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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

A Scientific Meeting was held at Conservatoire National des Arts et Métiers, Paris, France on 13–16 July 1994, at which the following papers were presented.

Feeding cholesterol blunts the proliferative effect of dietary guar gum. By M.M.A.K. KHATTAK and J.C. MATHERS. *Department of Biological and Nutritional Sciences, University of Newcastle upon Tyne, NE1 7RU*

Raised cell proliferation may be a risk factor for cancer (Preston-Martin *et al.* 1990). Consumption of soluble non-starch polysaccharides e.g. guar gum (GG) is associated with increased crypt cell proliferation (CCP) in animal models (Pell *et al.* 1992) but neither the mechanism for this response nor its long-term consequences are certain. The present study investigated the interaction between GG and cholesterol feeding on CCP in rats.

Four semi-purified diets containing no GG and no cholesterol (Basal), 10 g cholesterol (Ch), 100 g guar gum (GG) and 100 g guar gum plus 10 g cholesterol (GGCh)/kg diet respectively were fed to twenty male Wistar rats (five rats/diet) for 21 d). Two hours before killing, each animal was injected intraperitoneally with vincristine sulphate for the measurement of CCP (arrested cells/crypt per 2 h) by the crypt microdissection metaphase arrest technique (Goodlad & Wright, 1982) at 10 (A), 50 (B) and 90 (C)% of the length of the small intestine (SI), in the caecum and at 10 (A) and 90 (B)% of the length of the colon.

	Diets					Significance of the effect		
Variables	Basal	Ch	GG	GGCh	SEM	GG	Ch	GG X Ch
SI (A)	17.0	15.8	22.4	18.8	1.68	*	NS	NS
SI (B)	12.9	11.3	21.2	15.2	1.21	***	**	NS
SI (C)	10.5	13.1	19.4	16.8	1.84	**	NS	NS
Caecum	9.5	9.5	12.7	9.2	0.70	*	*	*
Colon (A)	7.6	7.8	12.3	9.0	1.02	*	NS	NS
Colon (B)	6.4	7.4	11.9	8.6	0.90	**	NS	*

NS, not significant, * $P<.05$, ** $P<.01$ and $P<0.001$.

As expected GG-feeding reduced caecal pH and doubled caecal short-chain fatty acid concentration (data not shown). CCP was highest in the proximal SI and declined to the distal colon. At all intestinal sites CCP was increased by GG. Addition of cholesterol to the basal diet had little effect on CCP but appeared to blunt the proliferative effect of GG. If raised CCP is a risk factor for colo-rectal cancer (Terpstra *et al.* 1987) the potential 'protective' effect of dietary cholesterol would bear further study.

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The role of glucose and pyruvate in the nutrition of preimplantation mouse embryos.

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Preimplantation mammalian development begins in the oviduct or the Fallopian tube at the fertilized one-cell stage (day 1) and culminates in the uterus with the formation of the blastocyst (about day 5) which implants after hatching from the zona pellucida. Two key morphological processes occur during this time; compaction, at the eight to sixteen-cell stage, whereby the individual cells or blastomeres flatten on each other, forming the morula; and fluid accumulation or cavitation, at the thirty-two-cell stage, to form the blastocyst, which consists of a fluid-filled cavity surrounded by an outer single layer of trophectoderm cells and an eccentrically placed group of inner-cell-mass cells. Mouse embryos of certain inbred strains are able to develop *in vitro* from the one-cell to the blastocyst stage in a modified Krebs Ringer bicarbonate solution containing pyruvate, lactate and glucose. Mouse embryos have an absolute requirement for pyruvate as an energy source for the first cleavage division, and consume this substrate preferentially to the morula stage (about 2-3 pmol/embryo per h), before uptake declines to low levels in the blastocyst. Glucose as sole energy substrate is unable to support development until the four to eight-cell stage but is consumed increasingly from the morula stage, rising from about 0.5 pmol/embryo per h to about 4 pmol/embryo per h by the blastocyst stage.

To investigate the importance of the switch in energy substrate preference from pyruvate to glucose, one- and two-cell mouse embryos from an inbred strain (CBA/Ca x C57BL/6) were cultured for 6 d in M16 medium containing 0.33 mM-pyruvate and 5 mM-D+L-lactate in the presence (M16+G) and absence (M16-G) of 1 mM-glucose. The rates of nutrient uptake were determined over this time, along with the percentage of embryos developing to the blastocyst and hatching blastocyst stages, as well as blastocyst cell numbers. Nutrient uptakes were determined non-invasively by incubating single embryos in 300-800 nl drops and measuring the depletion of nutrients using an ultramicrofluorescence technique.

Both one- and two-cell embryos cultured in M16+G developed to the blastocyst stage and exhibited the switch in energy substrate preference from pyruvate to glucose with development. However, one-cell embryos cultured continuously in M16-G only developed to the morula stage, before degenerating. In these embryos, pyruvate was readily consumed between days 1 and 3, before falling, from 2.77 pmol/embryo per h on day 3 to 0.045 pmol/embryo per h on day 5. Transfer of these embryos to M16+G on days 4 or 5 did not prevent them from degenerating. In contrast, embryos cultured from the two-cell stage in M16-G compensated for the lack of glucose by consuming increasing amounts of pyruvate; from 2.78 pmol/embryo per h on day 2, to 5.21 pmol/embryo per h on day 6. However, the absence of glucose between days 2 and 6 compromised development to the blastocyst stage (64 *v.* 96%), the hatching rate (4 *v.* 68%) and blastocyst cell numbers (51 (SEM 4) *v.* 110 (SEM 5)). When two-cell embryos cultured in M16-G were transfered to M16+G on days 4 or 5, glucose was readily consumed in preference to pyruvate.

In conclusion, mouse embryos from an inbred strain have an obligatory requirement for glucose before late on day 3, to develop. For optimal development to the blastocyst stage, exposure to glucose needs to be for a period of between 24 and 48 h before late on day 3. If the obligatory requirement for glucose is fulfilled, embryos are able to utilize pyruvate in the absence of glucose at the later stages of development. The importance of glucose before the morula stage may relate to the need to synthesize glycogen for later use, or glycoproteins involved in compaction.

The work was supported by the Medical Research Council.

Free and total carnitine concentrations in continuous ambulatory peritoneal dialysis patients. By D. P. J. KIRBY[1], D. CONSTANTIN-TEODOSIU[1], A. H. SHORT[1], R. P. BURDEN[2], A. G. MORGAN[2] and P. L. GRENHAFF[1], [1]Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham, NG7 2UH and [2]The Renal Unit, The City Hospital, Nottingham, NG5 1PB.

The most widely established role for carnitine is in the transport of long-chain fatty acids into the mitochondrial matrix. It has been shown that carnitine concentrations in plasma and muscle in renal failure patients undergoing haemodialysis are lower than in healthy controls (Böhmer *et al.* 1978). This finding is less conclusive in patients undergoing continuous ambulatory peritoneal dialysis (CAPD; Moorthy *et al.* 1983).

We investigated free and total (free + acyl) plasma carnitine concentrations in eight CAPD patients (treatment duration 29 (SEM 16) months) and eight age- and sex-matched healthy controls. Daily loss of carnitine was also quantified in the two groups, via analysis of urine and dialysis fluids. Carnitine concentrations were determined according to Cederblad *et al.* (1990).

The measurements of total plasma carnitine concentrations in CAPD patients and controls revealed no significant difference between the two groups (42.8 (SEM 1.6) *v.* 43.1 (SEM 2.3) μmol/l, respectively). The loss of total carnitine measured over a 24 h period also revealed no significant difference between the two groups (269.7 (SEM 30.0) *v.* 240.5 (SEM 33.0) μmol, respectively). However, the ratio of acylcarnitine (AC) to free carnitine (FC) in plasma and daily fluid losses (urine/dialysis fluid) was significantly different between the two groups (Table).

	Plasma (μmol/l)						Urine/Dialysis fluid (μmol/d)					
	AC		FC		AC/FC		AC		FC		AC/FC	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Controls	6.8	1.4	36.5	2.5	0.2	0.1	134.4	17.7	105.8	16.4	1.3	0.1
Patients	14.3**	1.6	28.5*	1.4	0.5**	0.1	93.7	15.9	175.8*	17.3	0.5***	0.1

Significantly different from controls using Student's unpaired *t*-test: *$P<0.05$, **$P<0.01$, ***$P<0.001$.

In conclusion, the present results demonstrate that plasma total carnitine concentration and the daily loss of total carnitine are the same when comparing CAPD patients with age- and sex-matched controls. However, the difference in the AC/FC ratio between CAPD patients and controls suggests that an alteration in acyl group metabolism is occurring. This could be attributable to either a functional impairment of ß-oxidation above the citric acid cycle in liver/skeletal muscle of CAPD patients, or to a differential loss of free carnitine/acylcarnitine across the peritoneal cavity.

This study was partly supported by the Wellcome Trust.

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Incorporation of fatty acids into macrophage lipids *in vitro*. By P. YAQOOB, E. A. NEWSHOLME and P. C. CALDER, *Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU*

Many macrophage functions, including secretion, phagocytosis and interaction with other cell types involve the plasma membrane; thus alteration of the fatty acid composition of macrophage phospholipids could potentially affect these functions. Furthermore, macrophages appear to be prone to accumulation of excess lipid leading to the formation of foam cells, which are implicated in the development of atherosclerotic plaques. Therefore it seems important to examine the uptake and fates of different fatty acids into both membrane lipids and storage lipids in macrophages.

Murine thioglycollate-elicited peritoneal macrophages were prepared as described elsewhere (Calder *et al.* 1990). They were cultured in Minimum Essential Medium supplemented with calf serum (50 ml/l) and 0.1 mM-[1-^{14}C]fatty acids; fatty acids used were palmitic, oleic, linoleic and arachidonic acids. After 64 h the cells were washed and the lipid extracted. Lipid classes were separated using thin-layer chromatography. Incorporation of fatty acids into each lipid class was determined by liquid scintillation counting.

Fatty acid	Incorporation nmol/mg protein		Incorporation (% of total)											
			TG		DG+MG		NEFA		PC		PE		PS+PI	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Palmitic	288	23	76.3	10.4	6.7	2.5	7.3	3.4	3.1	1.8	3.9	1.4	2.6	1.5
Oleic	352	40	76.1	7.7	7.3	2.4	8.0	2.6	2.5	0.7	3.3	0.8	2.1	0.9
Linoleic	291	8	79.4	3.7	6.2	1.9	5.8	1.4	3.9	1.6	4.1	0.6	2.6	0.9
Arachidonic	178	3	80.8	4.4	5.8	2.1	4.5	2.1	2.6	0.7	4.4	0.2	1.9	0.5

Data are means and standard errors of three separate cell preparations.
TG, triacylglycerol; DG, diacylglycerol; MG, monoacylglycerol; NEFA, non-esterified fatty acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PI, phosphatidylinositol.

The rate of incorporation of oleic acid into macrophages was the highest of the four fatty acids examined and that of arachidonic acid was the lowest. The rate of incorporation of palmitic and linoleic acids into macrophage lipids was similar. In contrast, lymphocytes incorporate linoleic and arachidonic acids at the highest rates (Calder *et al.* 1994). In macrophages, the major fate of each of the fatty acids is incorporation into intracellular triacylglycerol. This is also different from the situation in lymphocytes, where the major fate of each of the fatty acids is incorporation into the phospholipid fraction (Calder *et al.* 1994). This probably represents contrasting roles of the fatty acids in the two cell types; activated lymphocytes undergo complex biochemical and functional changes during blastic transformation, including *de novo* synthesis of membrane phospholipids, and remodelling of their fatty acid composition, whereas macrophages are terminally differentiated and therefore tend to store lipid when it is abundant.

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Mechanisms underlying induction of fatty acid binding protein genes by peroxisome proliferators along the small intestine. By *A. MALLORDY, P. BESNARD, H. POIRIER, I. NIOT and H. CARLIER . Laboratoire de Physiologie de la Nutrition, E.A. DRED 580, ENSBANA, Université de Bourgogne, 21000 Dijon, France.*

Fatty acid binding proteins (FABPc) are abundant cytosolic 14-15 kDa proteins that have a great affinity for saturated and unsaturated long chain fatty acids. Several drugs that affect lipid metabolism change the FABPc concentrations. Published reports (for a review, see Veerkamp et al. 1991) have established that peroxisome-proliferating hypolipidaemic drugs of the fibrate family are the strongest inducers of the FABPc production in the liver (L-FABPc). The transcriptional origin of this induction has been recently demonstrated in this organ (Besnard et al. 1993). Paradoxically, little is know about the relationship between these drugs and the 2 FABPc expressed in the gut, the L-FABPc and the intestinal FABPc (I-FABPc).

The effect of bezafibrate and clofibrate on I- and L-FABPc expression has been investigated along the small intestine in mice and rats force-fed for 7 d. They were found to increase highly both I-FABPc and L-FABPc mRNA levels in the duodenum. The size of the induction is similar in the duodenum and in the liver. However, the degree of this stimulation progressively decrease along the cephalo-caudal axis of the gut, more rapidly for I-FABPc than for L-FABPc. The efficiency of the intestinal absorption of these drugs may explain this phenomenon.

L-FABPc gene (*Fabpl*) is silent in the terminal ileum of mice. Nevertheless, a direct infusion of bezafibrate in to the ileum switches on the *Fabpl*.. Using this original model, the time course of induction of *Fabpl* by bezafibrate has been investigated. L-FABPc mRNA was first detected 4 h after fibrate infusion, reached a peak at 16 h then decreased at 24 h. This up-regulation was totally prevented by cycloheximide. A small rise in L-FABPc mRNA levels also occurred in this model when sunflower-seed oil or linoleic acid were infused. This strongly suggests that (1) *Fabpl* is transcriptionally induced by bezafibrate via *de novo* protein synthesis, (2) long chain fatty acids or their derivatives may activate the *Fabpl*, and (3) mouse terminal ileum provides a powerful, and useful tool for studying the regulation of *Fabpl* expression both *in vivo* and *in vitro*. Whether these effects are mediated by peroxisome proliferator activated receptor (PPAR) remains to be elucidated.

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Effect of animal or vegetable protein intakes (100, 200 or 300 g casein or soya-bean protein / kg) on membrane hepatocyte very-low-density lipoprotein (VLDL) receptors. By J. PROST[1], D. AIT-YAHIA[2], M.L. SUAREZ-REY[1], Z. ARRIBI[1] and J. BELLEVILLE[1], *[1]Unité de Nutrition Cellulaire et Métabolique. Université de Bourgogne, Dijon, France and [2] Institut des Sciences de la Nature, Université Es Sénia, Oran, Algérie*

The aim of the present study was to investigate whether protein source (animal or vegetable) and levels have an effect on hepatic VLDL receptors.

Twenty-four male Wistar rats weighing 80 (SD 5) g were divided into six groups and fed for 4 weeks on diets containing (g / kg) casein or soya-bean proteins 100, 200 or 300, soya-bean oil 50, cellulose 50, vitamins 20, minerals 40, (diets were completed to 1 kg with starch). [^{3}H] VLDL and total lipoproteins were prepared using the technique of Meghelli-Bouchenak *et al.* (1991). The hepatic membranes were obtained from 1 g liver: liver homogenates were divided and centrifuged in 10 mM-tris-HCl buffer (pH 7.50), 150 mM-NaCl, 1 mM-$CaCl_2$ (solution A). Proteins were measured in the pellet using the technique of Schacterle & Pollack (1973). For each assay, about 100 μg liver membrane proteins were incubated with [^{3}H] VLDL in a total volume of 150 μl solution A, together with 20 mg/ml bovine serum albumin, for 60 min at 0°. After incubation, portions were poured over a presoaked glass fibre filter (Whatman GF/C) under vacuum. The filters were rapidly washed with 25 ml incubation buffer, dried and counted (Ultimagold Packard). Saturation analysis was performed by membrane incubation with labelled [^{3}H] VLDL (25 μg protein / ml, specific radioactivity 500 Bq / μg protein) with or without unlabelled total lipoproteins (200 μg / ml). Specific binding was determined by subtracting non-specific binding ([^{3}H] VLDL + total lipoproteins) from total binding ([^{3}H]VLDL).

Rats fed on casein developed fewer hepatocyte membrane VLDL receptors than those fed on soya-bean proteins (15.98 (SD 2.25), 29.26 (SD 1.20), 13.26 (SD 2.20) μg protein-VLDL / mg hepatic membrane, for rats given 100, 200 and 300 g casein / kg, respectively, and 26.40 (SD 0.85), 63.94 (SD 10.28), 35.23 (SD 10.8) μg protein-VLDL / mg hepatic membrane protein, for rats given 100, 200 and 300 g soya-bean protein / kg, respectively). The use of 100 or 300 g casein or soya-bean protein / kg in the diet involved lower liver VLDL uptake.

These results indicate that soya-bean proteins enhanced more VLDL uptake by hepatocytes, i.e. VLDL receptor synthesis, than casein, which may partially explain the hypolipaemic effect of these vegetable proteins.

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Modifications in the lipolytic response to ß$_1$, ß$_2$ and ß$_3$-adrenergic agonists after the administration of a non-selective ß-agonist. By J. A. MARTINEZ[1], M. A. GARCIA-CALONGE[2], E. SIMON[2], A. S. DEL BARRIO[2] and M. P. PORTILLO[2], *[1]Physiology and Nutrition, University of Navarra, Pamplona and [2]Nutrition and Food Science, University of País Vasco, Vitoria, Spain*

Metabolic fuel selection is under neuroendocrine control. On the other hand, persistent stimulation of a variety of different receptors may result in decreased responsiveness (Etherton & Louveau, 1992). This process, named desensitization has been, widely reported for ß$_1$ and ß$_2$-adrenergic agonists (Portillo *et al.* 1993). However, information is scarce concerning the "atypical" ß$_3$-adrenoceptor, which is involved in thermogenic and lipolytic activities. Evaluation of desensitization in intact animals is of interest because data obtained from isolated adipocytes cannot be extrapolated to physiopathological conditions such as obesity, where long-term treatment could be needed (Revelli *et al.* 1992).

The aim of the present study was to assess *in vitro* the lipolytic response to ß$_1$ (Dobutamine), ß$_2$ (Salbutamol) and ß$_3$ (BRL 37344) adrenergic agonists after different *in vivo* treatment periods with a mixed ß-agonist (Metaproterenol), as well as to compare the desensitization processes for ß$_1$, ß$_2$ and ß$_3$ adrenoceptors, through lipolysis measurements.

Female Wistar rats weighing 220-225 g were subcutaneously injected with a daily single dose of Metaproterenol (2 mg/kg body-weight) or vehicle 12 h, 2 d or 10 d before the animals were killed. The *in vitro* lipolytic effect was assessed by determination of glycerol production after 90 min incubation (Langin *et al.* 1991) with 10^{-9} M to 10^{-5} M of the selected ß-adrenergic agonist.

Maximal *in vitro* lipolytic responses (U: µmol glycerol/100 mg lipids) for the assayed ß-adrenergic agonists and the lowest concentration with lipolytic activity (M) are shown in the Table.

			Metaproterenol *in vivo* treatments		
		Control	12 h	2 d	10 d
		(*n* 6)	(*n* 6)	(*n* 6)	(*n* 6)
ß-Agonist	Units	Mean SE	Mean SE	Mean SE	Mean SE
Dobutamine	U	2.18^a 0.07	1.78^b 0.17	1.90^b 0.11	1.80^b 0.10
	M	$5x10^{-7}$	$5x10^{-7}$	$5x10^{-7}$	10^{-6}
Salbutamol	U	1.78^a 0.09	1.34^b 0.14	1.17^b 0.10	1.06^b 0.16
	M	$5x10^{-7}$	$5x10^{-6}$	$5x10^{-6}$	$5x10^{-6}$
BRL 37344	U	2.16^a 0.12	1.91^a 0.11	1.97^a 0.10	2.28^a 0.12
	M	10^{-9}	10^{-9}	10^{-9}	10^{-9}

a,b Mean values in the same row not sharing a common letter are statistically different (p<0.05).

The decreases in Dobutamine and Salbutamol maximal *in vitro* lipolytic responses and the initial lipolytic concentrations after the *in vivo* treatments with the mixed ß-adrenergic agonist suggest a desensitization process, which was greater for ß$_2$ than for ß$_1$-adrenoceptors. By contrast, this situation was not observed for ß$_3$"atypical"-adrenoceptor since the maximal lipolytic response to BRL 37344 remained unchanged, which suggests that their ß$_3$-mediated lipolytic actions are specific and selective. Summing up, different regulatory patterns can be proposed for the three known adrenoceptors involved in the lipolytic adrenergic control, which may have implications for energy utilization.

