be assumed that the lipogenesis in cafeteria–fed rats must be at least partially inhibited, probably as a consequence of the enormous amount of fat ingested and the inhibitory effect of activated fatty acids upon lipogenic enzymes (24,25).

Obese rats show hyperphagia (26), which further potentiates the increase in intake that characterizes the cafeteria diets (5) by supplying the rat with huge amounts of fatty acids. As a result, a high-fat diet can very probably hamper the *de novo* synthesis of fatty acids to a large extent (27,28). The practical identity between the mean chain length and unsaturation of the fatty acids ingested by 30–45 day lean rats and those deposited is in agreement with this idea. However, this is not the case with older lean rats, in which the suggested shortcomings in the desaturation pathways result in the accretion of more saturated fatty acids.

Obese Zucker rats possess very active lipogenic activity (29), a mechanism linked to their ability to withstand cold exposure (*unpublished results*); the considerable importance of this pathway, as well as its eventual operation under prandial conditions, could help explain

the maintenance of net fatty acid synthesis even when the rat is supplied with a high–fat cafeteria diet and keeps accruing large amounts of fat.

The essential polyunsaturated fatty acid availability of both cafeteria and reference diets seems to be in large excess —in disagreement with some claims suggesting that the cafeteria diet could have a deficit in them (15)—, since they were degraded to a large extent in both diet groups. In addition, the actual loss of linoleic acid from the body stores, observed in rats fed the reference diet, occurred despite a very large amount of this fatty acid in the diet. Perhaps the actual net loss of C18:2 can in itself be a consequence of the large excess in the diet favouring its metabolic utilization (27). The excess polyunsaturated fatty acids in the diet were summarily oxidized or converted into other fatty acids.

In conclusion, this study has shown that fatty acid composition in growing lean and obese Zucker rats is only slightly affected by diet, in agreement with the deposition of fat in mature rats (30). Diet actually affects fat deposition quantitatively rather than qualitatively. The main difference between a fat–rich and carbohydrate–rich diet may lie in the stimulation/inhibition of net lipogenesis from other substrates. Genetic obesity enhanced the powerful drive to accumulate fat that typifies feeding on a cafeteria diet, resulting in an enormous accumulation of fat. Zucker rats, both lean and obese, show a defective unsaturation system that hampers the conversion of C18:2 into C20:4. This situation is aggravated by the high availability of fatty acids induced by cafeteria feeding, but it is also partly compensated by the direct incorporation of the diet fatty acids.

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Table 1

Fatty acid content of the foods offered to cafeteria–fed rats

Fatty acid	chow pellet	liver pâté	bacon	banana	biscuits	milk
saturated						
C10	0 00 ± 0 00	0 23 ± 0 11	0 88 ± 0 50	0 00 ± 0 00	6 40 ± 1 74	0 34 ± 0 01
C12	0 02 ± 0 01	0 47 ± 0 10	0 49 ± 0 14	0 00 ± 0 00	44 2 ± 5 6	0 37 ± 0 00
C14	0 14 ± 0 01	2 69 ± 0 29	3 59 ± 0 47	0 00 ± 0 00	14 9 ± 0 4	1 70 ± 0 12
C15	0 04 ± 0 01	1 39 ± 0 41	1 10 ± 0 62	0 00 ± 0 00	1 13 ± 0 17	0 27 ± 0 04
C16	4 14 ± 0 39	59 7 ± 9 5	72 2 ± 9 2	0 09 ± 0 03	24 7 ± 1 4	5 72 ± 0 03
C17	0 24 ± 0 05	4 60 ± 0 77	4 02 ± 1 21	0 00 ± 0 00	2 52 ± 0 25	0 38 ± 0 07
C18	1 11 ± 0 05	20 7 ± 2 1	26 1 ± 4 3	0 02 ± 0 01	14 2 ± 0 9	2 42 ± 0 21
C20	0 35 ± 0 09	2 76 ± 0 56	3 04 ± 0 68	0 01 ± 0 01	1 62 ± 0 28	0 14 ± 0 06
C22	0 79 ± 0 20	5 07 ± 0 05	4 76 ± 0 87	0 02 ± 0 01	2 54 ± 0 11	0 27 ± 0 12
C24	1 31 ± 0 03	7 03 ± 1 60	6 08 ± 0 62	0 07 ± 0 04	4 57 ± 0 47	0 65 ± 0 35
monounsaturated						
C14:1^{9}	0 04 ± 0 01	0 72 ± 0 24	0 61 ± 0 35	0 00 ± 0 00	0 80 ± 0 12	0 17 ± 0 01
C16:1^{9}	0 14 ± 0 01	5 00 ± 0 45	6 43 ± 0 87	0 01 ± 0 01	1 80 ± 0 09	0 25 ± 0 00
C18:1^{9}	4 66 ± 0 64	96 4 ± 12 7	117 1 ± 16 1	0 05 ± 0 03	28 3 ± 2 2	4 77 ± 0 09
C20:1^{11}	0 32 ± 0 03	3 54 ± 0 49	3 09 ± 0 81	0 01 ± 0 01	1 53 ± 0 14	0 21 ± 0 04
C22:1^{13}	0 23 ± 0 06	2 56 ± 0 41	2 69 ± 0 51	0 01 ± 0 01	1 03 ± 0 15	0 11 ± 0 09
polyunsaturated						
C18:2$^{9\ 12}$	11 1 ± 1 0	20 9 ± 2 3	32 5 ± 4 3	0 05 ± 0 02	8 85 ± 0 74	0 47 ± 0 01
C20:2$^{11\ 14}$	0 18 ± 0 01	2 92 ± 0 13	3 91 ± 0 59	0 01 ± 0 01	1 31 ± 0 11	0 10 ± 0 08
C18:3$^{9\ 12\ 15}$	0 84 ± 0 07	1 96 ± 0 23	2 50 ± 0 26	0 04 ± 0 01	1 15 ± 0 17	0 20 ± 0 01
C20:3$^{8\ 11\ 14}$	0 52 ± 0 24	2 46 ± 0 38	2 51 ± 0 45	0 02 ± 0 02	1 10 ± 0 14	0 14 ± 0 06
C20:4$^{5\ 8\ 11\ 14}$	0 39 ± 0 15	2 02 ± 0 33	2 49 ± 0 41	0 01 ± 0 01	0 78 ± 0 07	0 08 ± 0 04

All data are the mean ± SEM of 5 duplicate different samples for each food item. The data are expressed in mg of the fatty acid per gram of fresh food (per ml in the case of milk).

Table 2

Weights and lipid percentage of young lean and obese Zucker rats fed reference and cafeteria diets

age days	breed	diet	weight g	percentage of lipid
30	lean	reference	68 ± 2	6.2 ± 1.0
30	obese	reference	82 ± 3	17.5 ± 0.9
45	lean	reference	123 ± 3	6.2 ± 0.2
45	lean	cafeteria	147 ± 4	12.4 ± 1.2
45	obese	reference	174 ± 6	22.1 ± 0.7
45	obese	cafeteria	224 ± 7	30.1 ± 3.8
60	lean	reference	166 ± 4	9.6 ± 0.5
60	lean	cafeteria	209 ± 8	16.7 ± 1.6
60	obese	reference	279 ± 6	29.3 ± 0.7
60	obese	cafeteria	350 ± 7	34.4 ± 2.5

The data are the mean ± SEM of 12–20 rats for weight and 6 for lipid composition.
Significance of the differences between means (three–way ANOVA): there are significant ($P<0.05$) effects of time, diet and breed for the two parameters studied.