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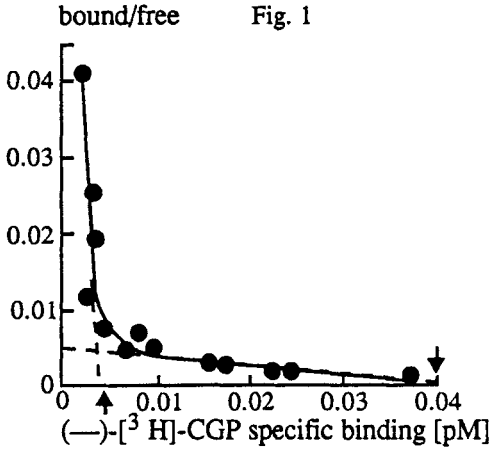
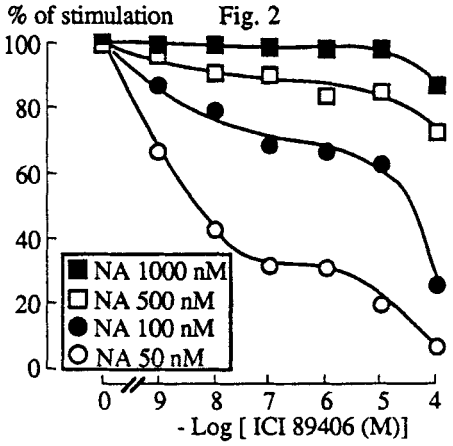
The physiological function of ß1- and ß3-adrenoceptors in rat brown adipocytes. By C. ATGIÉ, F. D'ALLAIRE, P.-M. SIMARD, P. MAURIÈGE, L. J. BUKOWIECKI. *Université Laval, Faculté de Médecine, Département de Physiologie, Québec, Canada, G1K 7P4.*

Molecular studies have revealed the presence of three subtypes of ß-adrenoceptors in rat brown adipose tissue, but their relative contributions in mediating the physiological effects of noradrenaline (NA) still remain to be defined. Scatchard analysis of specific [^{3}H]-CGP12177 binding to intact brown adipocytes revealed the coexistence of a major population of ß3-adrenoceptors (100 000 sites / cell), and a minor one of ß1-receptors (10 000 sites / cell) (Fig. 1). ß2-Adrenoceptors were undetectable.





The comparison of pD_2 values (an index of adipocyte sensitivity to agonists) with the corresponding pK_i values (dissociation constants of [^{3}H]-CGP12177 binding) revealed that the pD_2 values of all non selective agonists (isoproterenol, NA, adrenaline) were intermediate between the corresponding pK_i values for ß1- and ß3-adrenoceptors (see Table). This was not observed with the selective ß3-agonist CL 316 243. These results suggest that isoproterenol, NA and adrenaline stimulate lipolysis and respiration in brown adipocytes by acting via both ß1- and ß3- adrenoceptors.

	Binding (pK_i)		Lipolysis (pD2)	Respiration (pD2)
	ß1	ß3		
CL 316 243	5.4	7.8	8.3	8.9
Isoproterenol	10.3	4.5	7.9	8.3
Noradrenaline	9.2	4.2	7.1	7.7
Adrenaline	8.7	4.0	6.9	7.4

On the other hand, competitive inhibition experiments revealed that the lipolytic effect of low NA concentrations (<20 nM) were totally inhibited by a selective ß1-antagonist (ICI89406). However, ICI89406 only partially inhibited the effects of higher NA concentrations (≥100 nM) that are necessary to induce maximal lipolysis (Fig. 2). Similar observations were made when adipocyte respiration was measured. Thus, the conclusion that NA maximal lipolytic and respiratory effects are mediated via both ß1- and ß3-adrenoceptors is supported by competitive inhibition experiments as well as by the comparison of pD_2 with pK_i values. Under physiological conditions, plasma NA levels vary between 1 and 15 nM depending on the environmental temperature. At these concentrations, NA stimulates respiration in brown adipocytes mainly through ß1-adrenoceptors. ß3-Adrenoceptors may represent the physiological receptors for NA secreted from sympathetic nerve endings when NA concentrations in the synaptic cleft are very high and/or when the high affinity ß1-adrenoceptors are desensitized by prolonged adrenergic stimulation such as chronic cold exposure.

https://doi.org/10.1079/PNS19950058 Published online by Cambridge University Press

Alpha-2 adrenergic stimulation and resting energy expenditure in menopausal women before and during oestrogen therapy. By G. DEL RIO, R. MENOZZI, C. ZANDOMENEGHI, M.G. VENNERI, G. ZIZZO and A. VELARDO. *Department of Internal Medicine, University of Modena, Modena, Italy*

It is well known that α-2 adrenoceptor agonists are able to decrease resting energy expenditure (REE) and oxygen consumption in animals and humans. These effects may be mediated through a decrease of the sympathetic nervous system activity and/or other mechanisms that remain to be well defined. The catecholamine responses to α-2 adrenoceptor stimulation seem to be influenced by sex and a regulatory role of oestrogens on α-2 adrenoceptor number and activity has been reported. Menopause is associated with metabolic changes which would be expected to contribute to an increase in coronary heart disease in postmenopausal women. Weight gain is a common finding during menopause but it is not clear if this phenomenon is secondary to a reduction in oestrogen levels or is linked to age-related changes in REE that have also been described. Five menopausal women were recruited for a study where the cardiovascular and catecholamine responses to an α-2 adrenergic agonist like clonidine (CL:300 μg by mouth) were evaluated together with the variations in REE before and at the end of 4 months therapy with a patch containing 100 μg of oestradiol. Blood samples were drawn at -30, -15, 0, 120, 130 and 140 min after clonidine administration for determination of catecholamines (Noradrenaline and Adrenaline) by HPLC. Systolic, diastolic blood pressure (SBP, DBP), heart rate (HR) and REE were also measured at the same times. The results are shown in the Table.

	BEFORE OESTROGENS				AFTER OESTROGENS			
	Before CL		*During CL*		*Before CL*		*During CL*	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Weight (Kg)	66.9	6.0			67.2	6.7		
FFM (Kg)	42.6	3.4			42.9	3.5		
REE (MJ/24h)	5.51	0.22	4.48 *	0.48	6.31 §	0.51	5.27 *	0.61
Noradrenaline(pg/ml)	272	48	98 *	30	211 §	17	88 *	23
Adrenaline (pg/ml)	40	13	40	18	47	19	40	17
SBP (mmHg)	114	3	83 *	3	109	2	90 *	2
DBP (mmHg)	75	2	54 *	2	69 §	1	59 *	2
HR (beats/min)	62	2	56 *	2	63	3	57 *	3

* Significantly different from basal, $P<0.05$.

§ Significantly different from before oestrogens, $P<0.05$.

Oestrogen therapy increased REE and decreased plasma Noradrenaline and blood arterial pressure. The cardiovascular and catecholamine responses to CL were not however modified by oestrogen therapy. CL decreased REE both before and after therapy. These results suggest that alterations in the α-2 adrenergic tone do not contribute to the elevated plasma Noradrenaline during menopause and imply that the sympathetic nervous system secretion is not involved in the decrease of REE observed during lack of oestrogens.

Evidence of an association between blunted postprandial thermogenesis and insulin resistance. By C. MACOR, E. RELLA, S. FAVRO, R. VETTOR and G. FEDERSPIL
Institute of Semeiotica Medica, Patologia Medica III, University of Padua, Padua, Italy

Most studies carried out in man indicate that the increase of relative body fat is accompanied by decreased rates of postprandial thermogenesis, but whether blunted thermogenesis may be a consequence or a cause of the obesity remains still unclear. A major factor, which seems to influence the magnitude of diet-induced thermogenesis in obese individuals, is insulin resistance, but the mechanism by which insulin resistance may reduce the thermic effect of nutrients is not unknown. This study will focus on some aspects of the relationship between blunted glucose-induced thermogenesis and insulin-resistance. We investigated glucose-induced thermogenesis in different diseases showing an insulin resistant state, which is induced by different mechanisms, such as in acromegaly, in liver cirrhosis and in myotonic dystrophy of Steinert. In particular Steinert's disease is characterized by fewer type 1 fibres and by insulin resistance with postprandial hyperinsulinemia; our interest in this disease derives from recent researches suggesting that muscle morphology and the type of muscular fibres constitute an important factor in the aetiology of obesity. Eleven obese subjects, eight acromegalic, seven cirrhotic patients and nine subjects affected by Steinert's myotonic dystrophy were submitted to an oral glucose load. Blood glucose levels and insulin response after the load were determined. Before the test each subject was submitted to the measurement of body composition by bioelectrical impedance analysis to evaluate fat-free mass. Resting metabolic rate (RMR) and glucose-induced thermogenesis (GIT) were determined in each subject by indirect calorimetry using a canopy before and during 3 h of the test. The results were compared with those of a group of seventeen control subjects. We observed a reduced GIT in the obese, acromegalic, cirrhotic and in the myopathic patients in comparison with the response of thermogenesis to glucose in the control subjects (Table). All the groups presented hyperinsulinaemia after oral glucose load, even if the glucose profile demonstrated a normal glucose tolerance, suggesting the presence of an insulin-resistant state.

	CONTROL (*n* 17)		OBESE (*n* 11)		ACROMEGALIC (*n* 8)		CIRRHOTIC (*n* 7)		STEINERT (*n* 9)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
GIT	27.9	2.2	15.2**	2.7	5.5*	4.05	17.2*	5.4	14.8*	2.8

Statistical analysis by Student's *t* test (unpaired data): * $p < 0.001$, ** $p < 0.005$

In conclusion, our findings show that the reduced thermogenic response found in obesity can also be seen in many other pathological conditions in which there is an insulin-resistant state. Moreover, blunted postprandial increase of energy expenditure is also found in Steinert's disease where a similar alteration in skeletal muscle fibres, as in obesity, is present. Therefore these results show that reduced thermogenesis is linked to insulin resistance and they suggest that muscle fibres composition may play a major role in determining a reduced postprandial thermogenesis in obesity.

Insulin resistance in human pregnancy: a comparison of *in vivo* and *in vitro* measurement techniques.

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Physiological changes in normal human pregnancy result in a reduction of insulin sensitivity in the second half of gestation. Such changes have a profound effect on all classes of substrate and are therefore relevant to maternal and fetal metabolism. Our studies in human adipocytes revealed no change in insulin-receptor binding characteristics nor any post-receptor defect. We speculated that the change was likely to be caused by circulating insulin antagonists (Bruce *et al.* 1992).

Our *in vivo* experiments with hyperinsulinaemic euglycaemic clamps have been reported at the Nutrition Society previously (Stanley *et al.* 1993). Clamp techniques are of great value but are demanding of time and patients on the part of the research subjects. We have therefore explored the use of an *in vitro* technique with a mouse myoblast C2C 12 cell line which readily forms differentiated myofibres in culture which have many of the biochemical characteristics of striated muscle. These include a capacity to respond to growth in insulin-containing medium by increasing glucose uptake. Reductions in insulin sensitivity recorded with the two techniques and different groups of subjects are compared. Using clamp the higher the glucose infusion rate the higher is insulin sensitivity. The *in vitro* cell culture assays of 2-Deoxy glucose (2DG) uptake show that sera from pregnant women decreases the insulin responsiveness of the myofibres, with sera from late gestation having a greater effect than that from early gestation.

	CLAMP		CELL CULTURE				
	Glucose infusion (mg/ml per min)	Infusion rate (% non-pregnant value)	No insulin 2DG uptake (nmol/min per mg protein)		10^{-7}M-insulin 2DG uptake (nmol/min per mg protein		Increase in 2DG uptake due to insulin (% non-pregnant increase)
			Mean	SD	Mean	SD	
Non-pregnant women	283 *n* 5	100	20.5 *n* 8	4.0	31.3	6.9	100
Early Gestation	237 *n* 9	84	21.9 *n* 10	3.7	27.0	3.9	47
Late Gestation	139 *n* 7	49	29.7 *n* 6	7.1	32.6	2.8	27

The *in vitro* technique appears to be sufficiently sensitive to explore the implications of physiological and pathological changes in insulin sensitivity in human pregnancy.

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Assessment of physical fitness by submaximal exercise. By M. COX, N. R. GIBSON, M. KHALOUHA, K. MILLS and P.J. PACY, *Nutrition Research Unit, 4 St Pancras Way, London NW1 2PE*

Physical fitness, a negative coronary heart disease risk factor, is traditionally characterized by maximum oxygen consumption (VO_2 max) which is conventionally measured by indirect calorimetry using a mouthpiece. In athletes, familiar with this invasive procedure and working at or close to VO_2 max, this measurement poses few problems. However this technique is relatively limited for many sedentary individuals in the general population. The present study explored the use of a submaximal exercise test, designed not to raise the heart rate beyond 140 beats/min, to determine fitness levels over the widest population possible. Exercise consisted of progressively increasing (1 step/min) the stepping rate from 10 to 25 per min. The step height was 0.23 m although 0.12 m was used in functionally compromised individuals (obese). Oxygen consumption was measured continuously by ventilated-hood indirect calorimetry with a flow rate of 300 l/min and heart rate with a Sport tester PE3000. A predicted VO_2 max was calculated by extrapolating the regression line of O_2 against heart rate to the subject's population maximum heart rate, calculated from 220 minus age.

Three healthy adults (2M, 1F, age 35 (SD 12) years, weight 62 (SD 11) kg, body mass index (BMI) 22 (SD 2) kg/m^2) underwent repeated submaximal exercise tests over a 1 to 2-month period to assess reproducibility, during which time their weights and activities of daily living remained constant. The coefficient of variation in each individual was 6% (*n* 18, mean predicted VO_2 max 41 ml O_2 / kg per min), 5% (*n* 10, mean predicted VO_2 max 44 ml O_2 / kg per min) and 6% (*n* 13, mean predicted VO_2 max 29 ml O_2 / kg per min). We have subsequently used this protocol to determine fitness in several diverse groups of adults whose physical characteristics and level of fitness are tabulated below.

	Age (years)		Wt (kg)		BMI (kg/m^2)		predicted VO_2 max (ml / kg per min)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Females								
Sedentary (*n* 8)	29	6	59	8	22	2	28$^+$	4
Elite athletes (*n* 5)	25	2	63	3	21	1	47**	6
Obese (*n* 5)	30	4	114	14	40	4	20	3
Males								
Sedentary (*n* 6)	30	7	72	8	22	2	36	3
Elite athletes (*n* 5)	23	6	85	9	24	1	47**	6

** Significantly different from sedentary and obese, $P < 0.01$ (unpaired t test from sedentary & obese)

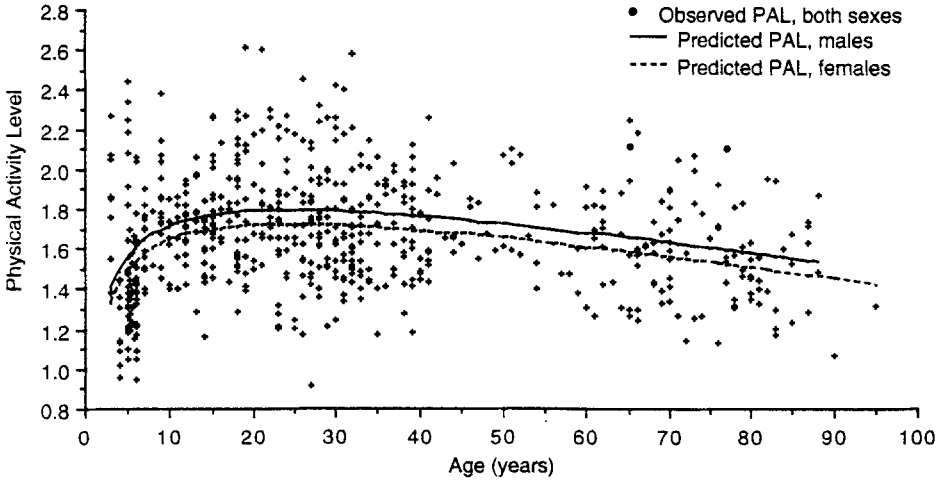
+ Significantly different from obese, $P < 0.05$ (unpaired t test)

https://doi.org/10.1079/PNS19950058 Published online by Cambridge University Press

Physical Activity Level in free-living persons measured by doubly-labelled water. By A.E.BLACK and T.J.COLE, *Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH.*

The total energy expenditure:basal metabolic rate (TEE:BMR) ratio, known as Physical Activity Level (PAL), is used as an expression of activity and energy requirements that controls for age and sex. Current recommendations for energy intake (FAO/WHO/UNU, 1985) are theoretical factorial estimates of PAL. Data from studies measuring TEE by doubly-labelled water (DLW) enable a comparison of real TEE and factorial estimates. Here meta-analysis of DLW studies has been used to derive PAL for different age-sex groups.

Individual data on age, sex, height, weight, observed sleeping metabolic rate (SMR), BMR or resting metabolic rate (RMR) and TEE by DLW were obtained (from papers or provided by authors) from thirty-three studies with 593 free-living persons aged 2 to 95 years. Excluded were infants, pregnant/lactating women, clinical patients, Third World studies, soldiers, athletes, and subjects with constrained or imposed activity. From studies with repeated DLW measurements, only the first, control, baseline or non-pregnant, non-lactating value was retained. BMR was taken as SMR x 1.05 or BMR or RMR as reported. Information on occupation or activity was scanty. Subjects were typically volunteers obtained by advertising among colleagues or in local media, mainly students, professional/white-collar workers or housewives. Three subjects only were identified as manual workers; seven elderly subjects were identified as non-ambulant. Sporting activity was indicated in some studies. Multiple regression analysis was used to predict PAL by age and sex. Weight and height had no significant effect on the regression.



The database reflects the world literature of DLW measurements. Subjects are highly selected and limited between ages 40-60 years and in manual occupations. Results for ages <7 years may be low due to inability to obtain basal conditions for BMR. Results at >60 years are dependent on the proportion of healthy active to sedentary infirm in the sample. The maximum predicted PALs (at age 19-31 years) were 1.79 in men and 1.72 for women. These are at or above the WHO recommendations of 1.78 and 1.64 for "moderate activity". This supports the WHO figure for men and suggests that women may be more active than assumed in factorial estimates.

Thanks are due to many colleagues around the world who generously made available unpublished data.

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^2H and ^{18}O natural abundance variations and their consequences in the doubly-labelled water measurement of energy expenditure. By P.RITZ[1,2], T.J. COLE[1] and W.A. COWARD[1], *[1]Dunn Nutrition Unit, Cambridge CB4 1XJ, and [2]Laboratoire de Nutrition Humaine, Clermont-Ferrand, France*

In current methods to estimate the internal precision of the doubly labelled water (DLW) method, the contribution of the uncertainty with which the natural abundance (NA) of ^2H and ^{18}O in body water is known, is ignored (Schoeller & Taylor, 1987; Cole & Coward, 1992). This would be acceptable if it could be demonstrated that within individuals ^2H and ^{18}O NA are covariant and have a known fixed slope. In these circumstances errors arising as a consequence of NA variation would cancel if the amounts of each isotope given were tailored to the slope of the covariance, the isotope turnover rates and the duration of the study (Schoeller, 1983; Coward & Cole, 1991). It is customary to assume that the slope of natural covariance is the same as that of the Meteoric Water Line i.e. $\partial_D = 8\partial_O + 10$ (Dansgaard, 1964). A relationship similar to this appears to exist across populations consuming differing water supplies and thus might be expected to occur when an individual moves from one location to another, but there is no evidence to indicate that this relationship applies for individuals in a single habitat. Modelling the system indicates that energy expenditure, respiratory quotient, and water intake will all affect the ^2H upon ^{18}O relationship. Even for subjects changing locations it is not correct to expect a slope the same as that of the Meteoric Water Line because the NA of atmospheric oxygen (about 20 % of total oxygen intake in man) is constant and does not vary with location in the same way that the NA of water does.

For the purposes of the present study 757 NA urine samples were collected from thirty-six adults and within-subject (within day and between days) and between-subject variability in ^2H and ^{18}O NA assessed. The Table shows the results.

All ∂^2 values relative to V-SMOW		Variance ^2H	Variance ^{18}O	Correlation	Slope
Within subjects	Within days	3.34	0.076	NS	
	Between days	2.08	0.051	NS	
Between subjects		10.8	0.343	$p<0.001$	5.6 (SE 0.37)

V-SMOW is Vienna-Standard Mean Ocean Water. NS is Not Significant.

This information shows that there is a need to provide a system for assessing the significance of NA variance in DLW studies to complement the error calculations of Cole & Coward (1992). The system involves for each isotope: normalization of enrichment for the dose, linear regression of the logged-enrichments and of their reciprocals with time, and knowledge of the NA variation in ^{18}O and ^2H, and of their correlation. This method was used in two DLW studies. In study 1, a single pre-dose sample only had been collected and so it was assumed that background variances and correlations were the same as that described above. In study 2 background variance was estimated from the analysis of six to eight baseline samples collected daily before the start of the experiments. In these studies errors arising from NA variations expressed as CV were 3.8 (SD 0.8) and 3.2 (SD 1.2) for studies 1 and 2 respectively.