Table 3

Fatty acid ingested with the diet and accumulated in the tissues of lean and obese Zucker rats fed reference or cafeteria diets from 30 to 60 days after birth.

	FATTY ACIDS INGESTED		FATTY ACIDS INGESTED		FATTY ACIDS ACCRUED		FATTY ACIDS ACCRUED	
fatty acid	Lean rats control diet	Lean rats cafeteria diet	Obese rats control diet	Obese rats cafeteria diet	Lean rats control diet	Lean rats cafeteria diet	Obese rats control diet	Obese rats cafeteria diet
C10	>7	533 ± 29	>12	1454 ± 99	–40 ± 3	6 ± 2	–70 ± 7	122 ± 5
C12	9 ± 0	2398 ± 211	15 ± 0	8631 ± 737	-60 ± 7	584 ± 23	41 ± 10	1582 ± 25
C14	63 ± 2	1847 ± 58	105 ± 2	4137 ± 218	163 ± 12	960 ± 38	943 ± 19	3019 ± 44
C15	18 ± 1	355 ± 7	30 ± 1	606 ± 22	96 ± 3	125 ± 5	231 ± 4	678 ± 15
C16	1852 ± 57	15330 ± 490	3091 ± 69	22980 ± 934	3870 ± 121	7943 ± 302	23980 ± 460	16680 ± 380
C17	107 ± 3	1013 ± 29	179 ± 4	1675 ± 63	358 ± 9	397 ± 15	451 ± 11	1410 ± 21
C18	497 ± 15	5797 ± 169	828 ± 18	9247 ± 364	637 ± 21	1246 ± 48	2710 ± 53	3513 ± 59
C20	157 ± 5	688 ± 21	261 ± 6	1113 ± 41	90 ± 3	118 ± 5	270 ± 20	804 ± 13
C22	353 ± 11	1182 ± 34	590 ± 13	1903 ± 66	419 ± 18	229 ± 9	795 ± 15	1746 ± 30
C24	586 ± 18	1760 ± 42	978 ± 22	2919 ± 100	674 ± 17	188 ± 7	972 ± 19	2196 ± 39
C14 1^{9}	18 ± 1	211 ± 4	30 ± 1	373 ± 14	56 ± 2	97 ± 4	211 ± 4	519 ± 9
C16 1^{9}	63 ± 2	1208 ± 46	105 ± 2	1803 ± 82	885 ± 24	1651 ± 62	5496 ± 108	6830 ± 114
C18 1^{9}	2084 ± 64	22520 ± 850	3479 ± 78	33120 ± 1470	3607 ± 102	12950 ± 480	27350 ± 540	44360 ± 780
C20 1^{11}	143 ± 4	757 ± 23	239 ± 5	1215 ± 44	173 ± 5	348 ± 13	664 ± 13	1251 ± 22
C22 1^{13}	103 ± 3	580 ± 20	172 ± 4	903 ± 34	153 ± 5	76 ± 4	185 ± 6	552 ± 8
C18 $2^{9\ 12}$	4970 ± 151	7232 ± 207	8295 ± 184	10390 ± 330	263 ± 26	–53 ± 13	1145 ± 23	9200 ± 173
C20 $2^{11\ 14}$	84 ± 2	737 ± 27	134 ± 3	1126 ± 48	130 ± 7	62 ± 5	329 ± 6	1146 ± 20
C18 $3^{9\ 12\ 15}$	376 ± 11	656 ± 15	627 ± 14	989 ± 30	47 ± 2	41 ± 2	271 ± 14	793 ± 14
C20 $3^{8\ 11\ 14}$	233 ± 7	615 ± 17	388 ± 9	954 ± 32	150 ± 7	62 ± 5	337 ± 34	918 ± 15
C20 $4^{5\ 8\ 11\ 14}$	174 ± 5	527 ± 17	291 ± 6	789 ± 28	90 ± 6	–36 ± 2	166 ± 4	899 ± 15

The values are expressed in mg and are the mean ± SEM of 6 different animals,

Significance of the differences between groups (two–way ANOVA):

There is a statistically significant difference ($P<0.05$) for the global effect of diet and breed on both intake and deposition. In addition, there are significant ($P<0.05$) effects of breed and diet for either intake or deposition of all fatty acids, with the sole exception of the non–significant ($P>0.05$) effect of diet on the deposition of lean rats fed the cafeteria diet for C17, C20, C20:3 and C18:3.

There are statistically significant ($P<0.05$) differences (Student's t test) between the amount of each fatty acid ingested and that accrued in all the experimental groups studied, with the only exceptions of: Lean rats/ reference diet: C10; Obese rats/ reference diet: C24 and C20:3; Obese rats/ cafeteria diet: C22, C20:1, C20:2 and C20:3.

Table 4

Fatty acid structure of the fat ingested and deposed by young lean and obese Zucker rats fed reference and cafeteria diets

	30–45 days				45–60 days			
parameter	lean reference	lean cafeteria	obese reference	obese cafeteria	lean reference	lean cafeteria	obese reference	obese cafeteria
FATTY ACID STRUCTURE OF INGESTED FATTY ACIDS								
mean chain length	18.2	17.4	18.2	17.1	18.2	17.5	18.2	17.1
mean double bonds	1.26	0.72	1.26	0.65	1.26	0.72	1.26	0.66
FATTY ACID STRUCTURE OF ACCRUED FATTY ACIDS								
mean chain length	18.0	17.6	17.6	17.1	17.6	16.7	16.9	17.5
mean double bonds	0.66	0.73	0.71	0.71	0.52	0.41	0.53	0.80
FATTY ACID COMPOSITION OF INGESTED FATTY ACIDS, IN %								
saturated	50.6	47.0	50.6	51.8	50.6	46.5	50.6	51.1
mono–unsaturated	20.3	38.1	20.3	34.8	20.3	38.6	20.3	35.5
polyunsaturated	29.1	14.9	29.1	13.4	29.1	14.9	29.1	13.4
ACCRUED FATTY ACID COMPOSITION (CHANGE), IN %:								
saturated	45.3	40.7	40.7	46.0	56.2	46.5	48.3	33.6
mono–unsaturated	45.4	48.4	51.0	41.3	39.8	63.4	51.0	55.2
polyunsaturated	9.3	10.9	8.3	12.7	4.0	–9.9[1]	0.7	11.2

The results presented have been calculated from the means of data shown in Table 3.
The data for ingested reference diet are identical for all age and breed groups.
[1] This negative value represents an absolute loss of polyunsaturated fatty acids from the rat fat stores.

Table 5

Fatty acid structure of the body lipid in young lean and obese Zucker rats fed reference and cafeteria diets

	30 days		45 days				60 days			
	lean	obese	lean	lean	obese	obese	lean	lean	obese	obese
parameter	reference	reference	reference	cafeteria	reference	cafeteria	reference	cafeteria	reference	cafeteria
mean chain length	17.0	17.0	17.5	17.5	17.4	17.3	17.6	17.1	17.2	17.4
mean double bonds	0.77	0.59	0.71	0.74	0.67	0.69	0.65	0.60	0.59	0.74
fatty acid composition in %:										
saturated	53.0	51.3	49.2	43.6	44.7	50.5	52.8	44.8	46.6	40.6
mono-unsaturated	25.3	41.6	32.2	43.0	47.4	37.3	37.6	52.1	49.3	48.1
polyunsaturated	21.7	7.1	15.6	13.4	7.9	12.2	9.6	3.1	4.1	11.3

The results presented have been calculated from the means of data of Figure 1.