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The measurement of energy expenditure in young children by the minute-by-minute heart-rate method: a validation study. By J. A. PAYNE[1], A. C. PAYNE[2], N. R. BELTON[1], M. M. ROLLO[3], and S. DOWNIE[3], [1]*Department of Child Life and Health, University of Edinburgh, Edinburgh EH9 1UW,* [2]*Department of Physiotherapy, Queen Margaret College, Edinburgh EH12 8TS and* [3]*Department of Anatomy and Physiology, Dundee University, Dundee DD1 4HN*

The minute-by-minute heart-rate (HR) method of measuring energy expenditure is gaining popularity as a means of investigating energy balance as, in comparison with the doubly-labelled water (DLW) method, it is inexpensive, less complicated and provides an assessment of patterns of habitual physical activity. Using the HR method, energy expenditure is estimated from the relationship between heart-rate (fHR), recorded on a minute-by-minute basis during the waking hours of the day and oxygen uptake (VO_2). The relationship is largely linear and unique to the individual. Calibration involves simultaneous determination of fHR and VO_2 at rest, (lying, sitting and standing) to obtain resting metabolic rate (RMR) and in three active phases. With a cohort of thirty-five children aged 5-9 years (seventeen boys, eighteen girls), the present paper validates two adaptations to the HR methodology of Livingstone *et al.* (1992) to provide a suitable and safe means of determining energy expenditure in young children. Rather than using a treadmill, a modified bicycle ergometer was used to exercise the children. Instead of measuring VO_2 with a gas analyser connected to a mouthpiece and noseclip, a ventilated-hood method was employed (Deltatrac Metabolic Monitor; Datex) with a hood specially designed for use in sitting and standing positions. Minute-by-minute fHR was measured during calibration and during the waking hours of the day (usually for 3 or 4 d, *n* 29) using Polar Sports Testers (PE4000; Polar Electro, Finland). A critical heart rate (CHR) differentiating periods of rest from activity was defined as the mid-point between the mean fHR standing and mean fHR at the lowest level of activity. Energy expenditure below CHR was determined from the average resting energy expenditure and above CHR from the regression of fHR with energy expenditure (from VO_2) during activity. Overnight energy expenditure was estimated from standard equations for basal metabolic rate (Schofield, 1985).

The HR estimate of total daily energy expenditure (EHR) was compared with energy intake (EI) assessed by the 7-d weighed intake method (Payne & Belton, 1992), using Tefal Microtouch Electronic Scales weighing in 1 g units to an accuracy of +/-1%, and analysed using COMP-EAT 4 (Nutrition Systems, London).

A highly significant relationship was found between EHR and EI (r 0.81; $P < 0.0001$, n 35).

	n	Mean	SD	Variance	Min.	Max.	Coeff. of variation (%)
EHR (MJ/d)	35	7.36	1.53	2.34	5.13	10.98	21
EI (MJ/d)	35	6.88	1.32	1.74	4.6	11.19	19
EDLW (MJ/d)	8	9.15	1.77	3.14	4.99	10.66	19
EHR (MJ/d)	8	8.83	1.68	2.82	6.62	10.98	19
EI (MJ/d)	8	7.95	1.25	1.53	6.79	10.48	16

Energy expenditure was also estimated by the DLW method (EDLW; Schoeller & van Santen, 1982) in a subgroup of eight children aged 7-8 years, for comparison with EHR and EI. A paired t test to compare EDLW with EHR found no significant difference between the two sets of data (t value 0.47; P 0.651). These results suggest that the adapted HR method can give a close estimate of the energy expenditure of young children.

Supported by the Scottish Home and Health Department.

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Components of total energy expenditure during refeeding in the rat. By P.C.EVEN, A.PELE and S.NICOLAIDIS, *Laboratoire de Neurobiologie des Régulations, CNRS URA 1860, Collège de France, Paris.*

We have shown that in mildly food-restricted rats (70% of spontaneous food intake) during 10-20 d, until body-weight gain is again close to that of *ad libitum*-fed controls, the decrease in total energy expenditure (TEE) is primarily due to "passive" phenomena such as the reduced thermogenesis due to the reduced feeding, the reduced spontaneous activity (related to underfeeding) and the loss of metabolically active tissues. As for the "active" economy, only a small reduction in basal energy expenditure per kg body-weight was found. On the other hand, Dulloo & Girardier (1990), using alimentary balances and assessments of body composition, demonstrated that during controlled refeeding (pair-feeding on age-matched control), rats that previously endured food restriction exhibited a 7 to 8% adaptative decrease in TEE. The purpose of the present study was to verify that this adaptative economy could be measured by the method of indirect calorimetry, and if so, whether it could be assigned to a particular compartment of TEE.

We observed that at the fifth day of refeeding after food restriction, TEE of the restricted-refed (RR) rats was reduced by 15.5% in comparison with age-matched controls, but was comparable to that of weight-matched controls. Spontaneous activity quantitatively measured with piezo-electric-force transducers was slightly reduced, primarily because food intake occurred in one or two large meals instead of eight to ten in control rats. Despite this difference in meal pattern, the thermic effect of food was not changed in the RR rats. Basal energy expenditure corrected for body-weight showed a significant decrease in comparison with age-matched but not with weight-matched controls. Further fitting of the relationship linking basal energy expenditure with body-weight in control rats was performed by weighing each of the main organs of the animal. Using only muscles and heart weights (selected from stepwise regression analysis) allowed us to predict for 98.9% of the weight-related changes of basal energy expenditure in the control rats. Comparison of the basal energy expenditure of the RR rats using the heart/muscles-weights relationship to basal energy expenditure demonstrated that RR rats had a 9% or 10.7% reduction of their basal metabolic rate compared with that of either weight-matched or age-matched controls respectively.

As a result, the adaptative reduction in basal energy expenditure of RR rats saves about 17 kJ or 7% of TEE. Behavioural adaptations in the form of hypoactivity and reduction of body mass save some additional energy accounting for 3% and 8.6% of TEE respectively. Taken together, these three different mechanisms of economy account for the usual observation of an increment of body-weight gain in RR rats even when they are pair-fed with age-matched controls.

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Components of heat production in pigs: effects of stage of growth and genotype. By Y. LE COZLER, S. DUBOIS and J. NOBLET, *INRA, Station de Recherches Porcines, 35590 St Gilles, France*

Under thermoneutral conditions, total heat production (HP) results from the addition of activity-free fasting HP (FHP), HP due to physical activity (HPact) and the thermic effect of ingested feed (TEF; Noblet *et al.*, 1993*a*). In order to evaluate the effects of genotype and stage of growth on the contributions of these three components to total HP, total HP (indirect calorimetry), physical activity (i.e. duration of standing) and feeding pattern were measured in three groups of Large White (LW; control), Piétrain (PP; lean) and Meishan (MS; fat) growing pigs which differ widely in their growth potential. Measurements were carried out at three stages of growth (1, 2 and 3; Table) on single pigs over 5 d under *ad libitum* feeding and the subsequent last 24 (stage 1) or 30 (stages 2 and 3) h on fasting. Mathematical analysis of HP measurements obtained over the fasting period allowed estimation of HPact and FHP; TEF was subsequently calculated as the difference between *ad libitum* HP (corrected for zero activity) and FHP; it was related to digestible energy (DE) intake. Least-square means (except for DE intake, no interaction between stage of growth and genotype) are presented in the Table.

	Stage of growth			Genotype			
	1	2	3	LW	PP	MS	RSD
Body wt (BW, kg)	21.3^{a}	37.9^{b}	57.1^{c}	36.0^{a}	42.2^{b}	35.7^{a}	3.8
FHP (activity-free; kJ/min/kg$^{0.5}$)	0.93	0.92	0.88	1.04^{a}	0.95^{a}	0.71^{b}	0.55
HPact (kJ/min activity)	3.01^{a}	4.46^{b}	6.34^{c}	4.74^{a}	6.43^{b}	2.51^{c}	1.24
Ad libitum feeding							
DE intake (kJ/min)	12.61^{a}	15.56^{b}	19.17^{c}	15.55	14.94	14.75	1.50
Total HP (kJ/min)	6.76^{a}	8.88^{b}	10.96^{c}	8.76^{a}	9.44^{b}	7.98^{c}	0.59
HPact (% of total HP)	6.7	6.2	6.3	5.5^{a}	8.1^{b}	5.4^{a}	1.5
TEF (% of total HP)	34.0	31.3	30.5	26.1^{a}	27.4^{a}	45.0^{b}	4.7
TEF (% of DE intake)	18.1	17.9	18.3	14.9^{a}	16.5^{a}	24.0^{b}	3.1
Respiratory quotient	1.17^{a}	1.11^{b}	1.11^{b}	1.09^{a}	1.10^{a}	1.21^{b}	0.04

RSD, residual standard deviation; ^{a,b,c} values with unlike superscripts within a row for each main factor were significantly different, $P<0.05$.

Results show that MS pigs had reduced FHP; FHP was comparable at the three stages when results are expressed per kg $BW^{0.5}$. HPact was also lower in the MS group; the highest value of HPact was observed in PP animals. The value of HPact in control LW pigs (0.29 kJ/kg $BW^{0.75}$) was comparable to that obtained in 210 kg adult sows (Noblet *et al.*, 1993*b*). TEF represented 18% of DE intake, on average; surprisingly, it was higher in MS pigs. The combined effects of higher TEF (% of DE) and reduced values of FHP and HPact resulted in a higher contribution of TEF to total HP in MS pigs. Variations of the respiratory quotient between genotypes are related to differences in feeding level and composition of energy gain. Further statistical analysis of the kinetics of HP in fed animals will provide a basis for studying the metabolic regulation of feed intake in growing pigs.

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Measuring energy expenditure in Polar explorers. By P. RITZ[1,2], W.A. COWARD[1], T.J. COLE[1], M.A. STROUD[3], and M.B. SAWYER[1], *[1]Dunn Nutrition Unit, Cambridge CB4 1XJ, [2]Laboratoire de Nutrition Humaine, Clermont-Ferrand, France, and [3]Army Personnel Research Establishment, Farnborough, Hants GU14 6TD*

A principle of tracer experiments for *in vivo* kinetics when stable isotopes are used is that appropriate corrections have to be made for isotopic natural abundance (NA). In the context of experiments to measure CO_2 production with doubly-labelled water (DLW) it is therefore customary to assume that a pre-dose sample represents NA, and to subtract pre-dose values from post-dose enrichments to calculate the slopes of mono-exponential disappearance curves. Data is fitted to the equation $C_t=C_0e^{-kt}$ where C_t and C_0 are pre-dose-corrected post-dose isotope concentrations at time t and 0 respectively, t is time after dose administration, k is a fractional rate constant. In most cases this is the correct approach but there are occasions when NA abundance changes are likely to occur during the course of DLW experiments because of changes in location, food and water supply or even body composition. Schoeller *et al.* (1986) have discussed indirect strategies in these circumstances. It can however be shown theoretically that daily measurement of ^2H and ^{18}O enrichment in body water following isotope dosing can be used to predict the NA values to which study subjects are depleting. In most circumstances daily measurements for 28 d would be expected to supply estimates of NA at least as precise as those obtained from a single pre-dose measurement. We have therefore applied this direct procedure in four DLW studies on two Polar explorers who made an unsupported 95 d crossing of Antarctica, exhibiting massive NA changes in the course of their travels. Total energy expenditure (TEE) values were calculated by this new procedure and compared with those obtained from precisely known food intake, and body-composition changes.

At days 0 and 50 isotope doses were given aiming at an excess enrichment above pre-dose of 150 ppm for ^2H, and 300 ppm for ^{18}O. Urine samples were obtained before dose administration and on every subsequent day. Post-dose isotopic data were fitted to the relationship $C'_t = C_e + (C'_0-C_e)\ R^t$ where C'_t and C'_0 are observed isotope concentrations at time t and 0, C_e is the value of NA at infinite time, and $-\ln R = k$. There were massive changes in NA in both men. ^2H in body water fell by 45 ppm and ^{18}O by 53 ppm in 80 d. Thereafter there were unexpected and unexplained increases in NA. Because of the latter phenomenon TEE was calculated for days 0-50 and 51-80 only. These are shown in the Table together with estimates of internal precision and corresponding values from food intake and body-composition changes. The values are comparable with those obtained in previous studies on the same subjects in the Arctic (Stroud *et al.* 1993).

	TEE (MJ/d) (1)	Energy intake (2)	Energy derived from body stores (3)	Difference (1) - (2÷3)
Subject (d)	Mean (SD)	(MJ/d)	Mean (SD)	(%)
MS (0-50)	29.1 (2.33)	19.7	8.81 (0.87)	+ 2.07
RF (0-50)	35.5 (2.10)	19.7	18.2 (0.62)	- 6.40
MS (51-80)	18.8 (2.41)	23.1	Not available	
RF (51-80)	23.1 (1.88)	23.1	Not available	

We can conclude that if appropriate consideration is given to circumstances prevailing in DLW experiments modifications can be made to make the method applicable and useful in remote and extreme (physiological and physical) conditions.

Schoeller, D.A., Kushner, R.F., and Jones P.J.H. (1986). *American Journal of Clinical Nutrition* 44, 291-298
Stroud, M.A., Coward, W.A., Sawyer, M.B. (1993). *European Journal of Applied Physiology* 67, 375-379

Changes in energy expenditure, glucose-induced thermogenesis and protein turnover during short-term head-down-tilt conditions. By K.J. ACHESON, J. DÉCOMBAZ, C. PIGUET-WELSCH, F. MONTIGON, B. DECARLI, I. BARTHOLDI and E.B. FERN, Nestec Ltd., Nestlé Research Centre, Lausanne, Switzerland

In an attempt to understand the changes which occur in glucose and protein metabolism during prolonged bedrest and microgravity (space flight), whole-body protein turnover, and energy expenditure before and during an oral glucose tolerance test (1 g/kg body-weight) were studied, on separate occasions, in six healthy young men before and during 3 d simulated microgravity using the 6° head-down-tilt (HDT) method (Volicer *et al.* 1976).

After 42-47 h HDT, basal insulin concentrations increased significantly from 9.4 (SD 1.9) to 13.1 (SD 2.1) μU/ml ($P<0.002$); however, no significant differences in glycaemia, insulinaemia or free fatty acid concentrations were observed in response to the oral glucose load. Basal, postabsorptive, resting metabolic rate increased by approximately 8% ($P<0.05$) during HDT, which was essentially due to an increase in lipid oxidation (33 (SD 2) to 51 (SD 5) mg/min; $P<0.02$).

Although the thermic response to glucose ingestion increased from 7.7 (SD 1) to 10.7 (SD 0.6)%, this was not statistically significant. Nevertheless, energy expenditure remained elevated at the end of the 4-h test in HDT conditions, whereas it had already returned to baseline values in the control, semi-supine study. Protein turnover calculated from the arithmetic mean of ammonia nitrogen and urea nitrogen flux was unchanged by HDT but a significant increase in the contribution of ammonia synthesis to nitrogen excretion was observed.

These preliminary results on protein and glucose metabolism in simulated microgravity suggest that a cephalic shift of body fluids increases energy requirements due to increases in both basal metabolic rate and the thermic response to food ingestion. The changes observed in substrate utilization accompanied by a small but significant increase in basal insulin concentrations, without abnormal glucose tolerance, further suggest that metabolic changes at the cellular level could be responsible for the development of glucose intolerance observed during prolonged bedrest and exposure to microgravity.

Volicer, L., Jean-Charles, R. & Chobanian, A.V. (1976). *Aviation, Space, and Environmental Medicine* 47: 1065-1068.

Energy expenditure in lean and obese prepubertal 9-10 year old children. By J.P.DELANY, G.A.BRAY, D.W.HARSHA, J.KIME and L.MELANCON, *Pennington Biomedical Research Centre, Baton Rouge, LA 70808, USA.*

The relationship between energy expenditure and obesity was examined in prepubertal children. All consenting 9 and 10-year-olds at local elementary schools were screened. Screening measurements included Tanner Staging and skinfolds. Subjects were selected for further study to obtain equal numbers of black and white, girls and boys with a wide range of body composition. Weight, total daily energy expenditure (TDEE) by doubly labelled water, resting metabolic rate (RMR), and body composition by dual-energy X-ray absorptiometry (DEXA) were measured. Since we do not yet have equal numbers in each of the groups, racial and gender comparisons cannot be made. Children were further subdivided into tertiles (T) of subscapular (SS) plus triceps (TC) skinfolds. The SS+TC tertiles did quite well in grouping subjects by degree of obesity as differences in percentage fat in each tertile were significantly different (Table).

T	*n*	Body wt (kg)		FEM (kg)		Body fat (%)		RMR (MJ/d)		TDEE (MJ/d	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
1	16	32[a]	1	27	1	15[a]	1	5.36	0.21	9.62	0.67
2	16	35[a]	1	27	1	22[b]	1	5.31	0.21	9.83	0.63
3	14	46[b]	1	29	1	36[c]	1	5.65	0.21	9.96	0.71

FEM = fat-free mass. [a,b,c] - Values within a column with unlike superscripts were significantly different: $p < 0.05$.

No differences in RMR or TDEE between the three groups were observed. A reduced RMR or TDEE could not explain differences in obesity in these prepubertal children. However, the fact that the heaviest children had the same TDEE as the leanest while weighing 14kg more, indicates that the obese may have a reduced activity level.

Supported by NIH, NICHHD #R01 HD28020-02

Total energy expenditure, net protein, carbohydrate and fat oxidation in pregnant and postpartum women. By N. BUTTE, J. HOPKINSON, J. MOON, A. ADOLPH, M. PUYAU AND F. VOHRA, *Department of Pediatrics, Baylor College of Medicine, USDA/ARS Children's Nutrition Research Center, Houston, TX, USA.*

Alterations in total energy expenditure (TEE) and substrate utilization imposed by pregnancy and lactation were investigated by room respiration calorimetry. Fifty-one women (40 lactators (L), 11 non-lactators (NL)) were confined for 24 h to a room calorimeter between 36-38 weeks of pregnancy and at 3 and 6 months postpartum adhering to the same protocol of low physical activity. Dietary intake (food quotient 0.87) was controlled to body weight with allowances for pregnancy and lactation. TEE,. net protein, carbohydrate (CHO) and fat oxidation were computed according to Livesey & Elia (1988). Fat-free-mass (FFM) was determined by underwater weighing, corrected for pregnancy.

	Pregnancy				3 months Postpartum				6 months Postpartum			
	L		NL		L		NL		L		NL	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TEE (MJ/d)	9.64	0.92	9.41	1.46	7.86	0.89	7.27	0.55	7.56	0.97	7.22	0.62
Respiratory quotient	0.88	0.02	0.88	0.02	0.87	0.02	0.86	0.02	0.88	0.02	0.87	0.02
Protein oxidation (%TEE)	16.1	2.8	12.6	2.8	19.8	3.0	16.9	3.3	19.9	4.0	16.3	3.0
Carbohydrate oxidation (%NPEE)	64.3	7.7	64.3	10.2	61.9	9.6	56.8	6.9	64.2	9.8	58.5	9.7
Fat oxidation (%NPEE)	35.7	7.7	35.7	10.2	38.1	9.6	43.2	6.9	35.8	9.8	41.5	9.7
BMR (MJ/d)	7.22	0.76	7.30	1.22	5.87	0.92	5.58	0.61	5.70	0.86	5.80	1.10
Respiratory quotient	0.83	0.03	0.82	0.05	0.80	0.04	0.79	0.06	0.80	0.04	0.80	0.05
Protein oxidation (%TEE)	21.2	3.7	16.2	3.7	26.7	4.5	22.2	4.8	26.5	5.6	20.8	5.0
Carbohydrate oxidation (%NPEE)	42.1	12.1	39.5	23.1	29.8	21.7	24.4	25.6	25.8	22.1	31.2	21.5
Fat oxidation (%NPEE)	57.9	12.1	60.5	23.1	70.2	21.7	75.6	25.6	73.3	27.5	68.8	21.5

NPEE, nonprotein energy expenditure; BMR, basal metabolic rate.

TEE and BMR were higher during pregnancy, even when controlled for weight or FFM ($P \leq 0.01$). Fetal demands for glucose and mammary gland requirements for glucose and fat altered 24 h net rates of CHO and fat oxidation ($P \leq 0.01$). CHO oxidation (24 h) was elevated during pregnancy and lactation. Fat oxidation (24 h) was higher among nonlactating, postpartum women.

Livesey, G. & Elia, M. (1988). American Journal of Clinical Nutrition 47, 608-628.