Table 6

Ratios of polyunsaturated fatty acids present in the lipids of Zucker lean and obese rats fed reference and cafeteria diets, as well as in the diet they ingested

age	breed	diet	C20:4/C20:3 ratio	C20:3/C18:2 ratio
30 days	lean		0.93	0.20
30 days	obese		0.87	0.23
60 days	lean	reference	0.73	0.33
		cafeteria	0.37	0.36
60 days	obese	reference	0.55	0.29
		cafeteria	0.96	0.11
30–60 day ingested diet:				
		reference	0.75	0.05
	lean	cafeteria	0.84	0.08
	obese	cafeteria	0.84	0.08

The results presented have been calculated from the means of data of Figures 1 and 2.

Captions to Figures

FIGURE 1

Fatty acid content in the body of lean and obese Zucker rats fed reference or cafeteria diets from 30 to 60 days after birth.

Upper panels, lean Fa/? rats; lower panels, obese fa/fa rats; left side, reference diet; right side, cafeteria diet. Dotted line and circles: 30–day old rats; Dashed line and diamonds: 45–day old rats; Solid line and squares: 60–day old rats. Each symbol is the mean of 6 different animals. The scale for fatty acids is logarithmic.

Significance of the differences between groups (three–way ANOVA):

There is a statistically significant difference ($P<0.05$) for global effect of time, diet and breed. In addition, there are significant ($P<0.05$) effects of time, breed and diet for all fatty acids, with the sole exception of the non–significant ($P>0.05$) effect of diet on lean rats for C22, C22:1, C20:3 and C20:4.

FIGURE 2

Percent fatty acid composition of lean and obese Zucker rats fed reference or cafeteria diets from 30 to 60 days after birth.

Percentage of each fatty acid versus the total fatty acid content in the saponifiable lipids of the whole rat. Dotted line and open circles: 30–day old rats; Solid line and black circles: 60–day old rats fed reference diet; Dashed line and black diamonds: 60–day old rats fed the cafeteria diet. Left, lean Fa/? rats; right, obese fa/fa rats.

FIGURE 3

Differences between the amount of each fatty acid ingested with the diet and that accumulated in the tissues of lean and obese Zucker rats fed reference or cafeteria diets from 30 to 60 days after birth.

Upper panels, lean Fa/? rats; lower panels, obese fa/fa rats; left side, reference diet; right side, cafeteria diet. Each symbol is the mean of 6 different animals. Positive values represent net synthesis, and negative values net degradation.

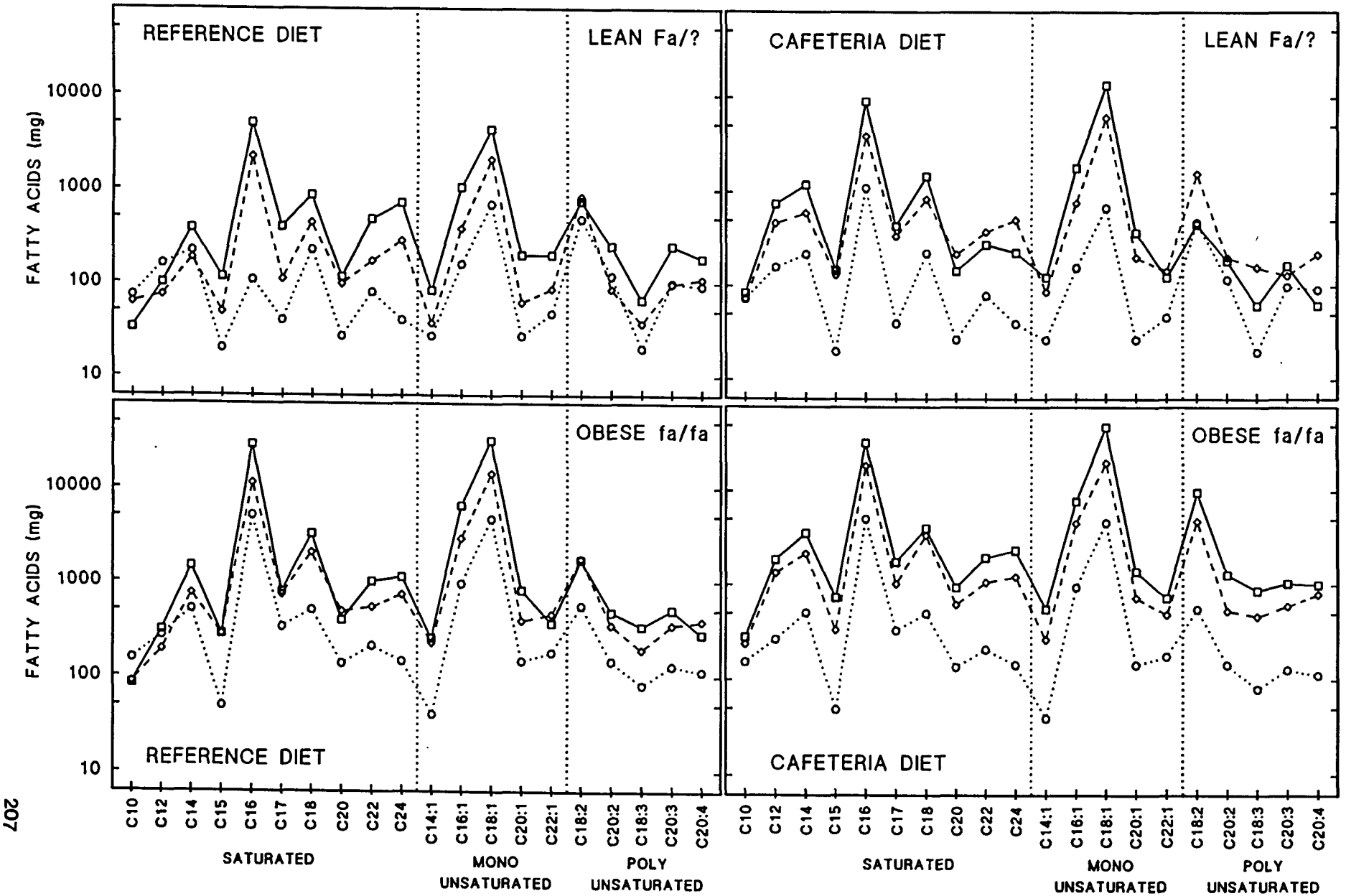
REFERENCE DIET
LEAN Fa/?
CAFETERIA DIET
LEAN Fa/?
OBESE fa/fa
OBESE fa/fa
REFERENCE DIET
CAFETERIA DIET
FATTY ACIDS (mg)
FATTY ACIDS (mg)
10000
1000
100
10
C10 C12 C14 C15 C16 C17 C18 C20 C22 C24 C14:1 C16:1 C18:1 C20:1 C22:1 C18:2 C20:2 C18:3 C20:3 C20:4
SATURATED
MONO UNSATURATED
POLY UNSATURATED