Total energy expenditure, basal metabolic rate and physical activity plus thermogenesis in a group of free-living elderly men aged over 75 years. By N.J. FULLER[1], W.A. COWARD[1], M.B. SAWYER[1], P. PAXTON[2] and M. ELIA[1], *[1]MRC Dunn Clinical Nutrition Centre, Cambridge CB2 2DH and [2]Lensfield Medical Practice, 48 Lensfield Road, Cambridge CB2 1EG*

Although recommendations for energy requirements in elderly subjects are based on very scanty data, a mean energy intake of 1.5 x basal metabolic rate (BMR) is suggested (or about 88 J/kg per min) in both the UK and USA. In contrast, a study with doubly-labelled water (DLW) in a group of fifteen elderly male subjects (mean age 69 (SD 1.8) years) in the USA (Roberts *et al.* 1992) suggested that total energy expenditure was significantly higher (1.75 x BMR) than recommended dietary allowances. However, the subjects of that study were not selected randomly from the population. Therefore, the purpose of the present study was to measure total energy expenditure (TEE) by DLW, and BMR by indirect calorimetry, in a group of elderly male subjects, randomly selected from a General Practice register, in order to establish whether or not current national recommendations are appropriate.

Twenty-three subjects were studied (mean age 82 (range 76-88) years; weight 72.4 (SD 10.5) kg; height 1.71 (SD 0.08) m; fat-free mass (FFM, measured by deuterium dilution) 50.2 (SD 6.2) kg). TEE was calculated assuming that the energy equivalent of CO_2 was 23.8 kJ/l, which applies to an assumed respiratory quotient of 0.85. The physical activity level ratio (PAL ratio = TEE : BMR) and physical activity plus themogenesis (TEE - BMR), for the whole body and per kg body-weight and FFM were calculated.

The Table shows values obtained for the various indices of energy expenditure.

	Mean	SD	Median	Range
Total energy expenditure :				
whole body (MJ/d)	9.2	1.4	9.3	6.3-12.5
per kg body-wt (J/kg per min)	89.4	14.2	89.2	65.1-114.2
per kg FFM (J/kg per min)	130.3	17.3	131.6	105.8-169.6
Basal metabolic rate :				
whole body (MJ/d)	6.0	0.5	6.0	4.3-7.0
per kg body-wt (J/kg per min)	58.2	6.3	57.2	47.5-71.0
per kg FFM (J/kg per min)	85.0	7.1	84.4	74.2-95.6
Physical activity plus thermogenesis :				
whole body (MJ/d)	3.2	1.2	3.1	0.9-6.1
per kg body-wt (J/kg per min)	31.2	12.2	29.3	9.4-56.0
per kg FFM (J/kg per min)	45.3	17.0	44.5	15.9-83.1
Physical activity level ratio	1.5	0.2	1.5	1.2-2.0

This is the first cross-sectional study of its kind to report values of TEE in a randomly selected group of free-living elderly subjects, who are over 75 years of age. Despite the relatively large inter-individual variation, these data add support to the use of current national recommendations for energy intake in elderly subjects, free-living in Western society.

Roberts, S.B., Young, V.R., Fuss, P., Heyman, M.B., Fiatarone, M., Dallal, G.E., Cortiella, J. & Evans, W.J. (1992). *International Journal of Obesity* **16**, 969-976.

Total and activity-related heat production of group-confined pigeons as affected by gender and space. By J. GORSSEN, P. KOENE, J.W. SCHRAMA and F. VAN DER VEEN, *Department of Animal Husbandry, Wageningen Agricultural University, The Netherlands*

During transport, racing pigeons are confined in groups consisting of males or females only. In the present study, the effect of available space (AS) and gender (G) on total (H_{tot}) and activity-related heat production (H_{ac}) was investigated by indirect calorimetry. In total, twenty groups of ten pigeons (male or female) were allotted to one of five AS-levels (cm^2/animal, see Table). Each group was placed in a climate-respiration chamber at a constant temperature of 36 ° with water available. Locomotor activity and H_{tot} were measured in 9-min intervals from 10.30 hours until 09.30 hours of the next day. Lights were off from 20.30 hours to 07.30 hours. H_{ac} per group was calculated by linear regression of H_{tot} on activity counts. Mean H_{tot}, H_{ac} (both in $kJ/kg^{0.75}$ per d), and proportion H_{ac}/H_{tot} (%) were calculated and subjected to analysis of variance for each photoperiod (Li, "lights on", or Da, "lights off") separately. The results for Li are shown in the Table (least square means, $\underline{n}$ 20).

AS	H_{tot}		H_{ac}		H_{ac}/H_{tot}	
	♂	♀	♂	♀	♂	♀
210	374.6	371.1	80.2	65.6	21.4	17.6
280	385.6	366.1	65.7	55.6	17.0	15.2
350	383.2	345.9	61.7	43.8	16.2	12.5
420	406.1	345.5	109.1	38.0	26.8	10.9
630	380.5	318.6	54.2	30.4	14.2	9.5
SE	13.0		7.3		1.7	

During Li, H_{tot} and H_{ac} were higher for males (386.0 and 74.2 $kJ/kg^{0.75}$ per d) compared with females (349.4 and 46.7 $kJ/kg^{0.75}$ per d, $\underline{P}<0.01$). H_{ac} was affected by AS ($\underline{P}<0.01$), whereas H_{tot} was not ($\underline{P}>0.20$). The proportion H_{ac}/H_{tot} was higher for males (19.2 $\underline{v}$. 13.1%, $\underline{P}<0.001$), and decreased with increasing AS ($\underline{P}<0.01$). For both H_{ac} and the proportion, a significant interaction between G and AS was present ($\underline{P}<0.05$). During Da, H_{tot} was not affected by treatments ($\underline{P}>0.10$), but H_{tot} of males was 7.0 $kJ/kg^{0.75}$ per d higher. H_{ac} and the proportion H_{ac}/H_{tot} were higher ($\underline{P}<0.05$) for males (26.4 $kJ/kg^{0.75}$ per d, 9.4%) compared with females (18.7 $kJ/kg^{0.75}$ per d, 6.8%), whereas AS had no effect. In female birds, the proportion H_{ac}/H_{tot} decreased with increasing AS during Li. No such effect was found during Da, where activity was low. We can conclude that the higher H_{tot} for male pigeons was mainly due to activity. Furthermore, the effect of AS on the proportion H_{ac}/H_{tot} Li resulted from differences in H_{ac}.

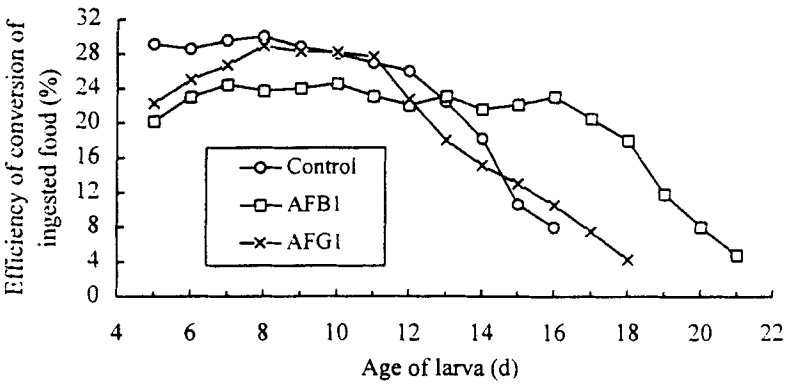
Food consumption and energy metabolism of *Spodoptera littoralis* Boisdoval (Lepidoptera, Noctuidae), feeding on an aflatoxin-containing diet. By M. WEDENIG, K. CRAILSHEIM and M. M. SADEK, *Institute of Zoology, Karl-Franzens University, A-8010, Graz, Austria*

Spodoptera littoralis feeds naturally on a wide variety of diets. All substances necessary for development and metamorphosis must be synthesized and stored during the larval stage. Hence, any qualitative or quantitative disturbance in feeding has consequences not only for the larval stage but also for successive stages of the life cycle. Aflatoxins are known to have repellent effects on insects (Beard & Walton, 1969) and some are thought to interfere with oxidative metabolism in caterpillars (Dowd, 1988).

In the present study we investigated the efficiency of *S. littoralis* in utilizing an aflatoxin-containing food and the effect of these toxins on oxygen consumption by the insect. Larvae were provided a meridic diet containing 3 ppm of aflatoxin B_1 (AFB_1) or aflatoxin G_1 (AFG_1).

AFB_1 affected the respiratory quotient (RQ) only slightly (0.913 and 0.896 for control and treated larvae respectively), but significantly ($P < 0.05$) inhibited the oxygen uptake of 8-d-old larvae. Treated insects consumed only 0.055 ml O_2/g per h in contrast to the 0.08 ml O_2/g per h of controls. Both control and treated larvae showed a slight increase in oxygen uptake during the dark phase of a 14h:10h light—dark cycle, when oxygen consumption was measured over 72 h.

The food consumed per larva averaged 4.53, 3.25 (significantly different from control) and 4.65 g over the feeding periods until pupation at ages of 16, 18 and 21 d in control, AFB_1 and AFG_1, respectively. The efficiency of conversion of ingested food (ECI) of larvae (following Waldbauer, 1968) is shown below.



Young larvae exhibited greater sensitivity to both toxins. In the case of AFG_1, the ECI was slightly affected in general while AFB_1 had a powerful effect on the ECI of the larvae; a significant difference was recorded between AFB_1 and control amongst all larvae between 8 and 11 d old. No conclusion can be drawn for larvae older than 12 d as the ECI of control larvae declines as they approach pupation.

Beard, R. L. & Walton, G. S. (1969). *Journal of Invertebrate Pathology* **14**, 53-59.
Dowd, P. F. (1988). *Entomologia experimentalis et applicata* **47**, 69-71.
Waldbauer, G. P. (1968). *Advances in Insect Physiology* **5**, 229-288.

Shivering is the main thermogenic mechanism in cold-exposed newborn pigs. By D. BERTHON, P. HERPIN and J. LE DIVIDICH, *INRA, Station de Recherches Porcines, 35590 St Gilles, France*

At birth, pigs are usually exposed to a cold environment and, therefore, must develop rapidly their thermoregulatory function. The newborn piglet is poorly insulated so maintenance of its homeothermic balance depends almost exclusively on its ability to produce heat (Berthon *et al.* 1993). Further, unlike many altricial mammals, it appears to have no brown adipose tissue (Trayhurn *et al.* 1989) but it shivers vigorously from birth (Mount, 1968).

In the present study, both the magnitude and the occurrence of shivering, taken as integrated electromyographic activity (EMG, mV/min) in *longissimus dorsi*, and metabolic rate (MR, kJ/h per kg) were simultaneously determined on pigs aged 2, 24, 48 h and 5 d, at ambient temperatures ranging from thermoneutrality to moderate cold, i.e. from 34° to 20°. The lower critical temperature (L_{ct}) and the threshold of ambient temperature for shivering (S_{tt}) were compared to question the presence of nonshivering thermogenesis. Further, the calculated shivering capacity at 25° (S_c=(MMR-MR at 25°)/ integrated EMG at 25°, where MMR is the minimal metabolic rate) and the relation between MR and shivering intensity were analysed to evaluate the development of the thermogenic capacity of shivering with age.

Age...	2 h		24 h		48 h		5 d		Age effect
	Mean	SE (n)	Mean	SE (n)	Mean	SE (n)	Mean	SE (n)	
MMR	13.3	1.2 (9)	16.3	0.9(*12*)	20.3	0.8(*13*)	23.9	0.6(*13*)	P<0.001
L_{ct}	33.9	0.3 (9)	32.9	0.8(*12*)	30.2	0.8(*13*)	30.4	0.5(*13*)	P<0.001
S_{tt}	34.9	0.3 (9)	33.4	0.6(*15*)	31.4	0.9(*15*)	30.9	0.5(*13*)	P<0.01
S_c	1.97	0.23(*6*)	2.78	1.46(*8*)	3.73	0.39(*6*)	12.57	4.57(*5*)	P<0.01

The early postnatal period was characterized by an 80% increase in MMR and a concomitant decrease in both L_{ct} and S_{tt} (P<0.01). At all ages, the lack of delay between the cold-induced increase in metabolic rate (L_{ct}) and the onset of shivering (S_{tt}), and the linearity of the relation between MR and integrated EMG activity disproved the existence of nonshivering thermogenesis, and confirmed the main role of shivering in neonatal thermogenesis in pigs. Moreover, the calculated S_c (Table) and the slope of the relation between MR and integrated EMG (0.08 (0.01) *v.* 0.17 (0.06) at 2 h and 5 d of life respectively) increased with age (P<0.01) which demonstrated the postnatal enhancement of the thermogenic capacity of shivering.

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Resting metabolic expenditure in patients with end-stage liver insufficiency. By J.C. MANELLI, C. BADETTI, V. BERNINI, J. di COSTANZO, C. CHAMPEAU and V. PELLEGRIN. *Départment of Anesthesiology, Hôpital de la Conception, 13385-Marseille Cedex 5 (France).*

Controversies in medical litterature lead us to study resting energy expenditure (REE) in patients suffering from end-stage liver insufficiency.

In patients proposed for hepatic transplantation and suffering from severe hepatic insufficiency, pre-transplantation evaluation included measurements of VO_2 and VCO_2 through a head canopy connected to a Deltatrac apparatus (Datex Inc.) for calculation of REE. In order to refer REE to fat free mass (FFM), anthropometric measurements of tricipital and subscapular skin thickness (method described by Durnin = ANT) and bioelectrical impedance measurement (method using 2 frequencies, described by Thomasset and Boulier = BEI) were performed simultaneously. BEI allowed also calculation of extra-cellular fluid volume (ECFV) and of metabolically active cell mass (MACM = FFM-ECFV). When transplantation was not performed, the previous measures were repeated each two months. Values observed by indirect calorimetry and by anthropometry in healthy subjects were used as a reference.

79 measurements were performed in 40 patients. Mean REE was 6753 (SD 912) kJ/day; corresponding to +4.2% of calculated basal metabolic rate (Harris and Benedict): this difference is statistically the same as the difference observed in healthy subjects (+3.7%).

Mean REE/FFM values were, in the patient group, 12.9 (SD 18.0 4,30) kJ/kg FFM (ANT) or 124.1 (SD 15.9) kJ/kg FFM (BEI). These values are statistically below those of healthy subjects (132.6 SD 15.5) kJ/kg FFM; $p < 0,02$).

No difference in REE/FFM values were found between the sub-group of patients with an increase in ECFV above 50% (measured by BEI) and the sub-group of patients with ECFV about normal range (ANT: 123.7 vs 119.4 kJ/kg FFM,N.S., and BEI: 126.1 vs 119.5 kJ/kg FFM, N.S.), although comparison with the healthy subject group shows that only the subgroup with normal ECFV has mildly significant lower values ($p < 0,05$).

On the contrary, REE/MACM values show difference between ECFV related sub-groups: 212.1 kJ/kg MACM in patients with increased ECFV, and 191.6 kJ/kg MACM in patients with normal ECFV ($p < 0,02$).

REE in patients with end-stage liver insufficiency is not different from healthy subjects.
REE/FFM values are significantly lower in patients with liver disease. In addition, patients with increase in ECFV shows higher REE than patients with ECFV about normal range.

Cold- and diet-induced thermogenesis in the pre-ruminant kid goat and lamb. By M.R. SANZ SAMPELAYO, I., PRIETO, J. FONOLLA, F. GIL EXTREMERA and J. BOZA, *Departamento de Nutrición Animal, EEZ, (CSIC), Profesor Albareda, 1, 18008 Granada, Spain*

The processes which constitute thermogenesis play a significant role in the regulation of animal energy balance (Trayhurn *et al.* 1982). The metabolic origin for thermogenesis has long been a matter of controversy and it seems that the particular animal metabolic type is important (Miller *et al.* 1979; Thurlby & Trayhurn, 1979).

The present experiment studied the nature of cold- and diet-induced thermogenesis, during the first 2 months of life in the pre-ruminant kid goat and lamb. It is well-recognized that both these animals have different corporal development, the former showing a relatively low capacity to fatten. Animals from both species were fed on a milk replacer with a high protein (30%) and fat (31%) content, at different intake levels, at 12 (cold temperature) and 30° (thermoneutral temperature). Energy balance was determined by the comparative slaughter method. As far as protein and fat utilization for heat production is concerned, three different situations were analysed according to environmental temperature and/or intake level (Table). Heat production from protein and/or fat was calculated as the difference between the corresponding metabolizable energy intake and energy retention, with the assumption that in these animals fat deposition is entirely of dietary origin (Pearce, 1983).

Animal	Temperature (°)	MEI*	HPp*	HPf*	Situation
Kid	12	900	154	342	1
Kid	30	900	156	309	2
Kid	30	650	113	264	3
Lamb	12	900	136	391	1
Lamb	30	900	145	247	2
Lamb	30	650	83	239	3

MEI, Metabolizable energy intake; HPp, Heat production from protein; HPf, Heat production from fat. *$kJ/kg^{0.75}$ per d.

From situation 1*v.* 2 it is possible to infer that for both kinds of animal, the thermogenesis induced by cold-temperature under the same intake level, would be practically associated with fat utilization only, this process being lower in the kid than the lamb. In the same way, from situation 2 *v.* 3 it is possible to deduce that the thermogenesis induced by diet at thermoneutrality, would be associated in the kid with fat and protein utilization. However in the lamb, this process, together with being lower than in the kid, would be practically associated with protein utilization only. The results suggest that the cold- and diet-induced thermogenesis processes analysed here, seem to be different types of thermogenesis. At the same time, these processes appear to be quantitatively and/or qualitatively different for pre-ruminant kid goats and lambs.

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Thurlby, P.L. & Trayhurn, P. (1979). *British Journal of Nutrition* **42**, 377-384.

Trayhurn, P., Goodbody, A.E.& James, W.P.T. (1982). *Proceedings of the Nutrition Society* **41**, 127-131.

Physiological interactions between proliferating lymphocytes and adjacent adipose tissue. By C. M. POND and C. A. MATTACKS, *Department of Biology, The Open University, Milton Keynes, MK7 6AA.*

Fatty acids are important to lymphocytes as an energy source, as components of membranes and as substrates for the synthesis of complex lipid-based cytokines such as leukotrienes, prostacyclins, thromboxanes and prostaglandins. *In vitro* experiments using single fatty acids indicate that they can act as both stimulators and inhibitors of lymphocyte proliferation (Calder *et al.* 1989). In mammals, lymph nodes always occur embedded in adipose tissue, which is the main source of fatty acids *in vivo*.

To investigate the functional basis for the anatomical relationships between adipose tissue and the lymphatic system, a method was devised to co-culture lymphocytes from adult female guinea-pigs with pieces of adipose tissue from areas adjacent to and far from the lymph nodes of seven depots that contain one or more lymph nodes and from near to or far from knots of blood vessels of two depots that lack lymph nodes. Adipose tissue was chopped into pieces about 1 mm^3 that were cultured for 48 h with lymphocytes isolated from the cervical, and if necessary the axillary or inguinal, lymph nodes of the same animal. Pilot experiments showed that lymphocytes from these nodes all behaved similarly. The tissues were co-cultured in normal medium (Roswell Park Memorial Institute 1640) plus 10% fetal calf serum, 2 mM glutamine, antibiotics and the mitogen, concanavalin A (40 μg/ml). Lymphocyte proliferation was measured as incorporation of [^{3}H]-thymidine and lipolysis as glycerol release. The fatty acids in the media after incubation and the triacylglycerol fatty acids (TAG FA) in adipose tissue taken from sites near those used for incubation with lymphocytes were separated and identified by gas chromatography.

Adipose tissue pieces functioned well under these conditions: the rate of incorporation of labelled glucose into lipids was only slightly impaired after 48 h. Adipose tissue inhibited lymphocyte proliferation to below 65% of the rate without adipose tissue, but site-specific differences were large, with samples from two small intermuscular depots being the most inhibitory and perirenal the least. Inhibition was antagonized by insulin: site-specific differences in samples' inhibitory capacity were proportional to the rate of lipolysis of isolated adipocytes (Pond & Mattacks, 1991). Samples from all adipose depots depressed lymphocyte proliferation by less than 10% in the presence of 500 IU insulin, suggesting fatty acids as the mediator of inhibition. Samples from near the lymph nodes were always more inhibitory than those of the same depot taken from far from nodes. Lipolysis was slightly higher without lymphocytes, and much higher with lymphocytes, in adipose tissue from near the lymph nodes than in that from elsewhere in the same depot. These observations show that proliferating lymphocytes stimulate lipolysis in adipose tissue, with that from near lymph nodes being more sensitive than that from far from nodes.

The unsaturation indices of TAG FA extracted from adipose tissue near lymph nodes were 5-18% higher than those of samples from far from nodes within the same depot, especially in the mesentery and intermuscular depots. The perirenal depot lacks both lymph nodes; the compositions of TAG FA were similar in samples taken from beside or away from a knot of blood vessels. This heterogeneity in lipid composition of adipose tissue in relation to the lymph nodes may be maintained by selective uptake and/or selective release of fatty acids.

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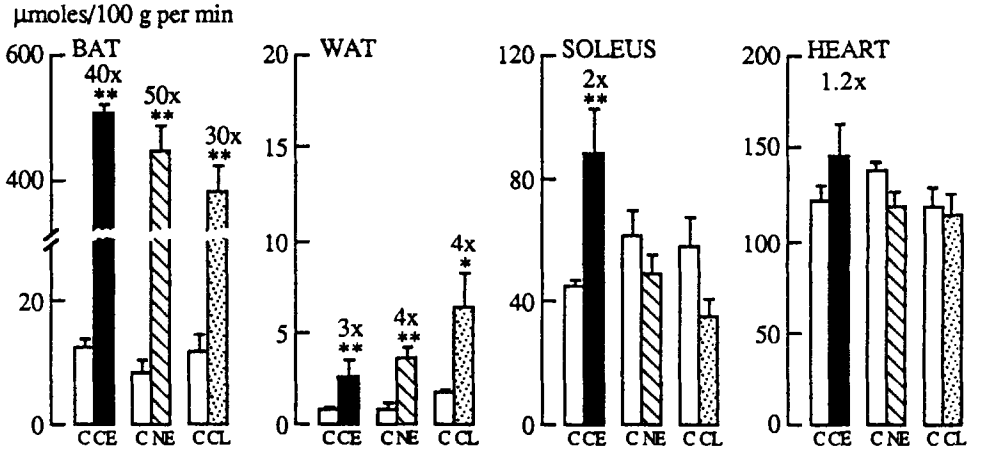
The new ß3-agonist CL 316 243 decreases plasma glucose levels and markedly stimulates glucose uptake in brown and white adipose tissues. By L. J. BUKOWIECKI, X. LIU, C. ATGIÉ, F. PÉRUSSE, *Université Laval, Faculté de Médecine, Québec, Canada, G1K 7P4*

Cold exposure stimulates the sympathetic nervous system, improves glucose tolerance, increases insulin sensitivity and reverses the diabetogenic effects of high fat feeding. Using the 2-[^{3}H]-deoxyglucose method, we reported that cold exposure (2 d at 5 °) stimulates glucose uptake in rat brown and white adipose tissues (BAT and WAT), heart and skeletal muscles in spite of the fact that it decreases plasma insulin levels (Vallerand et al., 1990). Considering that cold exposure stimulates the release of noradrenaline from sympathetic nerves, we tested whether noradrenaline (NA) (a mixed ß-agonist) or CL 316243 (a new ß3-agonist, see Bloom et al., 1992) would mimic the effects of cold exposure (4 d at 5 °) on glucose uptake in peripheral tissues when infused in nonanaesthetized rats (Sprague-Dawley, females, 250-300 g) maintained at 25 ° for 4 d (Liu et al., 1994). It can be seen in the Figure that cold exposure (CE), NA and CL 316243 all stimulated glucose uptake in BAT 30-50 times over controls (C) and WAT (3-4 times). No treatment significantly altered glucose uptake in the heart. Cold-exposure, but not drug infusion, significantly increased glucose uptake in the soleus muscle (and other types of muscle).



The capacity of BAT for NA-stimulation of glucose uptake (expressed per gram of tissue) was much higher than that of WAT (100 times), various types of white or red skeletal muscles (10-80 times) or the heart (3-4 times). Assuming that BAT represents 0.75 % of body weight and skeletal muscles 39 %, it can be calculated that glucose uptake by total BAT, when maximally stimulated represents approximately one third of the glucose utilized by the skeletal muscles. Thus, BAT possesses a remarkable capacity for utilizing glucose. CL 316 243 significantly decreased plasma glucose and free fatty acid levels in spite of the fact that it also decreased plasma insulin levels, suggesting that it increases the sensitivity of peripheral tissues to insulin. The selectivity of this agent for $ß_3$ receptors (abundant in WAT and BAT), its thermogenic and hypoglycaemic effects indicate that this agent has potential as a drug in the treatment of obesity and non-insulin-dependent diabetes. These results indicate that anti-diabetogenic effects of cold exposure (improvement of insulin sensitivity) can be mimicked in the warm by treating rats with adrenergic drugs.

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Seasonal changes of mass and lipid droplet size in brown adipose tissue of the Pygmy shrew *(Sorex minutus)*. By R.M. McDEVITT[1] and J.F. ANDREWS[2], [1]*Department of Zoology, University of Aberdeen, Aberdeen AB9 2TN* and [2]*Department of Physiology, Trinity College, Dublin 2, Republic of Ireland*

Soricine shrews have the highest metabolic rate per unit of mass of any group of species so far studied. It is therefore not surprising to find that on a simple weight basis brown adipose tissue contributes a relatively high percentage to total body-weight. An increase in multilocularity (number of lipid droplets per cell) with decreased lipid droplet size has been shown to indicate increased thermogenic capacity of brown adipose tissue (Ahlabo & Barnard, 1974).

We have studied one of the smallest species in this subfamily, the Pygmy shrew, with respect to the thermogenic capacity of its brown adipose tissue, and how that changes with season. We have examined changes in body-weight with season, the percentage contribution of interscapular brown adipose tissue (IBAT) to that weight and the degree of multilocularity by distribution in lipid droplet size (minimum of 1200 droplets measured per seasonal value). These findings are presented in the Table. Only in spring animals could sex be readily determined in live animals.

Season		*n*	Body-wt (g)	IBAT wt (g)	IBAT/wt %	Median droplet area (μm^2)
Winter	Mean	16	3.1** †	0.13*	4.0	106‡
	SEM	0.2	0.03	0.8		
Spring male	Mean	12	4.7	0.35	6.5	
	SEM	0.3	0.1	1.6		
						194§
Spring female	Mean	9	4.1	0.22	5.0	
	SEM	0.3	0.1	1.4		
Summer	Mean	16	3.1** †	0.14*	3.7	124‡
	SEM	0.1	0.05	1.1		

Mean values significantly different from spring males: *$p \leq 0.05$, **$p \leq 0.01$ (ANOVA).

Mean values significantly different from spring females: †$p \leq 0.05$ (ANOVA).

Mean values significantly different from spring animals: ‡$p \leq 0.05$ (two-paired Kolmogorov-Smirnov median test)

§Variable not separated on the basis of sex.

Winter shrews showed the smallest droplet size reflecting the highest relative thermogenic capacity. Spring shrews, especially the males, showed a considerable significant increase in body-weight from winter conditions. Despite the more temperate climate of spring this increase is matched by an increase in the relative and absolute mass of the IBAT. Droplet size, however, increased in spring animals. This shows that the increase in IBAT weight is due to increased triacylglycerol deposition. The weight increase is not due to an increase in thermogenic machinery, the reverse is true. Thus IBAT in the spring animals appears to have a fat storage role. One possibility is that this is preparatory to breeding and its energy demands, when the imperatives of breeding behaviour reduce foraging time so that the shrews have to rely more on body stores.

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Effect of maternal glucose infusion during late gestation on brown adipose tissue and liver iodothyronine 5'deiodinase activity in the newborn lamb. By L. CLARKE, M.A. LOMAX and M.E. SYMONDS, *Department of Biochemistry and Physiology, University of Reading, Whiteknights, Reading, RG6 2AJ*

Chronic maternal cold exposure alters fetal brown adipose tissue (BAT) and liver development over the final week of gestation (Clarke *et al.* 1993) which results in an improved ability of the newborn lamb to thermoregulate. These effects may be mediated by an increase in maternal glucose production and supply to the developing fetus. The present study investigates the effect of maternal glucose infusion over the final 5-7 d gestation on BAT and liver iodothyronine 5'deiodinase activity (I5'D) in the newborn lamb.

Nineteen unshorn ewes were given 60% of the energy requirements for maintenance and pregnancy (0.2-0.25 kg barley concentrate: 1.0-1.25 kg chopped hay) and infused with either glucose (G: *n* 10) at a rate of 0.62 mmol/min or saline (S: *n* 8) which increased plasma glucose concentrations by 153% (G: 3.92 (SEM 0.54); S: 2.57 (SEM 0.14) mmol/l ($P<0.05$)). All ewes lambed normally at term and after 30 minutes of life perirenal adipose tissue and liver were sampled and subsequently analysed for I5'D activity plus the thermogenic activity of BAT (Clarke *et al.* 1994).

	Tissue (g)				Iodothyronine 5'deiodinase (pmol I^- released/mg protein per h)					
					Type I				Type II	
	Liver		BAT		Liver		BAT		BAT	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
S	91	7	22.8	1.0	1857*	268	758*	67	0.52*	0.05
G	91	5	20.4	1.1	2655	186	1029	73	0.74	0.03

Significantly different from G (analysis of variance): * $P<0.05$.

There were no significant differences in lamb birth weight (S: 4.5 (SEM 0.4); G: 4.1 (SEM 0.2) kg), liver and BAT weight or BAT thermogenic activity (S: 91 (SEM 16); G: 103 (SEM 17) nmol/lamb) between the two groups. Maternal glucose infusion increased both hepatic and BAT I5'D without influencing plasma triiodothyronine concentration (S: 6.6 (SEM 0.5); G: 6.3 (SEM 0.5) nmol/l).

It is concluded that increasing maternal plasma glucose concentration stimulates I5'D activity in liver and BAT without any apparent benefical effects on the newborn lamb's ability to thermoregulate.

This work was funded by the Wellcome Trust, AFRC and a MRC studentship (L.C.).

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Variation of energy metabolism among ruminant muscles : low glucose utilization potential by oxidative muscles. By F. BORNES[1], J.F. HOCQUETTE[1], M. BALAGE[2], P. FERRE[3], B. PICARD[1] and M. VERMOREL[1], *INRA, [1]Laboratoire Croissance et Métabolismes des Herbivores et [2]Laboratoire d'Etude du Métabolisme Azoté, Theix, 63122 Saint-Genès Champanelle and [3]INSERM U342, Hôpital Saint Vincent de Paul, 75014 Paris, France*

It is well accepted that skeletal muscle uses a high proportion of absorbed energy. In ruminants, contributions of the different energy-yielding substrates to total oxidation in muscle have been estimated *in vivo* at 30–40% for volatile fatty acids (VFA), 15% for ketone bodies (KB), 30–57% for glucose and 5% for long-chain fatty acids (LCFA) (Pethick, 1984). However, data *in vivo* are difficult to interpret due to the heterogeneity of muscle tissue. Therefore, the aim of this work was to characterize *in vitro* energy metabolism of eight bovine muscles and five goat muscles to assess the heterogeneity of muscle tissue in ruminants.

Insulin-responsive glucose transporter (GLUT4) and DNA contents, lipoprotein lipase (LPL, *EC* 3.1.1.34), isocitrate dehydrogenase (ICDH, *EC* 1.1.1.42) and lactate dehydrogenase (LDH, *EC* 1.1.1.27) activities were measured in heart (H), *masseter* (MA), *diaphragma* (D), *rectus abdominis* (RA), *semitendinosus* (ST), *tensor fasciae latae* (TFL), *longissimus thoraci* (LT) and *cutaneus trunci* (CT) from seven 5-month-old Montbéliard calves. The same variables except LPL activity were also measured in MA, D, TFL, LT and *anconeus* (A) from six adult non-lactating goats.

DNA content, ICDH and LPL activities were higher in H, MA and D than in other muscles (RA, ST, TFL, LT and CT) from calves (DNA content : 1367–1903 *v.* 870–1115 μg/g wet tissue ; ICDH activity : 1.00–2.50 *v.* 0.15–0.23 nkat/g ; LPL activity : 211–748 *v.* 33–154 mU/g ; $P<0.05$ in each case). In contrast, oxidative muscles (H, MA and D) exhibited lower GLUT4 content and LDH activity than other skeletal muscles (GLUT4 content : <0.1 *v.* 0.4–1.0 densitometric units (DU)/g ; LDH activity : 17–34 *v.* 73–98 nkat/g]. The highest GLUT4 content and LDH activity and the lowest DNA content and LPL activity were observed in LT. Similar results were obtained with goat muscles especially for GLUT4 content (<0.3 DU/g in MA and D *v.* 0.7–1.0 DU/g in A, TFL, LT).

Mean values of GLUT4 contents, in the six bovine muscles in which GLUT4 was detected or in the five goat muscles, were positively correlated with mean LDH activities but negatively correlated with mean values of DNA contents, LPL and ICDH activities ($r > 0.83$; $P<0.05$).

In conclusion, it can be hypothesized that uptake of LCFA from triacylglycerol-rich lipoproteins (related to LPL activity) and catabolism of LCFA, VFA and KB (related to ICDH activity) are high in oxidative muscles as described in single-stomached animals (Pearson & Young, 1989). Our results also suggest that glucose uptake and conversion to lactate (related to GLUT4 amount and LDH activity respectively) are lower in oxidative muscles than in other muscles from ruminants. This is in contrast with the situation in rodents in which glucose uptake and GLUT4 content are higher in oxidative muscles than in glycolytic muscles (James *et al.* 1989). Therefore, oxidative muscles from ruminants may utilize VFA and ketone bodies in preference to glucose for use as energy sources.

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Mechanisms underlying muscle insulin resistance in late pregnancy. By M.J. HOLNESS and M.C. SUGDEN, *Department of Biochemistry, Queen Mary and Westfield College, University of London, London E1 4NS.*

Insulin resistance in late pregnancy is viewed as an adaptation to ensure an optimum glucose supply for the fetus. Hepatic and peripheral insulin resistance has been demonstrated using the euglycaemic-hyperinsulinaemic clamp technique in anaesthetized, late-pregnant rats (Leturque *et al.* 1984). However, anaesthesia suppresses glucose utilization (Rg) by the important oxidative skeletal muscles (Pénicaud *et al.* 1987).

The present study examined muscle Rg using the euglycaemic-hyperinsulinaemic clamp in combination with 2-deoxy[1-^{3}H]glucose administration (see Leturque et al. 1986 for details) in unrestrained, unanaesthetized late-pregnant rats at 19-20 d gestation. Pregnant and control (unrestrained, unanaesthetized virgin) rats were studied in the post-absorptive state (6 h-starved) and after 24 h starvation. Individual muscles of differing oxidative capacities and contractile activities in the resting state were studied, namely *tibialis anterior* (TA, non-working), *soleus* (SOL, working) and heart (working). The fibre profiles (fast oxidative glycolytic:fast glycolytic:slow oxidative) of TA and SOL were 66:32:2 and 0:0:100 respectively. Insulin was infused at 4.17 mU/kg per min to achieve physiologically-high circulating insulin concentrations. Variable rates of glucose infusion were used to maintain glycaemia at 4 mM during clamp.

	Insulin-stimulated Rg (ng/min per mg)											
	TA				SOL				Heart			
	6 h starved		24 h starved		6 h starved		24 h starved		6 h starved		24 h starved	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Virgin	13.3	1.4	31.7§	2.9	44.6	3.4	53.4	4.0	95.6	7.5	52.1§	5.5
(*n*)	(20)		(14)		(20)		(14)		(10)		(7)	
Pregnant	11.5	1.3	26.0§	3.5	37.0	1.7	42.7	4.3	47.2***	5.7	37.2	6.6
(*n*)	(16)		(9)		(16)		(10)		(8)		(5)	

*** Significantly different from 6 h starved $P<0.001$; §§§ significantly different from virgin $P<0.001$.

Prolonged (24 h) starvation was associated with a highly significant enhancement of insulin-stimulated Rg in TA, representing increased glucose storage. Insulin-stimulated Rg by TA was similarly enhanced by extending starvation in pregnant rats. Insulin-stimulated Rg was unaffected by prolonged fasting in SOL (where a high proportion of glucose is used to sustain muscle work): in SOL, pregnancy was associated with a modest (approximately 20 %) decline in insulin-stimulated Rg both after 6 h and after 24 h starvation. Insulin-stimulated Rg was severely impaired (by 46%) by prolonged 24 h starvation in hearts of virgin rats. This effect of prolonged starvation was mimicked even by short-term (6 h) fasting in pregnant rats. The results suggest that the insulin resistance of late pregnancy is achieved via decreased glucose utilization by muscle via effects exerted at the levels of the adipocyte and the liver which impair the ability of insulin to suppress the production of alternative lipid substrates.

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Synthesis and degradation of hepatic glycogen in uraemic rats. By Z. BAKKOUR, D. LAOUARI and C. KLEINKNECHT, *INSERM U251, Faculté Xavier Bichat, Paris, France,*

Hepatic glycogen (gly) is low in uraemia particularly with a sucrose-rich diet (S diet; Laouari D. *et al.* 1994).To explain this effect, we compared uraemic (U) rats on S diet with pair-fed control (C) rats and studied: (1) gly degradation at 2, 7 and 18h after feeding (study I), (2) gly synthesis at 30 and 60 min after either a glucose (G) or a fructose (F) load in rats fasted for 18h.

We measured changes in gly (Δ gly), glucose-6-phosphate (G6P) an activator of gly synthesis, activities of gly synthase (gly S; EC.2.4.1.11) and phosphorylase (gly P; EC.2.4.1.1) involved in gly synthesis and degradation respectively.

During fasting, gly decreased with time in U and C rats but the decrease was greater in U than in C rats so that gly of U rats was 76, 26 and 15 % that of C rats at 2, 7 and 18 h respectively. Gly P rose at 7 h and gly S rose at 18h to the same extent in the two groups.

Time post-meal	Gly		Gly S		Gly P	
(h)	µmol/g		µmol/min per g		µmol/min per g	
	C	U	C	U	C	U
2	430	330*	0.07	0.05	5.6	4.7
7	240	65*	0.05	0.05	11.0	9.5
18	27	4*	0.13	0.11	10.3	9.0

* Significantly different from control : P < 0,05.

Gly synthesis was greater after the F than after the G load, consistent with higher gly S, lower gly P and higher G6P. The G load produced comparable gly storage in U and C rats despite different gly P and gly S activities. The F load produced greater gly synthesis in U than in C rats associated with lower gly P.

Time post-load (min)	Δ Gly µmol/g		Gly S µmol/min per g		Gly P µmol/min per g		G6P nmol/g	
	C	U	C	U	C	U	C	U
G-30	30	24	0.7	0.3*	8.9	7.7	111	124
G-60	38	42	0.5	1.0*	12.8	7.0*	202	187
F-30	31	42*	1.0	1.1	11.2	6.2*	268	144*
F-60	60	79*	0.9	1.1	8.9	5.4*	188	135

* Significantly different from control : P < 0,05.

In conclusion, glycogenolysis was enhanced in uraemia during fasting. This cannot be ascribed to changes in gly P activity but may be involve *in vivo* activators of gly P. Gly synthesis was not reduced in uraemia after sugar loads and, on the contrary, improved after a F load consistent with reduced gly P and unrelated to synthase activity and G6P level. The role of glycogenolysis in gly depletion of U rats can be suggested.

Laouari D, Jurkovitz C, Burtin M, Bois B, Vassault A and Kleinknecht C. *Metabolism* 43, 403-412,1994.

https://doi.org/10.1079/PNS19950058 Published online by Cambridge University Press

Differential handling of substrates in rat strains that vary in their sensitivity to dietary obesity.
By H. NAGASE, D.A. YORK and G.A. BRAY, *Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, USA*

Osborne-Mendel (OM) rats develop obesity when fed on a high-fat (HF) diet whereas S5B/Pl rats remain thin. Previous observations had shown that S5B/Pl rats had higher circulating levels of ketone bodies and higher rates of ketone body uptake into the brain.

We have investigated the effects of pyruvate and lactate on food intake, serum insulin, hepatic glycogen levels and hepatic pyruvate dehydrogenase (PDH) (EC 1.2.4.1) activity in OM and S5B/Pl rats. Male OM and S5B/Pl rats were adapted over a 12-d period to a HF or low-fat (LF) diet providing 56% or 10% of energy as fat respectively. Both pyruvate and lactate (5 mmol/kg intraperitoneally [i.p.]) inhibited food intake of overnight-fasted OM rats fed on either the HF or LF diet. In contrast, the responses to pyruvate and lactate were attenuated in S5B/Pl rats fed on the LF diet and absent in S5B/Pl rats fed the HF diet. Serum pyruvate and lactate levels were increased in S5B/Pl rats fed *ad lib* compared with OM rats. After overnight starvation pyruvate and lactate levels fell to the same levels in both strains but β-hydroxybutyrate (βOHB) rose to higher levels in S5B/Pl rats. There were no dietary effects on serum pyruvate levels in either strain and no differences in the pyruvate:lactate ratios. After i.p. injection of pyruvate, serum pyruvate and lactate rose to higher levels in S5B/Pl rats and βOHB remained elevated. Hepatic glycogen levels were not different between strains.

Both the total activity of hepatic PDH and the activity of the dephosphorylated active form of the enzyme were assayed. Total PDH activity was similar in both OM and S5B/Pl rats. However, the proportion of the active dephosphorylated form was increased in fed OM rats compared with S5B/Pl rats. This difference was particularly pronounced after pyruvate injections. Serum insulin was increased in OM rats. Pyruvate increased serum insulin levels in OM rats fed on both diets but had no effect in S5B/Pl rats fed on either diet.

The results suggest that S5B/Pl rats have a reduced clearance of pyruvate. This may reflect the reduced PDH activity that results from the combined effects of lower insulin and higher βOHB concentrations. The absence of pyruvate stimulated insulin secretion may enhance fatty acid oxidation and reduce carbohydrate oxidation in S5B/Pl rats and contribute to their resistance to HF-diet-induced obesity.