Figura 1

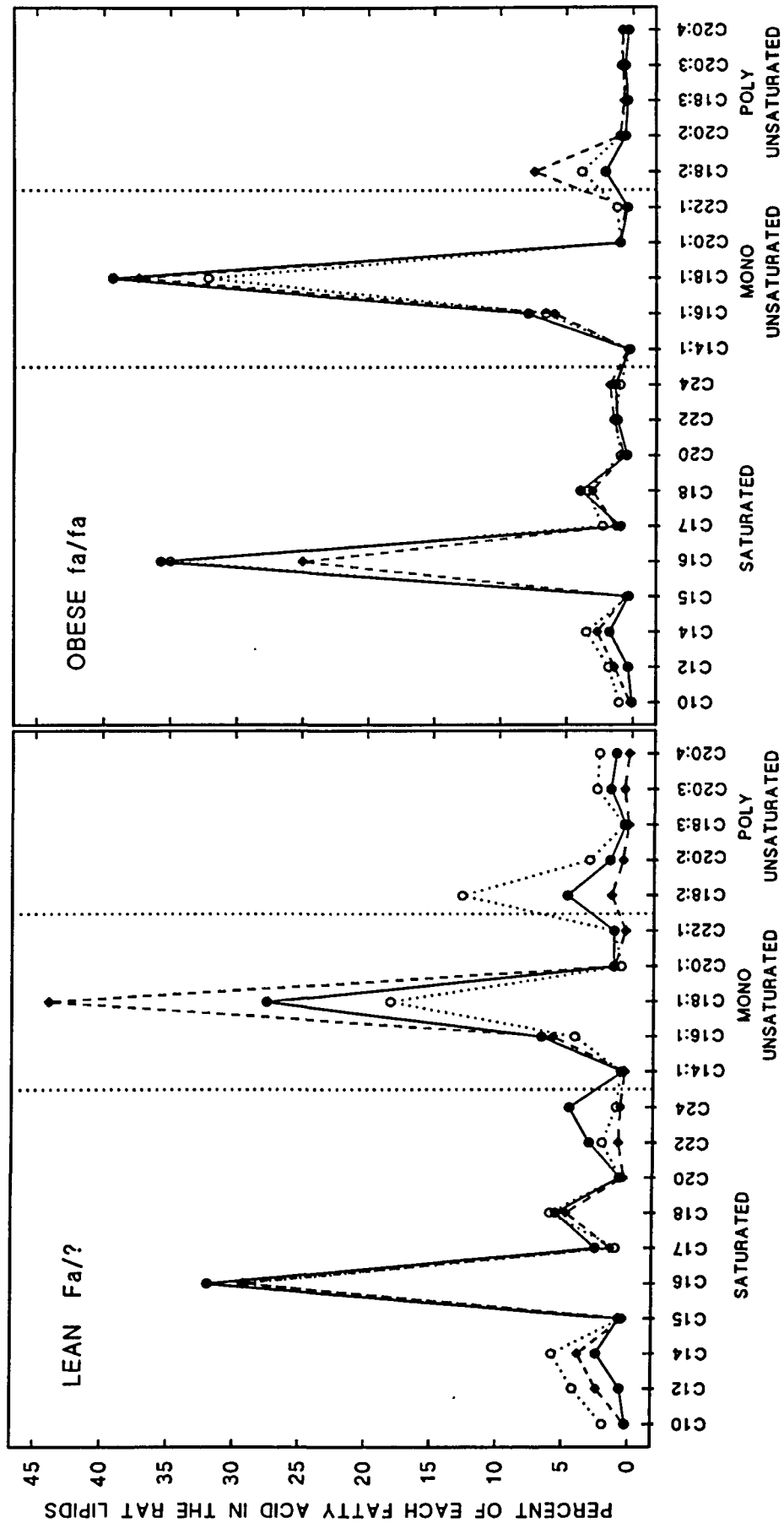
LEAN Fa/?
OBESE fa/fa
PERCENT OF EACH FATTY ACID IN THE RAT LIPIDS
0
5
10
15
20
25
30
35
40
45
C10
C12
C14
C15
C16
C17
C18
C20
C22
C24
C14:1
C16:1
C18:1
C20:1
C22:1
C18:2
C20:2
C18:3
C20:3
C20:4
SATURATED
MONO UNSATURATED
POLY UNSATURATED

Figura 2

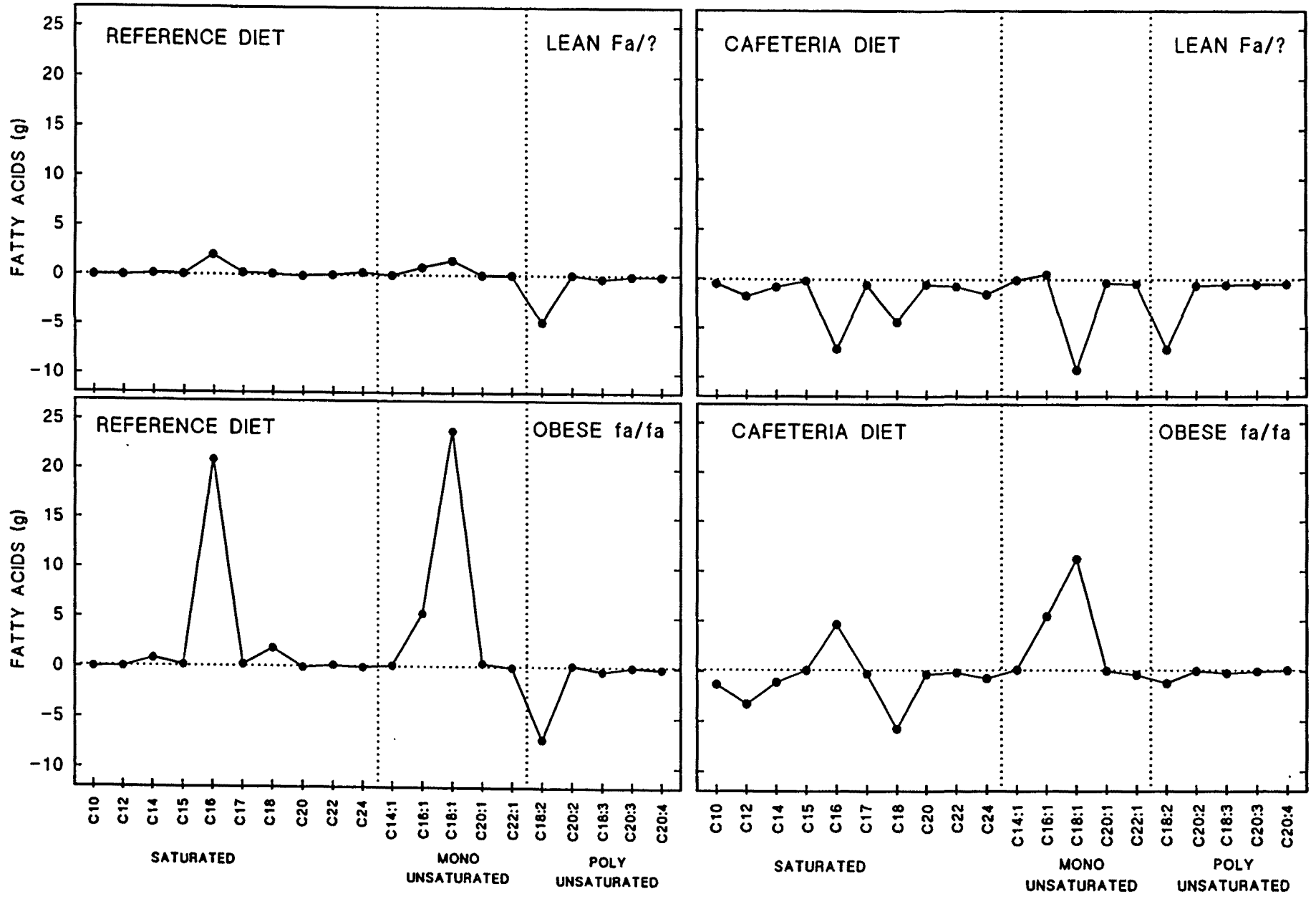
REFERENCE DIET
LEAN Fa/?
CAFETERIA DIET
LEAN Fa/?
REFERENCE DIET
OBESE fa/fa
CAFETERIA DIET
OBESE fa/fa
FATTY ACIDS (g)
25
20
15
10
5
0
-5
-10
C10
C12
C14
C15
C16
C17
C18
C20
C22
C24
C14:1
C16:1
C18:1
C20:1
C22:1
C18:2
C20:2
C18:3
C20:3
C20:4
SATURATED
MONO UNSATURATED
POLY UNSATURATED

Figura 3

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Lipid synthesis: a thermogenic mechanism in cold-exposed Zucker fa/fa rats

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SUMMARY

The oxygen consumption and carbon dioxide production of Wistar and Zucker lean (Fa/?) and obese (fa/fa) rats was measured at 4, 10, 20 and 30 °C. These data allowed the estimation of a net synthesis of lipid at the expense of carbohydrate in Wistar rats at 20 °C, with active lipid oxidation at 4 °C, and increasing heat production with lowering temperature. Zucker lean rats also showed this trend.

Zucker fa/fa rats synthesized lipid at 4, 10 and 20 °C, showing a less marked increase in heat production with lowering temperature. It is postulated that Zucker obese rats synthesize lipids as a way to obtain residual metabolic heat to maintain their body temperature. This is part of a process —fully functional in Wistar and Zucker lean rats, and truncated in Zucker obese rats— in which liver lipogenesis can combine with brown adipose tissue lipolysis to generate enough heat to maintain body functions under a cold environment.

INTRODUCTION

Exposure to cold induces the activation of thermogenic mechanisms in normal rodents (Rothwell & Stock, 1980). The main site of non-shivering cold-induced thermogenesis in rats is the brown adipose tissue (Smith & Horwitz, 1969), which envelope the core tissues and help maintain their temperature by means of very active oxidation of substrates (Nicholls, 1976). The mechanisms that control this brown adipose tissue thermogenesis have been elucidated (Nicholls, 1977) and consist of noradrenergic signal—induced higher tissue blood flows (Foster & Frydman, 1979), as well as the uncoupling of mitochondrial oxidative phosphorylation (Sundin & Cannon, 1980). The result is a powerful heating of the whole rat, allowing it to maintain its body temperature at the expense of a severe drainage of the available energy resources (Trayhurn & James, 1981); the rats react to this increase in demand and compensate for this loss by eating very large amounts of food (Hardeveld *et al.*, 1979). Brown adipose tissue can oxidize practically any substrate available under conditions of cold–exposure (Depocas & Masironi, 1960), but under cold acclimation the main substrates oxidized are lipids (Lafrance *et al.*, 1980).

Genetically obese Zucker fa/fa ("fatty") adult rats show a very poor response to cold exposure (Trayhurn *et al.*, 1976), they are

seldom able to withstand severe cold, and thus cannot adapt to low temperature environments (Levin *et al.*, 1980). Obese fa/fa rats often die in the cold because they cannot maintain their body temperature (Kraul *et al.*, 1985), since their thermogenic system is faulty or incomplete (Godbole *et al.*, 1978). The fa/fa rat brown adipose tissue is not able to oxidize the large amounts of substrates that other rats do under uncoupling conditions (Levin *et al.*, 1984).

Despite this inability to cope with the cold, there are several reports in the literature showing a certain ability of the fa/fa rats to manage under milder cold conditions (Refinetti, 1989). We have studied the effects of environmental temperature on the overall oxidative energy production, as well as on the type of substrate oxidized in three series of rats; Zucker fa/fa and Fa/?, as well as Wistar controls, in order to determine the different effect of cold in lean and obese animals in relation to type and amount of substrate used and heat produced.

MATERIALS AND METHODS

Female Zucker lean (Fa/?) and obese (fa/fa) rats, weighing, respectively, 190-220 and 250-300 g (from Charles River heterozygous parents) and female Wistar rats weighing 200-250 g (Charles River) were used. The animals were housed under standard conditions (21-22 °C, 70-80 % relative humidity, lights on from 08.00 to 20.00) in solid-bottomed polypropylene cages with wood shavings as bedding material. They were fed standard chow pellets (type A04 from Panlab, Barcelona; 17 % protein, 59 % metabolizable carbohydrate and 3 % lipid) and had free access to tap water.

The experimental setup consisted of four groups of six animals for each strain (Zucker lean and obese and Wistar). Gas exchange measurements were performed individually for every animal for two hours (within the 12.00 to 16.00 hour period). The rats were introduced into a hermetic 1200 ml all-glass chamber connected to a gas analysis system. This system contained two matched flowmeters for measurement of inflow and outflow from the chamber, two infra-red gas analysis systems (one for water and the other for carbon dioxide) as well as a paramagnetic oxygen monitor (all from Leeds & Northrup, UK), connected serially. The chamber contained a metal grid for comfortable support of the rat; this mesh separated the animal from a cellulose compress. The gas was provided from a cylinder of compressed air (N_2/O_2 mixture: 79% / 21%); the flow was adjusted to 21 $ml \cdot s^{-1}$. The chamber was kept in a thermostat controlled water bath. The inflowing air was circulated through a copper coil (also immersed in the water bath) before entering the chamber. The measurements were performed at 4, 10, 20 and 30 °C. Some experiments were carried out at 40 °C but were discontinued since some rats died shortly after they were maintained at these temperatures.

Analogic continuous recordings were computed for 5 series of minute-intervals. Carbon dioxide production, as well as oxygen consumption were calculated from the *in* and *out* air flows, temperatures and efflux gas composition (the influx air composition was known and fixed). Variations in outflow gas volume because of changing composition and temperature were corrected by assuming that the inflowing mass of nitrogen was identical to that leaving the chamber (Haldane, 1989).

The urinary nitrogen output of the rats kept at different temperatures was determined (in sets of three rats each) for 24 hours in standard plastic metabolic cages (Tecniplast, Gazzada, Italy). Data from the

Zucker rats measured at 10 °C were used also for the 4 °C group, since long exposure of fa/fa rats to a temperature of 4 °C often resulted in deep hypothermia and death (Closa *et al.*, 1992) The urine samples were used for the estimation of total nitrogen with a Carlo Erba NA-1500 elemental analyzer. The mean values of nitrogen excretion (for 24 hours) per unit time were used in standard equations linking the measured changes in O_2 and CO_2 with substrate consumption (McLean & Tobin, 1987) for the estimation of carbohydrate and lipid consumption/ synthesis at any given time. The energy output of the rats was likewise calculated from the standard biological heat of oxidation (McLean & Tobin, 1987) of the amounts of lipid, carbohydrate and protein oxidized per unit time. Statistical comparisons between means were performed using a standard ANOVA program (Dixon, 1987).