Selective in vivo **and** in vitro **mobilization of individual fatty acids during fat store depletion.**

By R. GROSCOLAS, E. MIOSKOWSKI and T. RACLOT, *Centre d'Ecologie et Physiologie Energétiques, CNRS, 23 rue Becquerel, 67087 Strasbourg, France*

Adipose tissue is the reservoir of a wide spectrum of fatty acids differing in their chain length, unsaturation and positional isomerism. In contrast to what has been generally assumed, we recently demonstrated that the *in vitro* release of fatty acids by isolated fat cells is a selective process. As a rule, fatty acids are more readily released when they are short, unsaturated and have double bonds close to the methyl end of the chain (Raclot & Groscolas, 1993). However, whether such a selectivity operates *in vivo* and whether it has a physiological relevance is unknown.

In the present study, the mobilization of thirty-eight individual fatty acids from retroperitoneal adipose tissue (RP) of rats fasted for 1, 7 or 10 d was studied. RP was previously enriched in very-long-chain mono- and polyunsaturated fatty acids by feeding rats on a fish-oil diet. At each stage of the fast, the composition of RP triacylglycerols (TAG), the total mass of each individual RP fatty acid and the composition of free fatty acids released from isolated RP cells under conditions of stimulated lipolysis (noradrenaline 1 μmol/l) was determined by GLC.

The mass of total RP fatty acids decreased by 55 and 93 % after 7 and 10 d fasting, respectively. Throughout the fast, RP became progressively and relatively enriched (by 1.5 to 2-fold) in very-long-chain saturated and monounsaturated fatty acids, and relatively depleted (by 1.5 to 4-fold) in polyunsaturated fatty acids with 4–5 double bonds and 18–20 carbon atoms. Between days 1 and 7 of the fast, 20 % (22:1*n*-11) to 90 % (20:5*n*-3) of the initial store of individual fatty acids was mobilized. Actually, the *in vivo* relative mobilization of fatty acids (the ratio of their weight % in removed fatty acids to their weight % in RP TAG) was dependent on their molecular structure. At a given chain length, relative mobilization increased exponentially with unsaturation. At a given unsaturation, it decreased with increasing chain length. On average, a 2-carbon atom displacement of double bonds towards the methyl end of the chain also resulted in a 26 (SEM 8)% increase in the *in vivo* relative mobilization. Very similar relationships were found between the molecular structure of fatty acids and their *in vitro* relative mobilization from RP (% in released free fatty acids / % in fat cell TAG). A very significant direct relationship was found between *in vivo* and *in vitro* relative mobilization, indicating that on average the *in vivo* and *in vitro* relative mobilization of individual fatty acids was the same.

In conclusion, this study demonstrates that the selectivity of the release of fatty acids from fat cells is operating *in vivo*, and that it entirely accounts for the selective removal of individual fatty acids during a fast-induced fat store depletion. The selectivity of the removal is based on the molecular structure of fatty acids, but does not seem to be related to any demand of the body for particular fatty acids.

Raclot, T. & Groscolas, R. (1993). *Journal of Lipid Research* **34**, 1515–1526.

Fish oil attenuates long-term enhancement of hepatic pyruvate dehydrogenase kinase activity by dietary saturated fat. By M.C. SUGDEN, M.J. HOLNESS, K.A. ORFALI and L.G.D. FRYER, *Department of Biochemistry, Queen Mary and Westfield College, University of London, London E1 4NS.*

Work from others has examined the effects of manipulation of dietary subtype on whole-body insulin resistance in the rat (Storlein *et al.* 1987, 1990). Providing a diet high in safflower oil (18:2*n*-6) or saturated fat leads to whole-body insulin resistance which is no longer observed if a portion of the fat component is replaced by long-chain *n*-3 fat (fish oil). The present study examined the effects of administration of a diet high in lipid on the activity of hepatic pyruvate dehydrogenase (PDH) kinase (EC 2.7.1.99).

Rats were maintained for 4 weeks on a standard (high-carbohydrate) diet (8 % energy as lipid) or one of two isoenergetic high-fat diets (47 % energy as lipid). The first high-fat diet (SatFAT) contained 42 % saturated long-chain fatty acids (FA; mainly 16:0 and 18:0), 43 % monounsaturated FA (mainly 18:1) and 15 % polyunsaturated FA (PUFA), mainly *n*-6. The second high-fat diet (Sat FAT/long *n*-3) was identical to the SatFAT diet except that long-chain *n*-3 from marine oil replaced 7 % of the FA component of the SatFAT diet. Hepatic PDH kinase was assayed in extracts of hepatic mitochondria (Denyer *et al.* 1986). Hepatic glycogen was measured in extracts of freeze-clamped liver samples (Holness *et al.* 1988).

	Standard		SatFAT diet		Sat FAT/long n-3	
	Mean	SE	Mean	SE	Mean	SE
Hepatic glycogen (mg/g)	98.6	13.1	72.2	7.6	55.2*	4.0
(*n*)	(3)		(6)		(6)	
PDH kinase activity (/min)	0.092	0.010	0.192***	0.022	0.109	0.012
(*n*)	(13)		(6)		(6)	

Significantly different from rats maintained on the standard diet: * $P<0.05$; *** $P<0.001$.

The provision of the SatFAT diet for 28 d was associated with a significant, stable enhancement of hepatic PDH kinase activity to 211.0 (SE 24.2) % (*n* 6) of that of rats maintained on standard diet without a significant fall in hepatic glycogen. Substitution of only 7 % FA with long chain *n*-3 PUFA from marine oil completely reversed the effects of consumption of the SatFAT diet to evoke a stable increase in hepatic PDH kinase activity. The quality of dietary lipid therefore has a direct and specific effect of the activity of a key hepatic enzyme regulating the ability of the liver to utilize pyruvate for oxidation or lipid synthesis.

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Recycling of the carbon skeleton of polyunsaturates during lipid synthesis in the neonatal rat brain. By S.C. CUNNANE[1], S.S. LIKHODII[1] and M.A. CRAWFORD[2], [1]*Department of Nutritional Sciences, University of Toronto, Toronto, M5S 1A8 Canada and* [2]*Institute of Brain Chemistry & Human Nutrition, Queen Elizabeth Hospital for Children, Hackney, London E2 8PS.*

Polyunsaturated fatty acids (PUFA) are needed for normal neonatal brain development but they are also readily oxidized to CO_2. We have investigated whether, in addition to being chain-elongated and desaturated, the carbon skeleton of shorter chain PUFA may be partially oxidized but reutilized during lipid synthesis. For instance, although cholesterol is an important component of brain lipid composition, the origin of the carbon used in its synthesis is largely unknown. Carbon-13 (^{13}C) labelled PUFA were used to trace a possible link between dietary PUFA and brain cholesterol.

A mixture of four closely related 96% uniformly ^{13}C-enriched PUFA methyl esters (about 25% each of hexadecadienoate (16:2*n*-6), hexadecatrienoate (16:3*n*-3), linoleate (18:2*n*-6) and α-linolenate (18:3*n*-3); Martek, Columbia, MD, USA) were injected intragastrically into 9-day-old rat pups (40 mg/pup). Rat pups were euthanized 1, 7 or 14 d later. Total lipids were extracted from whole brain, liver and remaining carcass using chloroform-methanol (2:1). Portions were analysed by ^{13}C nuclear magnetic resonance (NMR) spectroscopy to determine the distribution of ^{13}C in cholesterol and long-chain fatty acids and by isotope ratio mass spectrometry (IRMS) to determine actual ^{13}C enrichment in the lipid extracts. Brain fatty acid composition was measured by capillary gas-liquid chromatography.

One day after ^{13}C PUFA injection, ^{13}C enrichment measured by IRMS increased by 25-30% above background abundance in each of brain phospholipids, free fatty acids and free cholesterol. Over the next 14 days, ^{13}C enrichment remained high in the phospholipids but declined by 30% in brain cholesterol. ^{13}C enrichment in individual brain fatty acids was present primarily in docosahexaenoate (22:6*n*-3), arachidonate (20:4*n*-6), palmitate (16:0), oleate (18:1*n*-9), and stearate (18:0) and paralleled the developmental changes in brain fatty acid composition. Using ^{13}C NMR, ^{13}C-enrichment in brain cholesterol 7 d after injection of the ^{13}C PUFA was shown to be present in a highly specific pattern which could be accounted for by ^{13}C-enriched carbons being incorporated into cholesterol as both ^{13}C singlets and ^{13}C pairs, both presumably derived from ^{13}C-enriched acetate or ketone bodies following ß-oxidation of the injected ^{13}C-labelled PUFA.

We conclude that, in the neonatal rat brain, dietary 16 and 18-carbon PUFA are not only elongated and desaturated but the PUFA carbon skeleton is recycled and utilized during de novo synthesis of long-chain saturated and monounsaturated fatty acids and cholesterol (Cunnane *et al.* 1994). Whether the ^{13}C-enriched cholesterol was synthesized in the brain or transported to it awaits clarification.

Supported by the Ontario Ministry of Colleges and Universities.

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Lipoprotein lipase activity in bovine and ovine adipose tissue explants: effects of insulin and/or dexamethasone. By Y. FAULCONNIER and Y. CHILLIARD, *Laboratoire Sous-Nutrition des Ruminants, INRA, Theix, 63122 Ceyrat, France*

Effects of insulin (I; 2 mU/ml) and/or dexamethasone (D; 100 nmol/l) were studied on perirenal adipose tissue (AT) from non-lactating non-pregnant cows (n = 5) and ewes (n = 5) fed on a restricted diet (that provided 20-22% of energy maintenance requirement, EMR) for 8-10 d and then overfed (188 or 228% of EMR, for cows or ewes) for 21 (cows) or 10 (ewes) d until slaughter. Explants of AT were cultured in sterile conditions with or without hormones for 2 or 7 d (Faulconnier *et al.*, 1994). The lipoprotein lipase activity (LPL; *EC* 3.1.1.34) was measured using a detergent (Deoxycholate-Nonidet P_{40}) extraction procedure.

The LPL activity was lower after 2 d culture (Table) than in fresh AT (241 and 331 nmol fatty acids/min per 10^6 adipocytes for cows and ewes respectively). In control conditions, LPL activity was similar in the two species and declined from day 2 to day 7.

	LPL (Day 2)*				LPL (Day 7)*			
Species	Control	I	D	I+D	Control	I	D	I+D
Bovine	34.1^{c}	53.4^{b}	44.6^{bc}	70.2^{a}	18.0^{b}	50.6^{a}	21.2^{b}	39.3^{ab}
Ovine	35.8^{b}	41.6^{b}	46.6^{b}	86.2^{a}	18.0^{b}	23.6^{b}	20.6^{b}	69.2^{a}

*Adipose tissue LPL activity (nmol/min per10^6 adipocytes) after 2 or 7 d culture.
a, b, c, d Values within a row bearing unlike superscripts were significantly different, $P < 0.05$.

Insulin increased ($P < 0.05$) LPL activity in bovine AT (+57 and +181% on days 2 and 7) but did not significantly change this activity in ovine AT. The D alone had no significant effect on LPL activity, although it tended to increase (+31% on day 2) this activity in the two species. The addition of D to the insulin-supplemented medium was different according to the species (interaction species x insulin x D, $P < 0.001$). D addition to the insulin-supplemented medium increased ($P < 0.05$) LPL activity in ovine AT after 2 (+107%) and 7 (+193%) d culture; in bovine AT the effect of D on day 2 was small (+31%) and became negative (-22%) on day 7 of culture.

This study shows large differences in the response to insulin of LPL activity in ovine and bovine AT explants, and in its interaction with dexamethasone.

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The effects of meal frequency on lipoprotein lipase activity. By C. HALLETT, S.G. ISHERWOOD, J.A. LOVEGROVE and M.C. MURPHY, *Nutritional Metabolism Research Group, School of Biological Sciences, University of Surrey, Guildford, Surrey, GU2 5XH*

It has been suggested that the metabolic consequences of a given diet may depend partially on the frequency of meals eaten. Increasing meal frequency has been shown to decrease fasting blood total and low-density-lipoprotein cholesterol levels (Jenkins *et al.* 1989) but the postprandial responses to a standard mixed meal after the chronic ingestion of a nibbling diet (12 meals/d) or a gorging diet (2 meals/d) have not been fully investigated.

Eleven female subjects (aged 22 (SD 0.89) years , body mass index 23.6 (SD 2.77)) were used for the present study. The study was a cross-over design, where the subjects were allocated in random sequence to either the nibbling or gorging diet for 2-week periods, with a 3-week washout period between the diets. Identical foods were eaten on each diet. The day before commencing the study (representing the normal diet), and after each 2-week diet, a postprandial study was performed. Eight hours following the consumption of a mixed meal, subjects were given a bolus dose of heparin (7500U) intravenously, and two postheparin blood samples were collected after 5 and 15 min. Lipoprotein lipase (LPL; *E C* 3.1.1.34) activity was measured by the detection of free fatty acids from a labelled triolein substrate emulsion (Nilsson-Ehle & Schotz, 1976). The LPL activities are shown in the Table, expressed in mU/ml where 1mU represents 1nmol fatty acids released/ml per min at 37°.

	Postheparin LPL Activities (mU/ml)					
	Baseline 5 min	Baseline 15 min	Gorging 5 min	Gorging 15 min	Nibbling 5 min	Nibbling 15 min
Mean (*n* 11)	129.14	283.90[a]	157.19	254.40	102.20	204.30[b]
SEM	36.51	38.32	43.21	41.58	37.89	47.38

a, b Mean values were significantly different ($P < 0.05$), paired comparisons.

LPL activity of all groups was significantly higher at 15 min postheparin when compared with 5 min postheparin ($P < 0.05$). No significant differences were seen in 5 min postheparin LPL activities following the nibbling or gorging diet, nor in 15 min postheparin LPL activities after consuming the gorging diet, when compared with basal levels. However, the 15 min postheparin LPL activity was significantly lower after the nibbling diet compared with the baseline measurement ($P < 0.05$). Fifteen min postheparin LPL activities were lower than baseline following either period of dietary intervention.

We therefore conclude that the lower response to a standard meal in the nibblers may have been a conditioning effect due to previous small meals. It may indicate a reduced capacity to clear a high fat meal once accustomed to a nibbling diet.

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Lipoprotein lipase activity in muscular and adipose tissues from normal and double-muscled growing calves. By J.F. HOCQUETTE[1], S. BRAZI[1], T. OLIVECRONA[2], G. BENGTSSON-OLIVECRONA[2] and Y. GEAY[1], *[1]Laboratoire Croissance et Métabolismes des Herbivores, INRA, 63122 Saint-Genès Champanelle, France, and [2]Department of Medical Biochemistry and Biophysics, University of Umea, S-90187 Sweden*

The rate-limiting step for delivery of long-chain fatty acids (LCFA) from triacylglycerol-rich lipoproteins to peripheral tissues is usually lipoprotein lipase (LPL ; *EC* 3.1.1.34)-catalysed hydrolysis of triacylglycerols. There is evidence in single-stomached animals for a correlation between local production of LPL by heart, skeletal muscles and adipose tissues and the uptake of LCFA from lipoprotein by these tissues (Olivecrona *et al.* 1991). In the same way, LPL activity in bovine adipose tissues is positively related to the metabolic activity of these tissues (Robelin & Chilliard, 1989). To our knowledge, LPL has never been studied in ruminant muscle. Therefore, the aim of the present study was to characterize LPL activity in oxidative and glycolytic bovine muscles in comparison with rat muscles, and to study the influence of the muscular hypertrophy gene on bovine LPL activity in muscles and adipose tissues.

Conditions of LPL assay were described by Peterson *et al.* (1985). Briefly, the substrate used was the commercial emulsion Intralipid® into which [^{3}H]oleic acid-labelled triolein had been incorporated by sonication. Heat-inactivated sera from fasted rat or calf were used as sources of activator. Incubation time was 1 h at 25°. LPL activity was partly inhibited by 1M NaCl in the incubation medium and completely abolished by antibodies raised against LPL from bovine milk. LPL activity was highest in oxidative muscles from rat (4000 and 2200 mU/g wet tissue in heart and soleus respectively) and calf (748 (SE 35) and 674 (SE 118) mU/g in heart (H) and masseter (MA) respectively ; values from four 5-month-old Montbéliard calves) and low in other skeletal muscles from the two species (28-152 mU/g). LPL activity was positively related to isocitrate dehydrogenase (*EC* 1.1.1.42) activity and negatively related to lactate dehydrogenase (*EC* 1.1.1.27) activity in bovine muscles.

LPL activity was measured in H, MA, omental and subcutaneous adipose tissues (OAT, SCAT) from six normal and six double-muscled (DM) Belgian Blue calves slaughtered at 9-11 months of age. DM cattle exhibited muscular hypertrophy (+22.5% of total muscle mass in carcass, $P<0.05$), and a lower fat deposition (-44.3%, $P<0.003$). Regardless of the expression of the results (in mU/g wet tissue or in U/total tissue mass), LPL activity tended to be higher in oxidative muscles and lower in adipose tissues from DM calves (H: +25.2% in mU/g ($P=0.25$) and +21.5% in U/tissue ($P=0.32$) ; MA: +23.4% ($P<0.05$) and +46.4% ($P<0.05$) ; OAT: -8.5% (not significant) and -52.6% ($P=0.19$) ; SCAT: -32.8% ($P=0.47$) and -81.5% ($P<0.05$)). LPL activity in other muscles was too low to show any difference between the two groups.

In conclusion, our results show large variations of LPL activity among bovine muscles. Our data also suggest that a specific regulation of LPL activity in peripheral tissues from DM calves might favour LCFA uptake by muscles for catabolism rather than by adipose tissues for fat deposition.

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Nutritional manipulation of perirenal adipose tissue development in hand-reared postnatal lambs. By J.J. GATE[1], J.A. BIRD[1], M.J. BRYANT[2], L. CLARKE[1], M.A. LOMAX[1] and M.E.SYMONDS[1], *Departments of [1]Biochemistry and Physiology and [2]Agriculture, University of Reading, PO Box 228, Reading RG6 2AJ*

During postnatal development the rate at which brown adipose tissue (BAT) adopts the characteristics of white adipose tissue is delayed by hand-rearing in a cool ambient temperature (Symonds & Lomax, 1992). The present study investigated if this process may be futher manipulated by altering the level of feed intake.

Fourteen lambs of similar birth weights (3.62 (SEM 0.25) kg), from triplet pregnancies were removed from the ewe within 6 h of birth and individually housed at an ambient temperature of 10-15°. Each lamb was fed on milk replacer at either a high (H; 450 g/d) or low (L; 225 g/d) level. At 35 d of life the lambs were humanely killed and the perirenal adipose tissue was removed for measurement of thermogenic activity (guanosine diphosphate (GDP) binding) and types I and II iodothyronine 5'deiodinase (I5'D) activity (Clarke *et al.* 1994).

								I5'D			
		Weight (g)		Lipid (g)		GDP binding (pmol/mg mitochondrial protein)		Type I (pmol/mg protein per h)		Type II (fmol/mg protein per h)	
Group	*n*	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
H	7	187	19	46	10	151	27	225	36	171	52
L	6	24***	7	9***	3	115	19	144	28	102	21

Significantly different from Group H (Students t test): ***$P<0.001$.

H lambs grew 46% faster (H 312 (SEM 33); L 213 (SEM 16) g/d), deposited 5-fold more lipid in perirenal adipose tissue, which exhibited 60-70% higher type I and II I5'D activity than those reared on a low plane of nutrition. However, there was no difference in thermogenic activity of BAT between groups.

It is concluded that in hand-reared lambs the level of feed intake influences the rate of perirenal lipid deposition and I5'D activity, but not the BAT thermogenic activity.

This work was funded by the Wellcome Trust and AFRC (JB), MLC (LC) and MAFF (JG) studentships.

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Could linoleate be a significant metabolic fuel during fluctuating but adequate energy intake?

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At the minimum adequate intake level for linoleate (2-3 % energy), <40% of dietary linoleate is accumulated as linoleate or the *n*-6 long-chain polyunsaturates (LCP) derived from linoleate. Thus, under adequate growth conditions, >60% of dietary linoleate is still oxidized to CO_2 (Chen & Cunnane, 1993). Fasting reduces the oxidation of linoleate but refeeding after fasting and chronic undernutrition both increase linoleate oxidation (Chen & Cunnane, 1993; Cunnane *et al.* 1993). To test the hypothesis that repeated fasting-refeeding (weight cycling) would increase whole-body oxidation of linoleate, the intake, excretion and accumulation of linoleate and *n*-6 LCP was determined in young rats that underwent four cycles of 24 h fasting with each fasting cycle followed by 72 h refeeding.