RESULTS

Figure 1 presents the rates of carbohydrate consumption per unit of time during the two hours studied on Wistar and Zucker lean and obese rats. IN Wistar rats, the maximal carbohydrate consumption was found at 10 °C, followed by 4 °C, a pattern similar to that found in Zucker elan rats. Zucker obese rats, however, showed highest carbohydrate oxidation rates at 4 °C, followed by 10 °, then 20 °C and, finally, 30 °C.

The rates of oxidation or synthesis of lipid are shown in Figure 2. The pattern for Wistar and Zucker lean rats is similar —as for carbohydrate—, with highest oxidation found in the 4 °C group, and lowest (actually there was net lipid synthesis, more marked in Wistar rats) for the 10 °C series. Again, Zucker obese rats presented a different pattern, with net lipid synthesis in all temperature series except for 30 °C. The maximal synthesis rates were observed for the 4 °C series, 20 °C and 10 °C following closely.

The energy generated in the net oxidation of substrates by the three experimental groups is presented in Figure 3. For Wistar and lean Zucker rats (their patterns were similar), the maximal heat output was at 4 °C, followed by 10 °C; the power output at 20 °C was lower and that at 30 °C lowest. In Zucker obese rats, however, the maximal heat output was observed at 4 °C and 10 °C, followed by 30 °C, and showing the lowest values for 20 °C.

Figure 4 shows the mean 2-hour values for each rat strain and temperature values of lipid and carbohydrate oxidized as well as their heat production. For easiest comparison, the rates of carbohydrate and lipid oxidation have been expressed in power units by using standard equivalence values (McLean & Tobin, 1987). Zucker lean, as well as Wistar rats increased their oxidative energy output with lowering ambient temperature, doubling heat production from 30 to 4 °C. Zucker obese rats showed very similar values for 20 and 30 °C and for 4 and 10 °C heat production, the latter being somewhat higher than those of 20 and 30 °C.

Wistar rats oxidized little lipid at 30 °C, but at 20 °C they oxidized lipid in a small proportion in relation to carbohydrate oxidized (comparable to that of lipid present in the diet). At 10 °C the Wistar rats actually synthesized a significant proportion of lipid, so that more than almost half the carbohydrate oxidized was used for the synthesis of lipid. At 4 °C the situation was reversed, since now lipid oxidation was high, coinciding with somewhat lower carbohydrate oxidation. There was a wide variation in lipid synthesis/oxidation and carbohydrate consumption for individual rats. In general, those showing higher carbohydrate oxidation rates also had the highest lipid synthesis. Zucker lean rats showed a similar pattern, but here,

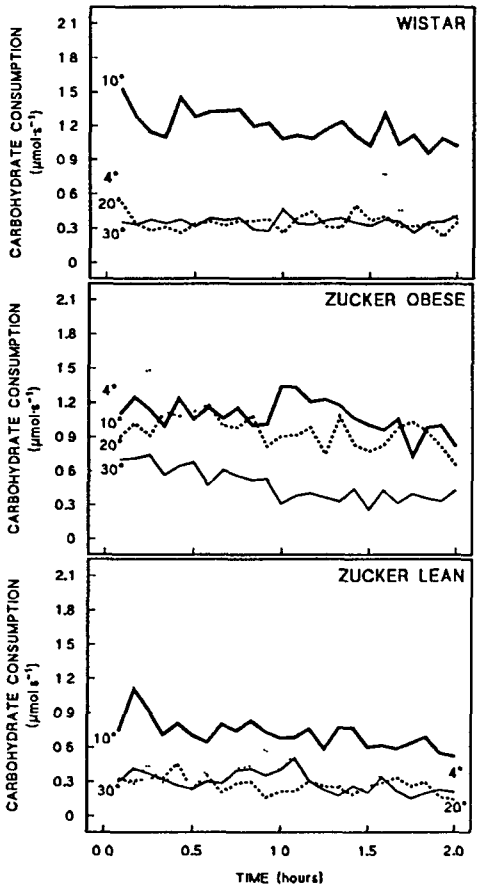

Figure 1

CARBOHYDRATE CONSUMPTION RATES IN WISTAR, ZUCKER OBESE AND ZUCKER LEAN RATS MAINTAINED AT DIFFERENT TEMPERATURES FOR TWO HOURS.

The data are the means of 5-6 different animals in each group.

Statistical analysis of the differences (2-way ANOVA):

parameter and group	df	F	P
temperature	3,49	16 75	0 0000
Wistar/temperature	3,49	14 52	0 0000
lean rats/temperature	3,49	14 00	0 0000
obese rats/temperature	3,49	9 77	0 0000
obesity in Zucker rats	1,49	43 24	0 0000
strain in lean rats	1,49	7 32	0 0094

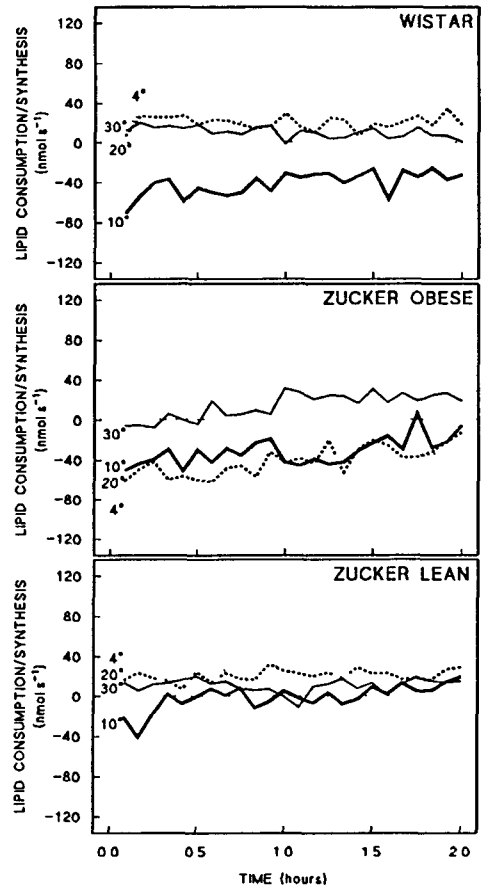

Figure 2

LIPID CONSUMPTION / SYNTHESIS RATES IN WISTAR, ZUCKER OBESE AND ZUCKER LEAN RATS MAINTAINED AT DIFFERENT TEMPERATURES FOR TWO HOURS.

The data are the means of 5-6 different animals in each group.

Statistical analysis of the differences (2-way ANOVA):

parameter and group	df	F	P
temperature	3,49	4 98	0 0043
Wistar/temperature	3,49	1 98	0 2093
lean rats/temperature	3,49	8 42	0 0001
obese rats/temperature	3,49	5 93	0 0016
obesity in Zucker rats	1,49	39 62	0 0000
strain in lean rats	1,49	2 09	0 1551

the net synthesis of lipid was lower, and thus the oxidation of carbohydrate at 4 °C was less marked that in Wistars. In addition, the energy derived from lipid at 4 °C was practically the same as that obtained form carbohydrate.

Zucker fa/fa rats behaved differently; at 30 °C there was a practically no

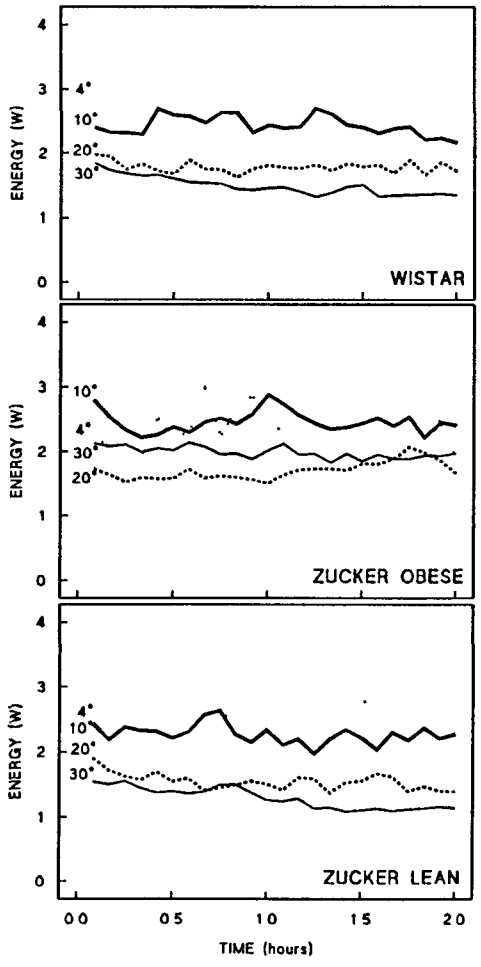

Figure 3

RATES OF CONVERSION OF NUTRIENT ENERGY CONVERTED INTO HEAT IN WISTAR, ZUCKER OBESE AND ZUCKER LEAN RATS MAINTAINED AT DIFFERENT TEMPERATURES FOR TWO HOURS.

The data are the means of 5-6 different animals in each group.

Statistical analysis of the differences (2-way ANOVA):

parameter and group·	df	F	P
temperature	3,49	64 83	0 0000
Wistar/temperature	3,49	53 19	0 0000
lean rats/temperature	3,49	58 75	0 0000
obese rats/temperature	3,49	1 56	0 2107
obesity in Zucker rats	1,49	6 28	0 0156
strain in lean rats	1,49	13 14	0 0007

synthesis of lipid at the expense of carbohydrate. With lowering temperatures there was a somewhat higher carbohydrate consumption and increased mean net lipid synthesis, maximal at 4 °C. The individual variation was also considerable, showing the same carbohydrate consumption/ lipid synthesis relationship observed for Wistar rats. The oxidative energy production data showed much less individual variation.

DISCUSSION

The application of standard available equations linking gasometric data and actual substrate oxidation give valuable and direct information on very general metabolic processes in otherwise intact animals (McLean & Tobin, 1987). The main drawback is the need for reliable nitrogen excretion (protein oxidation) data (Ferrannini, 1988).We have tried to circumvent this difficulty by incorporating the data from 24-hour nitrogen excretion rates. The problem could not be solved otherwise, since the rats do not emit urine at precisely timed intervals, and we had no other way to probe their protein oxidation rates for a given period.

The possible errors incurred by the assumption of uniform nitrogen excretion rates (Weir, 1949) could not, in effect, significantly change the outline of the results presented, since under the dietary conditions used, the RQ is practically unaffected by these values (Weir, 1949). In addition, the RQ for fa/fa rats was constantly and consistently higher than unity, implying large-scale synthesis. This could not be an experimental artifact, because of the accurate calibration of the system and the redundant correction of measurement errors; in addition, RQ and heat production data for standard Wistar rats at 20 °C were both as described (Rafecas *et al.*, 1989), and the consumption of carbohydrate and lipid bore relation to their proportions in the pellet diet. The fact remains, then, that fa/fa rats synthesized large

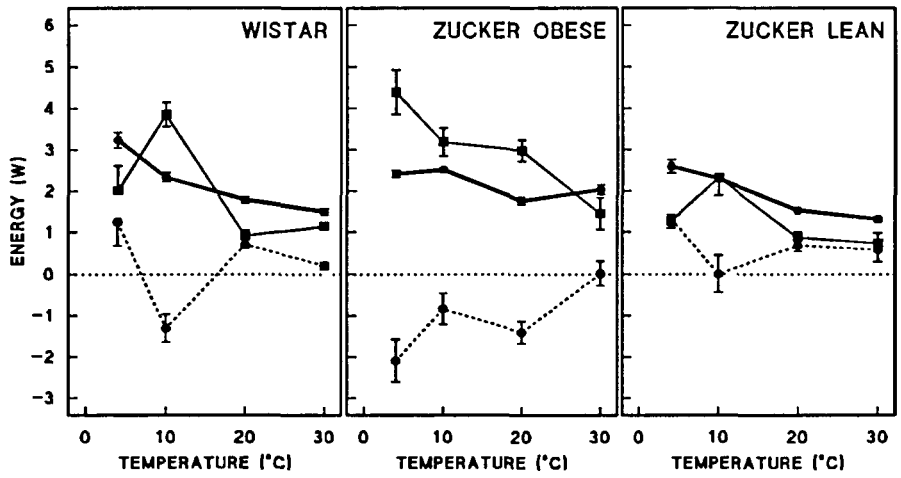

Figure 4

MEAN 2-HOUR HEAT PRODUCTION, AND CARBOHYDRATE AND LIPID OXIDATION RATES FOUND IN WISTAR AND ZUCKER RATS KEPT AT DIFFERENT TEMPERATURES.

The data are the means of the data presented in Figures 1, 2 and 3. Carbohydrate and lipid oxidation/synthesis are expressed in W for easier comparison with the overall heat production.

amounts of lipid, at room and lower temperatures, using inordinate amounts of carbohydrate to do so. This is fully in line with their known ability to synthesize and store fat (Zucker & Antoniades, 1972). However, there are no reports linking the exposure to the cold and increased fat synthesis at the expense of increased carbohydrate consumption.

The rat, upon exposure to the cold, actively mobilizes its fat reserves (Hannon & Larson, 1962) in order to cope with the additional burden of maintaining its core temperature (Brück & Zeisberger, 1987). Most studies on normal rat exposure to the cold have been carried out at 4 °C, and the active lipid oxidation, essentially through brown adipose tissue (Nedergaard *et al.*, 1980), has been duly noted (López-Soriano *et al.*, 1988). In close relation with this, several reports claim that the liver has a key role in thermogenesis in the rat (Ma *et al.*, 1987). Other studies ascribe a large portion of the overall heat output to the liver (Casado *et al.*, 1990) or show strong potential thermogenic ability for the liver because of its temperature, higher than that of the circulating blood (Closa *et al.*, 1992). The net lipid synthesis observed in Wistar rats kept at 10 °C suggests that tissues other than brown adipose tissue, e.g. the liver, can play a significant role in adaptive heat production under cold-exposure. At even lower temperatures, the net lipid oxidation found in Wistar rats agrees with the onset of the massive lipid oxidation process taking place mainly in brown adipose tissue (Nedergaard & Lindberg, 1982).