Five-week-old rats consumed rodent chow containing 10 % energy as fat and 3.4 % energy as linoleate. Control rats had free access to the rodent chow while the weight cycled rats had free access for 3 out of 4 d. Food intake and faecal output were measured daily. Four to five rats were killed at the beginning of the study and from each group during the fasting and refeeding phases. Total lipids in liver, adipose tissue and carcass were extracted and their long chain fatty acid profiles determined quantitatively (Chen & Cunnane, 1993).

Food intake in the weight-cycled group was the same as during free feeding. Whole-body fatty acid balance calculated during the 16 study period showed that 19% of dietary linoleate accumulated in the weight-cycled rats compared with 34% in the free-fed controls ($p<0.01$; Student's paired *t* test). Arachidonate (20:4*n*-6) and total *n*-6 LCP also accumulated to lower levels in the weight-cycled rats than in the controls. In contrast, as a percentage of dietary intake, whole-body accumulation of palmitate (16:0), stearate (18:0) and oleate (18:1*n*-9) was not different between the weight-cycled group and the free-fed controls.

Thus, whole-body fatty acid balance analysis, which accounts for total fatty acid intake, excretion and net accumulation (but not *de novo* synthesis), showed that the disappearance of total polyunsaturates (both *n*-6 and *n*-3 families), especially linoleate and α-linolenate, was significantly higher in weight-cycled rats than in controls. This occurred even though food intake during the refeeding periods in the weight-cycled rats was equivalent to that consumed during free feeding. Since linoleate was the main dietary fatty acid consumed and, in this study, weight cycling caused 81% of it to be completely oxidized, we wonder whether linoleate can be a more metabolic fuel under conditions of fluctuating energy intake than it is under free-feeding conditions.

Supported by the Natural Sciences and Engineering Research Council of Canada.

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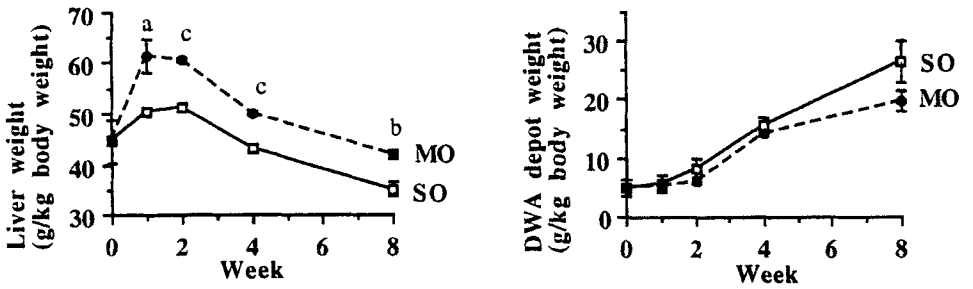
Time course of the effect of diets rich in *n*-6 or *n*-3 polyunsaturated fatty acids on serum cholesterol levels, liver weights and adipose deposition in the rat. By E. J. SHERRINGTON, N. M. JEFFERY and P. C. CALDER, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU

Dietary fat consumption is believed to be associated with the development of disorders such as cardiovascular disease and diabetes mellitus. Recently however, inclusion of polyunsaturated fatty acid (PUFA)-containing oils in the diet has been proposed for the prevention or therapy of these and other diseases (Horrobin, 1990; Drevon *et al.* 1993). We have reported differences in serum lipid levels, liver weights and adipose deposition between rats fed on different types of high-fat diets for 10 weeks (Yaqoob *et al.* 1993*a*,*b*; Sherrington *et al.* 1994). In particular, there were differences between rats fed on diets rich in *n*-6 or *n*-3 PUFA (safflower oil (SO)) and menhaden oil (MO) respectively). MO-fed animals had lower serum cholesterol levels, larger livers and less adipose deposition than animals fed on SO. It was considered important to investigate what period of feeding was required before these differences became apparent.

Male weanling Lewis rats were fed for between 1 and 8 weeks on diets containing 200 g/kg either SO or MO; the diets were identical in all other respects. After slaughter serum was prepared and the liver and dorsal wall of abdomen (DWA) adipose depot were dissected out and weighed. The DWA depot was chosen as it has been shown to be more sensitive to differences in the fatty acid composition of the diet than other adipose depots (Sherrington et al. 1994). Serum cholesterol concentrations were determined enzymically. Data are from three animals at each time point.

Serum total cholesterol concentrations (mg/ml) were significantly lower ($P < 0.02$) in animals fed on the MO diet (1.00 (SEM 0.02), 0.79 (SEM 0.08), 0.97 (SEM 0.05) at weeks 2, 4 and 8) than in SO-fed animals (1.22 (SEM 0.02), 1.46 (SEM 0.13), 1.30 (SEM 0.05) at weeks 2, 4 and 8). The livers from the MO-fed animals were significantly larger than those from the SO-fed animals at all time points (see Figure). The DWA depot was smaller after 2, 4 and 8 weeks of feeding the MO diet than after feeding the SO diet; however this difference was not statistically significant (see Figure). In contrast, after 10 weeks of feeding, DWA weight is significantly greater in SO-fed animals (Sherrington *et al.* 1994).



Statistical significance is indicated as: a $P < 0.05$, b $P < 0.02$, c $P < 0.01$.

The present study shows that the different effects of feeding diets rich in *n*-6 or *n*-3 PUFA on serum cholesterol level and liver weight become apparent within 2 weeks of beginning the dietary regimen; the effect on adipose deposition occurs later. The increase in liver size in MO-fed animals compared with those fed on the SO diet is believed to be a result of an increase in lipid deposition, due to a defect in lipoprotein assembly or secretion and in peroxisome number, resulting in enhanced PUFA oxidation (unpublished results). These two effects of *n*-3 PUFA would be expected to result in lowered serum lipid levels and less adipose deposition, as observed here.

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Rates of triacylglycerol and cholesterol synthesis and the effects of fitness. By M.L.THOMASON, M.J.RENNIE and P.WATT, *Department of Anatomy and Physiology, University of Dundee, Dundee, Scotland, DD1 4HN.*

Endurance-trained individuals are reported to have higher-high-density lipoprotein cholesterol, lower triacylglycerol (Thompson *et al.* 1991) and lower total cholesterol concentrations (Stray-Gunderson *et al.* 1991) when compared with sedentary controls. It may be postulated that these differences in the lipid profiles could be due to a reduced synthetic rate in the endurance-trained (ET) compared with the untrained (UT). In order to test this hypothesis, we compared the fractional synthetic rates (FSR) of triacylglycerol and cholesterol in male subjects differing in aerobic fitness, as indicated by their maximal aerobic capacity ($\dot{V}O_2$ max.). Subjects were refed hourly after an overnight fast, with a nutritionally complete liquid diet plus 0.059 mmol/kg per h [1-^{13}C] -acetate given orally. Venous blood samples were taken before dosing and every 2 h thereafter over the 10 h study period. Lipids were extracted from plasma using solvents, separated by thin layer chromatography then subjected to combustion isotope ratio mass spectrometry to determine ^{13}C:^{12}C ratio. Venous plasma acetate enrichments were obtained using gas chromatography-mass spectrometry and were taken to represent enrichment of the percursor pool for triacylglycerol and cholesterol synthesis.

	Age (years)		% Body fat (sum of skinfolds)		$\dot{V}O_2$ max. (ml/kg per min)		FSR cholesterol (%/h)		FSR triacylglycerol (%/h)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
ET (*n* 3)	22	2.1	9.77	1.75	49.8	1.1	1.060	0.752	0.413	0.126
UT (*n* 3)	20	0.3	20.37*	1.77	39.3*	0.4	1.013	0.279	2.973*	1.045

* Significantly different from ET, $P<0.05$.

Triacylglycerol FSR was significantly lower in the ET group compared with the UT group ($P<0.05$) and showed a significant positive correlation with percentage body fat and a negative correlation with $\dot{V}O_2$ max. (both $P<0.05$). Cholesterol FSR was not significantly different between the two groups and was not found to be significantly correlated with the subjects' body fat or $\dot{V}O_2$ max.

In conclusion, the results suggest that the lower plasma cholesterol levels found in ET subjects are not due to a lower FSR in these subjects. However, the reduced triacylglycerol concentrations in ET individuals may be partly the result of a lower rate of *de novo* synthesis. Whether or not this reduction is mediated primarily by aerobic fitness or lower body fat proportions requires further investigation, but nonetheless has important implications for the field of preventative medicine.

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Effect of carbohydrate overfeeding and underfeeding on *de novo* lipogenesis and insulin levels.

By J.-M. SCHWARZ, R. A. NEESE, D. DARE, S. T. TURNER and M. K. HELLERSTEIN, Department of Nutritional Sciences, University of California at Berkeley and San Francisco, USA

De novo lipogenesis (DNL) has been shown to be an important pathway in rodents (Assimacopoulos-Jeannet & Jeanrenaud, 1976). In man, however, the fraction of the body fat that comes from DNL may be less but this is controversial, because it has been difficult to measure DNL accurately *in vivo*. Indirect calorimetry measures only net DNL, the difference between fatty acid synthesis and fatty acid oxidation (respiratory quotient RQ>1). Using traditional tracer methodology it is not possible to determine the true precursor pool enrichment, which is necessary to calculate DNL. We recently developed a new tracer method called *mass isotopomer distribution analysis* (MIDA) by which the precursor pool enrichment and fractional DNL can be determined *in vivo* (Hellerstein & Neese, 1992).

We have previously reported that DNL is significantly correlated with excess energy intake (r^2 0.59, P <0.01) over a broad range of intakes during a 7d *ad lib.* diet. In this study, four lean (weight 77 (SE4) kg) and three non insulin resistant overweight (104 (SE7) kg) subjects were admitted to a metabolic ward for 15 consecutive days during which they ate three controlled diets of varying energy content, for 5d. The difference in energy intake was achieved by altering the carbohydrate (CHO) content of the diet: (standard, 100%; high-energy, 150%; and low-energy 50% of calculated energy requirement). At the end of each phase, [1-^{13}C]acetate (0.09-0.13 mmol/kg per hr) was infused over 16 h from 02.00 until 18.00 hours. Body weight remained stable during the standard diet, increased by 1-3 kg during the high-energy diet, and decreased by 1-3 kg during the low-energy diet. The RQ remained below unity in both fasting and postprandial conditions indicating no net DNL during the three feeding phases. Very-low-density lipoproteins were isolated by ultracentrifugation and transmethylated. The palmitate methyl ester isotopomer abundances were measured by gas chromatography/mass spectrometry (m/z 270-274). MIDA was used to calculate the precursor enrichment (acetyl-CoA) and fractional DNL (see Table).

Diet...		Standard				High-energy				Low-energy			
Subjects		f DNL (%)		Insulin (μU/ml)		f DNL (%)		Insulin (μU/ml)		f DNL (%)		Insulin (μU/ml)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE			Mean	SE
Lean	fasting	1.5	0.4	4.8	0.6	20.0*	4.6	9.5*	0.5	<1		4.8	0.9
	postprand.	2.7	0.9	-		26.2*	5.3	-		<1		-	
Obese	fasting	2.4	0.9	8.0	1.0	9.6*	0.4	14.7*	3.2	<1		7.3	1.5
	postprand.	11.3	3.3	-		20.9*	4.6	-		<1		-	

f DNL, fractional *de novo* lipogenesis; postprand.,-postprandial.
* Significantly different from standard and low-energy diets, P<0.05.

Fasting and post-prandial fractional DNL were significantly increased by the high-energy diet and reduced by the subsequent low-energy diet in both lean and obese subjects. The high-energy CHO diet increased plasma insulin compared with the standard or low-energy diet in both groups.

In conclusion, DNL varies remarkably as a function of the short-term dietary energy balance. Moreover, DNL may be a biomarker of recent dietary energy intake and changes in DNL may precede changes in tissue insulin sensitivity. The relative influence of total *v.* CHO energy and dose-response relationships of energy balance *v.* DNL remain to be determined.

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Hellerstein, M. K. & Neese R. A. (1992). *American Journal of Physiology* **26**, E988-E1001.
Hellerstein, M. K., Neese, R. A., Faix, D., Schwarz J-M., Cesar, D. & Shackleton, C. (1993). *Diabetes* Suppl. 1, **42**, a442.

De novo lipogenesis and energy expenditure before and after food restriction in morbid obesity.

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Fasting *de novo* lipogenesis (DNL) is increased in hyperinsulinaemic obese men and is positively correlated with fasting insulin levels (Schwarz *et al.* 1993). Furthermore in normal men DNL is increased during excess energy intake with *ad libitum* diets (Hellerstein *et al.* 1993). From these findings it has been hypothesized that fasting DNL may be a marker for both hepatic insulin resistance and excess energy intake.

In the present study we examined the effect of food restriction on DNL and energy expenditure in morbid obesity treated by gastric bypass surgery. Fasting DNL was measured in two obese women *in vivo* by infusing [1-^{13}C]acetate (0.09-0.13 mmol/kg per h) for 9 h overnight.. The plasma enrichment of very-low-density-lipoprotein triacyglycerol was measured by gas chromatography-mass spectrometry, and the rate of triacyglycerol synthesis determined by using mass isotopomer distribution analysis (Hellerstein & Neese, 1992). Measurements were made before gastric bypass surgery and 2 weeks after the surgery. Resting energy expenditure (REE) and the respiratory quotient (RQ) were measured by indirect calorimetry.

Subjects	Body wt (kg)	f DNL (%)	REE (MJ/d)	RQ
1				
Pre-operation	137	6.9	10.60	0.77
Post-operation	125	<<1	10.10	0.73
2				
Pre-operation	142	8.4	8.48	0.80
Post-operation	131	<<1	8.30	0.71

f DNL, fractional *de novo* lipogenesis.

Two weeks after surgery weight loss was approximately 9% for both subjects. REE was reduced by 2% to 5%, and RQ by 5% to 11%. Fractional DNLwas reduced by 90% for both subjects.

Based on these preliminary results, the elevated fasting DNL characteristic of obesity with insulin resistance is dramatically reduced by short-term food restriction, and this reduction is not reflected in the gross changes of energy metabolism measured by indirect calorimetry. These results demonstrate that changes in substrate metabolism due to food restriction precede alterations in body composition and gross energy expenditure.

Hellerstein, M. K. & Neese, R. A. (1992). *American Journal of Physiology* **26**, E988-E1001.
Hellerstein, M.K., Neese, R.A., Faix, D., Schwarz, J-M, Cesar, D. & Shackleton, C. (1993).. *Diabetes* **42**, Suppl. 1, a442.
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The effects of meal frequency on postprandial plasma triacylglycerol and non-esterified fatty acid concentrations. By J.A. LOVEGROVE, S.G. ISHERWOOD, C. HALLETT and M.C. MURPHY, *Nutritional Metabolism Research Group, University of Surrey, Guildford, Surrey GU2 5XH*

Increasing meal frequency has been shown to decrease fasting blood total and low density lipoprotein cholesterol levels (Jenkins *et al.* 1989) but the postprandial responses to a standard mixed meal after the chronic ingestion of diets with various meal frequencies have not been studied. Prolonged or magnified postprandial lipaemia profiles may be associated with increased risk of coronary heart disease (Zilversmit, 1979) and therefore the study of factors affecting lipaemia is clinically relevant. In the present study eleven female volunteers aged 22 (range 21-24) years with a body mass index of 23.6 (range 20.6-28.4) were randomly allocated to receive identical food as twelve or two meals a day corresponding to a nibbling or a gorging diet respectively. The volunteers received either diet for a period of 2 weeks in a crossover design with a washout period of 3 weeks between the diets. On the day immediately following each diet a test meal was given. This consisted of a cheese sandwich, milk shake and orange juice and contained 4.4 MJ energy, 82 g fat, 63 g carbohydrate and 20 g protein. Subjects were cannulated at 08.30 hours after a 12-h fast and two preprandial blood samples were taken before the consumption of the mixed meal. Samples were then taken at 30 min and hourly for an 8-h period postprandially. Plasma samples were analysed for plasma triacylglycerol (TAG) and non-esterified fatty acids (NEFA) and the area under the time response curve (AUC) was computed for both variables.

	Fasting TAG (mmol l^{-1})		Peak TAG (mmol l^{-1})		Total AUC TAG (min.mmol l^{-1})		Fasting NEFA (mmol l^{-1})		30-240min AUC NEFA (min.mmol l^{-1})		240-480min AUC NEFA (min.mmol l^{-1})	
Diet	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Normal	1.07	0.11	2.12	0.29	753	80	0.763	0.082	98.9	8.7	191.3	13.3
Gorging	1.09	0.08	2.52	0.34	756	68	0.524 *	0.066	84.1 *	8.6	159.5 *	11.3
Nibbling	1.10	0.10	2.30	0.42	788	86	0.475 *	0.058	77.9 *	6.6	178.9	10.6

*significantly different from normal diet ($P<0.02$)

As shown in the Table there were no significant differences in the fasting and postprandial TAG concentrations when the three diets were compared. This supported the findings of Jenkins *et al.* (1989) who reported no significant effect of changes in meal frequency on fasting TAG concentrations. However, significant differences were shown in the fasting NEFA levels when nibbling or gorging were compared with the normal diet ($P=0.02$). There was also a significant suppression in the NEFA AUC from time 30-240 min postprandially in the nibbling and gorging diets compared with the normal diet ($P=0.02$), with the NEFA AUC for the gorging diet remaining significantly lower ($p=0.01$) from time 240-480 min. The late postprandial NEFA release after the gorging diet may be due to the up regulation of the esterification or uptake of NEFA by adipose tissue and/or an increase in the use of NEFA by muscle as an energy source. These results in combination with the levels of other metabolic substrates and hormone levels, to be analysed, will provide a fuller picture of the possible effects of altering meal frequency on the metabolic responses to a mixed meal.

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Dietary fat oxidation in lean and obese women: effect of energy status. By B.J. SONKO[1], A.M. PRENTICE[2], P.R. MURGATROYD[2] and G.R. GOLDBERG[2], *[1] MRC Dunn Nutrition Group, Keneba, The Gambia and [2] Dunn Clinical Nutrition Centre, Hills Road, Cambridge CB2 2DH.*

The present study investigated whether post-meal utilization of ingested fat differs in lean and obese subjects, and whether any differences are related to the subjects' existing state of energy balance.

Six lean (weight 58.6 (SD 5.6) kg; body mass index (BMI) 20.8 (SD 1.3) kg/m^2) and six obese women (weight 101.1 (SD 10.8) kg; BMI 36.7 (SD 5.1) kg/m^2) were each studied three times using whole-body indirect calorimetry and $^{13}CO_2$ breath tests the day after 24 h manipulation of energy intake under standardized resting conditions as follows: underfeeding (UF) 50%, control (C) 100%, and overfeeding (OF) 150% of maintenance energy expenditure estimated as 1.4 x basal metabolic rate (BMR). The manipulated diet provided a fixed proportion (40:46:14) of energy from fat:carbohydrate:protein. After an overnight fast following the manipulation day each subject consumed a test meal providing 21 kJ/kg body-weight (BW) and containing 0.4 g/kg BW ^{13}C-palmitate-enriched maizeoil and 0.34 g/kg BW carbohydrate (CHO). Breath samples were taken at baseline and at 30-min intervals for 420 min after the meal. Total fat and CHO oxidations were estimated from non-protein gas exchanges and balances were calculated as the difference between intake and oxidation. Exogenous fat oxidation was estimated from $^{13}CO_2$ recovery without retention corrections. Results are expressed as means with their standard errors over 420 min following consumption of the test meal.

	Lean						Obese					
	UF		C		OF		UF		C		OF	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
CHO oxidation (g)	19	1	29	2	37	2	21	3	32	5	42	4
Exogenous fat oxidation (g)	4	1	4	1	4	1	5	1	4	1	4	1
Total fat oxidation (g)	36	1	32	2	29	1	49	3	42	4	37	4
Fat balance (g)	-13	0	-9	2	-5	2	-9	2	-2	4	+4	3

In both lean and obese subjects there was a strong positive influence of dietary pre-treatment on post-meal CHO oxidation (ANOVA, $P<0.001$). This effect was presumably mediated by glycogen status since the previous manipulated meal was 16 h before the test meal. Exogenous fat oxidation was about 10% of total fat oxidation and was unaffected by the pre-treatment. Total fat oxidation was reciprocally related to the level of pre-feeding (ANOVA, $P<0.001$), and fat balance altered accordingly. The difference in fat balance between UF and OF was 8 g in the lean women and 13 g in the obese (ANOVA, $P<0.001$ for both), but the treatment x group interaction was not significant. The suppression of fat oxidation and resultant positive energy balance following an identical test-meal provided the day after overfeeding can be viewed as counter-regulatory. The reverse effect would have been needed to re-establish fat balance. These results confirm that auto-regulatory adjustments in CHO oxidation, necessary to maintain glycogen homeostasis, dominate fuel selection and override any drive to maintain fat balance. The results also indicate powerful downstream effects of antecedent diet on fuel selection and macronutrient balance after subsequent meals.