The possible significance of the lipid synthesis observed under mild cold exposure (Wistar) and at practically all temperatures in fa/fa rats must lie in the low net energy efficiency of lipid synthesis from carbohydrate (Reeds *et al.*, 1982). Fourteen glycosyl residues are needed to synthesize a tripalmitin molecule (McGilvery, 1979), i.e. 42.1 J of carbohydrate must be metabolized, to yield 31.6 J worth of stored fat, an efficiency of c. 75 %. This process takes place essentially in the liver (Trayhurn, 1979). The

efficiency of glycogen storage or lipid resynthesis is much higher. The remaining 25 % of the carbohydrate energy is lost as heat. Wistar —and to a lower extent Zucker lean— rats also seem to rely on this 'hepatic' heat production process to confront a mild cold exposure. It has the advantage that only a quarter of the energy is lost and a ready–store of fat is prepared in order to cope with —expected continued or more intense— cold through the thermogenic lipid oxidative pathway in brown adipose tissue. In fact, lipid synthesis can be a more successful adaptation to mild cold than pure lipid oxidation in the brown adipose tissue. In fact, both processes can work fully in conjunction at low temperatures, since the liver can synthesize fat from dietary carbohydrate and brown adipose tissue can further oxidize the fat. This has some additional advantages: the machinery of both tissues is geared more efficiently to a single metabolic purpose; brown fat uses mainly one fuel, fat; and glycemia is less affected, and the heat production sites are more dispersed for a more efficient heat distribution.

The fa/fa rats may be obese because they lack the second part of this heat producing scheme, since their brown adipose tissue is inoperative as a massive heat production site. Thus, their liver remains as a main —only limitedly adaptive— thermogenic center, and they must maintain their temperature with only residual metabolic heat (Himms-Hagen, 1989). Upon cold exposure they somehow adapt and produce more heat through lipogenesis, but they cannot extend this process further, because they are unable to generate heat by oxidizing inefficiently this fat, and thus could not withstand severe cold.

In short, the obese Zucker fa/fa rats may store fat because they obtain heat through the relatively inefficient process of lipogenesis from carbohydrate. Both Wistar and Zucker lean rats show adaptive metabolic response to cold in the form of increased lipogenesis at 10 °C, a trait that is present in Zucker obese rats at 20 °C, 10 °C and 4 °C.

ACKNOWLEDGEMENTS

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COMENTARI GENERAL: BLOC III

En aquest tercer i últim grup ens hem centrat en l'estudi de la utilització dels lípids des d'un punt de vista del balanç d'àcids grassos, i des del punt de vista d' utilització de substrats *in vivo*, realitzat mitjançant la calorimetria indirecta.

Generalitzant, les dades obtingudes indiquen que la composició en àcids grassos dels lípids corporals de la rata segueix un patró molt uniforme, independent de la soca, l'estat d'obesitat i fins i tot del tipus de dieta (control o de cafeteria), tenint uns efectes més quantitatius que qualitatius; amb una predominància clara dels àcids palmític i oleic, seguits del linoleic. Això s'aconsegueix per vies diferents en les dues dietes, de manera que mentre que en la dieta control s'observa una síntesi activa, processos d'elongació de la cadena i un desenvolupament de l'activitat Δ^9-desaturasa; en els animals sotmesos a la dieta de cafeteria és probable que existeixi certa inhibició de la lipogènesi pels lípids de la dieta, afavorint-se una incorporació més directa d'aquests, fet que implica una situació més semblant a la de l'alletament, amb un greix més similar —pel grau d'insaturació— al dels trenta dies, i que potencia el creixement i una eficiència més gran en l'aprofitament de la dieta. Per altra banda ambdues dietes semblen subministrar àcids grassos poli-insaturats essencials en excés, doncs els oxiden en una elevada proporció, indicant que la dieta de cafeteria no és deficitària tal i com en ambdós casos sovint es suggereix. Finalment, l'obesitat mostra una forta tendència a l'acumulació de greix, amb un síntesi generalitzada de la major part d'àcids grassos, malgrat que ja hi ha una ingesta molt gran de greixos. Aquesta marcada síntesi de lípids en les rates obeses també s'ha observat mitjançant la utilització de la calorimetria indirecta, ja que aquests animals presenten quocients respiratoris superiors a la unitat, tant a temperatura ambient com a temperatures baixes. Aquest fet contrasta amb la mobilització de lípids que es dona en les rates primes exposades a 4 °C, en les quals aquests lípids són oxidats principalment al TAM per poder mantenir així la seva temperatura corporal. Aquests mateixos animals sotmesos a temperatures menys extremes (10 °C), mostren una síntesi de lípids, que suggereix que altres teixits diferents al TAM, com per exemple el fetge, poden jugar un paper important en la producció de calor adaptativa sota condicions de fred. Aquest sembla ser el mecanisme en les rates Zucker fa/fa, doncs aquesta síntesi de lípids es dona amb més intensitat en l'exposició al fred, indicant que donat que no disposen d'un TAM funcional, aquest podria ser un mecanisme funcionalment efectiu de manteniment de la temperatura corporal. Aquest mecanisme es basaria en la relativa ineficiència de la via de síntesi de lípids a partir dels glúcids (sols s'aprofita com a greix un 75% de l'energia dels glúcids emprats). Però a més de funcionar a baixes temperatures en els animals obesos, també es dona a temperatura ambient, de manera que mantenen una ingesta elevada —a canvi de poder tenir un cert control sobre la seva producció de calor— fet que podria explicar per què són hiperfàgics i per què esdevenen obesos.

CONCLUSIONS

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1) L'administració de sacarosa produeix efectes variables sobre la TID i no provoca necessàriament un augment específic de pes o de lípids dipositats en les condicions estudiades.

2) Les rates obeses, tant les que presenten obesitat genètica com induïda per la dieta, tenen una eficiència de deposició energètica més alta que les rates control, però la combinació d'ambdues té efectes semblants a l'obesitat genètica sola, doncs aquests animals ja es troben en el límit de la seva capacitat de deposició. Malgrat això aquesta eficiència podria veure's matisada si es confirma la menor eficiència d'absorció intestinal que semblen tenir els animals obesos, tal com es suggereix pel volum de pèrdues d'aigua i femta.

3) Es confirma l'existència en la rata de ritmes supradians en la producció de calor, correlacionats, però no totalment coincidents, amb els canvis de la temperatura central.

4) En les rates genèticament obeses ha adquirit preeminència l'homeostasi tèrmica en front de l'energètica, sent la síntesi de lípids un mecanisme important de generació de calor per mantenir la temperatura corporal a baixes temperatures. Però aquest mecanisme també actuaria a temperatura ambient, fent que l'elevada ingesta necessària per mantenir-lo i l'acumulació del greix així format, que no pot ser oxidat al TAM puguin ser la causa directa de la seva l'obesitat. Aquestes dades apunten al fetge com a principal òrgan responsable de la generació de calor en condicions en les que el TAM no és funcional.

5) Les fòrmules emprades per realitzar els càlculs de calorimetria indirecta han tenir en compte la composició dels substrats ingerits i l'existència d'una part significativa del N provenint de les proteïnes oxidades que no s'excreta per l'orina. Les dades del balanç energètic, de calorimetria indirecta i de calorimetria directa tenint en compte l'aigua evapo-transpirada són plenament coincidents i es validen mútuament.

6) Els àcids grassos majoritaris dels lípids corporals dels animals estudiats són el palmític i l'oleic, amb un patró de composició molt uniforme i pràcticament inalterat per efecte de la dieta de cafeteria o de l'obesitat genètica. Si bé els animals sotmesos a la dieta control han de sintetitzar-ne *de novo*, a partir dels balanços globals hem vist que les rates alimentades amb dieta de cafeteria dipositen alguns àcids grassos de la dieta i oxiden la resta. Les rates obeses, però, mantenen una síntesi activa de greixos malgrat ser alimentades amb dieta de cafeteria.