Fatty acid turnover and oxidation during starvation and in response to infused adrenaline in man. By J. WEBBER[1], J. TAYLOR[1], H. GREATHEAD[2], J. DAWSON[2], P. J. BUTTERY[2] and I. A. MACDONALD[1], *[1]Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH and [2]School of Agriculture, University of Nottingham, Sutton Bonnington LE12 5RD.*

There is a progressive increase in plasma fatty acid concentrations and in whole-body utilization of fat as an energy source during starvation. The relationships between plasma fatty acid turnover and oxidation and between plasma and tissue fat oxidation are not clear, but may be altered by fasting. It is known that there is potentiation of some adrenoceptor-mediated processes in response to fasting. The present study was designed to examine the effects of fasting and of infused adrenaline on fatty acid turnover and oxidation. The thermogenic and cardiovascular results from this study have previously been reported (Webber & Macdonald, 1992).

Nine healthy, non-obese subjects were recruited (mean age 22.9 (SE 1.2) years; four men, weight 64.2 (SE 4.2) kg, body mass index 22.0 (SE 0.80) kg/m^2, fat-free mass (50.2 (SE 3.2) kg) for the study. Each subject attended on three occasions, after 12, 36 or 72-h fasts, with the visits in random order. Blood and expired air samples were taken for background plasma [1,^{13}C]palmitate and air $^{13}CO_2$. A prime of NaH^{13}CO3 was then given followed by a continuous infusion of potassium [1,^{13}C]palmitate and samples of blood and expired air were obtained between 45 and 60 min of the infusion. The [1,^{13}C]palmitate was continued whilst adrenaline was infused at 25 ng/min per kg ideal body weight for 90 minutes. Further blood and air samples were taken at 10-min intervals and indirect calorimetry was carried out throughout the infusions. Mass spectrometry was used to measure plasma palmitate concentration, plasma [1,^{13}C]palmitate enrichment and expired air $^{13}CO_2$ enrichment.

	Duration of fasting (h)					
	12		36		72	
	Mean	SE	Mean	SE	Mean	SE
Basal plasma palmitate turnover (μmol/min per kg body wt)	1.48	0.22	1.95**	0.34	2.26**	0.33
Basal plasma palmitate oxidation (μmol/min per kg body wt)	0.64	0.09	0.85*	0.16	0.83*	0.11
Basal palmitate turnover oxidized (%)	44	2	46	5	42	4
Thermogenic response to adrenaline (J/min per kg fat-free mass)	14.6	1.7	16.6	1.8	22.6**	1.6
Peak plasma palmitate turnover in response to adrenaline (μmol/min per kg body wt)	2.31	0.36	2.79**	0.67	2.97**	0.73
Plasma palmitate turnover oxidized over last 30 min adrenaline infusion (%)	34	3	36	4	25*	4

Significantly different from 12-h fast: *P<0.05, **P<0.01, (paired t test).

Basal palmitate turnover increased with duration of fasting as did the thermogenic response to adrenaline, but the lipolytic response to adrenaline was unaffected. Adrenaline reduced the percentage of plasma palmitate turnover which was oxidized. This suggests that adrenaline infusion favours intratissue lipid oxidation and may be responsible for skeletal muscle thermogenesis which is likely to contribute to the whole-body enhanced thermogenic response which occurs after fasting.

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Regulation of the plasma non-esterified fatty acid concentration after meals. By J. CALLOW, R. OWEN, B.A. FIELDING, J.S. SAMRA and K.N. FRAYN, *Oxford Lipid Metabolism Group, Radcliffe Infirmary, Oxford OX2 6HE*

Elevated concentrations of non-esterified fatty acids (NEFA) in the plasma may be deleterious. After a meal containing carbohydrate, the plasma NEFA concentration is usually suppressed for 2 h or more. Ingestion of large amounts of fat may lessen the suppression of plasma NEFA concentrations in the postprandial period (Griffiths *et al.* 1994; Isherwood *et al.* 1994). Such studies are almost always conducted in subjects in the postabsorptive state. This state only arises once a day in normal daily life. In the present studies we have investigated the effects of ingestion of a fatty meal (61 g fat) following 5 h after an earlier meal (54 g fat) on the plasma NEFA concentration.

Seven normal subjects ate a fat-free meal before 19.00 hours and then fasted until breakfast at 07.00 hours, which contained 54 g fat (safflower oil), 140 g carbohydrate and 12 g protein in the form of flapjacks (baked oat 'biscuits'). Five hours later the subjects ate a meal containing 61 g fat (olive oil and avocado), 35 g carbohydrate and 13 g protein. Blood samples were taken before the lunch, and at intervals afterwards for 5 h. The results have been compared with those of two earlier studies in which normal subjects after an overnight fast ate meals containing either 33 g fat, 93 g carbohydrate and 22 g protein ('mixed meal'; Coppack *et al.* 1990), or 80 g fat, 80 g carbohydrate and 18 g protein ('high-fat meal'; Griffiths *et al.* 1994).

After the lunch, plasma NEFA concentrations rose during the first 3 h in all subjects (mean peak value 730 (SE 90) μmol/l, $P < 0.02$) despite elevation of plasma insulin concentration (mean baseline, 16 (SE 5) mU/l; peak at 40 min, 29 (SE 11) mU/l). They then stayed relatively steady until 5 h after the meal. In contrast, after both the mixed meal and the high-fat meal (given after overnight fast) plasma NEFA concentrations fell during the first 2 - 3 h (Table).

	Plasma NEFA concentration, μmol/l									
	Before		30 min		60 min		120 min		300 min	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Present study	490	70	530	120	580	100	490	60	490	110
Mixed meal	490	70	320	80	70	10	80	10	310	30
High-fat	450	60	350	19	190	20	350	62	508	80

We speculate that the postprandial increase in NEFA concentration reflects the rapid elevation of chylomicron-triacylglycerol (TG) concentration after lunch in the present study (mean peak at 50 min; mean peak chylomicron-TG after mixed meal at 4 h, after high-fat meal at 2 - 4 h). We conclude that the effects of successive fatty meals on plasma NEFA concentrations are not the same as those of a single meal given after an overnight fast. This may have relevance for those indulging in a traditional English breakfast, or a traditional French lunch.

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Origin of the early postprandial triacylglycerol peak. By B.A.FIELDING, R.OWEN, J.CALLOW and K.N.FRAYN, *Oxford Lipid Metabolism Group, Radcliffe Infirmary, Oxford, OX2 6HE*

Elevated postprandial plasma triacylglycerol (TAG) concentrations have been linked with coronary heart disease (Patsch *et al.* 1992). After the ingestion of fat by normal subjects in the postabsorptive state, the level of plasma TAG usually rises to reach a broad peak after about 180 min, and is then rapidly cleared from the circulation. However, two or three peaks of plasma TAG have been noted under certain circumstances and in the present study we have investigated the origin of an early postprandial plasma TAG peak, as reviewed by Sethi *et al.* (1993).

We have used two sequential test meals with specific fat composition to enable natural dietary fatty acids to be traced into the plasma chylomicron TAG profile. Seven normal subjects (two male) ate a fat-free evening meal the day before the study. After an overnight fast of at least 12 h, the subjects consumed a high-fat breakfast consisting of 54 g fat (safflower oil), 12 g protein and 140 g carbohydrate as a cooked oat 'flapjack'. Five hours later at 12.00 hours, the subjects ate a second test meal containing 61 g fat (olive oil and fresh avocado), 13 g protein and 35 g carbohydrate. The predominant fatty acids in the first test meal, expressed as g per 100 g of total fatty acids, were C18:2, 68% and C18:1, 19% and those in the second meal were C18:2, 8% and C18:1, 74.6%.

After the second test meal, the mean concentration of plasma chylomicron TAG demonstrated an early peak at 50-60 min and the fatty acid content of this peak was found to reflect the fat of the first meal whereas at later time points (after 1 h) the fat from the second meal appeared to predominate (Table). In a control experiment in which the first test meal was a fat-free meal, the early plasma TAG peak was absent (results not shown).

	Plasma chylomicron TAG fatty acid concentration (μmol/l)							
	0 min		50 min		180 min		300 min	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
C18:1	56	11	146	56	186	35	114	20
C18:2	234	80	538	193	143	62	19	7

These results agree with a recent study (Peel *et al.* 1993) which measured chylomicron apo-B and retinyl palmitate label in postprandial plasma samples and suggest the existence of a pool of dietary fat, possibly as preformed chylomicrons, which may be secreted in response to further meals.

We thank the Oxfordshire Health Authority District Research Committee for financial support.

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Plasma lipoprotein composition and amounts in patients with chronic renal failure on haemodialysis. By K. MEKKI[1], M. MEGHELLI[1], M. REMAOUN[2], J. PROST[3], H. MERZOUK[1] and J. BELLEVILLE[3], *[1] Laboratoire de Physiologie Animale et Nutrition, ISN, Université d'Oran, Algérie, [2] CHU-Hémodialyse de Sidi Bel Abbès, Algérie and [3] Unité de Nutrition Cellulaire et Métabolique, Université de Bourgogne, Dijon, France*

The aim of this study was to test whether different plasma lipoprotein levels and compositions are altered in patients with chronic renal failure (CRF) on haemodialysis.

Fifty eight patients (34 women, 24 men) with a mean age of 42 (SE 13) years, having plasma creatinine and urea values of 1026 (SE 256) µmol/l and 35 (SE 9) mmol/l respectively, were studied. This population was divided into three equal groups according to the haemodialysis duration : G1 < 1 year; G2 1 to 5 years; G3 5 to 13 years. A control group of twenty two adults (13 men, 9 women) with a mean age of 40 (SE 7) years was used as a reference. Plasma lipoproteins (VLDL, LDL, HDL_2 and HDL_3) were prepared according to Gambert *et al.* (1988) and their lipid and apoprotein (apo) compositions were measured. Apo electrophoresis was assayed according to Irwin *et al.* (1984).

The amount of VLDL was significantly higher in the three groups of patients, particularly in G3 while an increase in LDL amounts was noted in G1, then a progressive decrease was noted in G2 and G3 groups. HDL_3 amounts were decreased in the three groups, whereas HDL_2 values were similar in the three groups of patients. VLDL composition showed that triacylglycerol (TG) and apo values were higher, particularly in G3 group. A decrease in apo B100 and in unesterified cholesterol (UC) and an increase in TG were observed in LDL of the three groups. In HDL_3, TG and UC were increased whereas apo and phospholipids (PL) were diminished. In HDL_2, cholesteryl ester (CE) were decreased in the three groups. In VLDL, apo A4 and C3 levels were increased in the three groups. Apo B100 and apo E levels and apo C2 : apo C3 ratio values of VLDL were diminished. In HDL_2 and HDL_3, apo E was increased, apo A1 value and apo C2 : apo C3 ratio were diminished. Apo C3 Lp no B : apo C3 Lp B was increased in the three groups.

It could be suggested that altered composition of lipids was a consequence of impaired apo distribution, in particular in apo C isoforms (increase of apo C3, decrease of apo C2, respectively inhibitor and activator of lipoprotein lipase (EC. 3.1.1.34) which probably involved a decrease in lipolytic activity.

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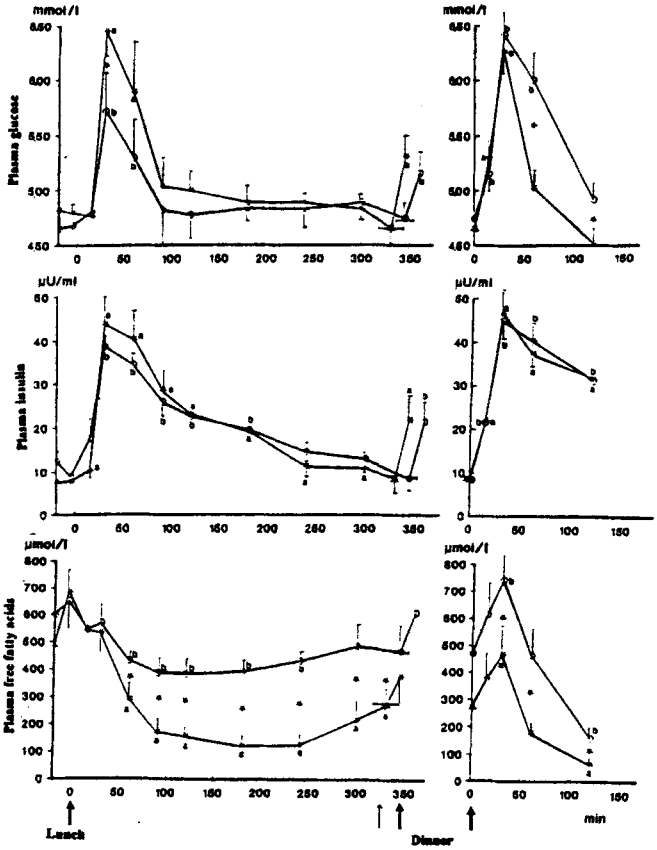
Effects of low-fat versus high-fat meals on hunger ratings, plasma hormone and metabolite levels, and energy intakes at the next meal. By A.HIMAYA, J.LOUIS-SYLVESTRE, L.BRONDEL, J.M.ANTOINE and M.FANTINO, *Neurobiologie de la nutrition EPHE, INSERM U286-UFR X Bichat B.P.416 - 75018 Paris, France*

The effect of low-fat and high-fat lunch meals on hunger ratings, plasma glucose, insulin, glucagon, lipid levels and energy intakes at dinner were examined in twelve normal-weight young men, tested over four experimental sessions. The lunch served at 13.00 hours consisted of a high carbohydrate pasta dish with either 50 g fat-reduced butter substitute (lunch A, 2107 kJ) or 50 g butter (lunch B, 3628 kJ). Hunger ratings were obtained before and at 30 min intervals after lunch, until the subjects spontaneously asked for the dinner. On two days, the subjects consumed a dinner *ad libitum*. On the other 2 d, the subjects were given a fixed standard dinner ; blood samples were taken at intervals for up to 2 h after dinner. Some results are shown below.



Plasma glucose, insulin and free fatty acid responses to pasta meal served with either 50 g butter subtitute (*—*) or 50 g butter (°—°). Values are means with standard errors indicated by vertical bars. Significantly different from basal values : « a » lunch A, « b » lunch B ($p < 0.05$) *Significant difference between lunches A and B, ($p<0.05$) .

The addition of 1558 kJ from butter to the pasta dish had no impact on post-lunch hunger ratings, and did not delay the onset of dinner. However, the amount of energy consumed at dinner was reduced by 305 kJ. High-fat lunches led to higher plasma triacylglycerol and fatty acid levels that persisted past the dinner meal and led to post-dinner glucose intolerance at normal insulin levels. These results may help explain the partial and delayed adjustments in energy intake following a high-fat meal.

https://doi.org/10.1079/PNS19950058 Published online by Cambridge University Press

Portal appearance of orally administered free and peptide-bound amino acids in rats. By C.J. SEAL, GAYLE B. SPILLER and D.S. PARKER. *Biological and Nutritional Sciences, University of Newcastle, Newcastle upon Tyne, NE1 7RU.*

Net uptake of intact peptides across the gastrointestinal tract *in vivo* is not well documented, although recent evidence from ruminants (Seal & Parker, 1993; Webb *et al*, 1993) suggests that luminally derived peptides may contribute to portal α-amino nitrogen flux. The present experiment was designed to investigate the appearance of free amino acids and intact peptides in portal and peripheral plasma of rats dosed by gastric intubation with either leucyl-glycyl-glycine (LGG) and prolyl-methionine (PM) or equivalent amounts of the free amino acids (FAA).

Groups of three female rats (mean weight 213 g) with *ad lib* access to a standard rat chow and drinking water received the following test solutions by tube directly into the stomach: 1 ml distilled water (Control), 1 ml 0.1 M-peptide mixture (PEP) or 1 ml of an equivalent mixture of FAA. Heart (H) and portal (P) blood was removed under pentobarbitone anaesthesia after 30 (Control, PEP30, FAA30) or 60 min (PEP60, FAA60). FAA and peptides were analysed in plasma by HPLC after filtration through 10K molecular weight filters (Seal & Parker, 1991). Statistical analysis was by ANOVA in a split-plot design comparing treatment and vessel effects.

Plasma FAA concentrations (μmol/l) and main plot treatment effects averaged across vessel are shown in the Table below. Portal plasma amino acid concentrations were higher than corresponding heart values indicating net uptake across gastrointestinal tissues.

		Treatment						Effect of Contrast†			
		Control	PEP30	PEP60	FAA30	FAA60	EMS	1	2	3	4
Glycine	H	187	278	218	266	221	5898	*	NS	*	*
	P	280	488	340	533	355					
Leucine	H	116	133	116	159	128	1227	NS	**	NS	*
	P	137	144	130	175	153					
Proline	H	174	285	227	332	230	5201	*	NS	NS	*
	P	160	378	285	419	283					
Methionine	H	57	133	126	170	114	307	***	NS	NS	*
	P	60	156	130	178	119					

EMS, main plot error mean square. NS, Not significant.
* $P<0.05$, ** $P<0.01$, *** $P<0.001$.
†, Control *v.* Rest; 2, peptide *v.* free amino acid; 3, PEP30 *v.* PEP60; 4, FAA30 *v.* FAA60.

Intact LGG and PM peptides could not be detected in portal or heart plasma at either sampling time. Plasma amino acid levels were numerically higher in rats dosed with FAA than those receiving peptides and in both groups the concentration of amino acids fell between 30 and 60 min after dosing. These results suggest that LGG and PM are hydrolysed in the gut lumen or during passage across the gut wall and are not absorbed intact into portal blood. Low-molecular-weight peptides appearing in portal plasma (Seal & Parker, 1993) may arise from protein turnover within gut tissues and are not peptides generated during protein digestion in the lumen.

This work is funded through the AFRC Link Research Fund.

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The influence of starvation and amino acid administration on whole-body protein kinetics in obese adults. By P. J. PACY[1], N .R. GIBSON[1], and D. HALLIDAY[2], [1] *Nutrition Research Unit, 4 St Pancras Way, London NW1 2PE and* [2] *Nutrition Research Group, Clinical Research Centre, Harrow, Middlesex HA1 3UJ*

It has been reported (Nair et al. 1987) that 3 d starvation in lean adult men resulted in significantly increased protein degradation (31%), leucine oxidation (46%) and protein synthesis (28%). The present study was conducted to establish whether a similar response occurred in obese adults and to document the effects of amino acid provision during the first 6 d starvation. Four obese adults (3F, 1M; age 43 (SD 8) years; body mass index 40 (SD 2) kg/m^2) were studied after 0, 1, 3 and 6 d starvation by a primed continuous 8 h infusion of L-[1-^{13}C]leucine (0.5 mg/kg per h). No amino acids were supplied during the initial 4h (PA) while throughout the following 4h Vamin 14 (V) was infused at a leucine intake of 38 (SD 2) μmol/kg per h. Whole body protein (WBP) kinetics were calculated using the steady-state approach from plasma [^{13}C] α-ketoisocaproic acid and $^{13}CO_2$ enrichment between 2 - 4 and 6 - 8 h and CO_2 production measured by indirect calorimetry for 60 min during each 4-h period. The results are shown in the Table. Units of WBP kinetics are μmol/kg per h while those for insulin are μU/ml.

	Degradation				Synthesis				Oxidation				Insulin			
	PA		V		PA		V		PA		V		PA		V	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Day 0	91	12	71*	13	78	9	86*	10	16	3	26*	2	16	3	20*	13
Day 1	91	6	77*	6	78	3	91*	5	17	3	28*	2	12	9	18*	14
Day 3	92	11	78*	9	74	8	92*	6	17	3	27*	2	10	4	12	7
Day 6	93	13	74*	10	79	7	88*	4	17	6	28*	4	7+	2	10+	3

* Significantly different from PA, $P < 0.05$ (paired t test)

\+ Significantly different from day 0, $P < 0.05$ (paired t-test)

The weight of the subjects declined progressively (104, 102, 101 and 99 kg) while plasma leucine concentration was 164, 211, 231 and 229 μmol/l (PA) and 295, 337, 347 and 343 μmol/l (V). Plasma insulin levels declined in both the PA and V phases during the period of starvation.

The present study failed to demonstrate any alterations in whole-body protein metabolism over the first 6 d of starvation in severely obese adults which contrasts with responses in lean subjects. The effect of provision of amino acids, which resulted in inhibition of protein degradation and promoted oxidation and synthesis, also remained constant during this period.

Nair, K.S., Woolf P.D.,Welle, S.L. & Matthews, D.E. (1987). *American Journal of Clinical Nutrition* **46**, 557-562.

Energetic cost of protein metabolism in young and elderly healthy humans. By Y. BOIRIE, P. RITZ, P. GACHON, and B. BEAUFRERE, *Laboratoire de Nutrition Humaine, CRNH Auvergne, Clermont-Ferrand, France*

Protein metabolism, particularly protein turnover, is an important component of resting metabolic rate in healthy humans. However, the contribution of protein metabolism to energy expenditure (EE) has not been investigated in relation to age and gender, and in response to feeding compared with fasting. EE and whole-body protein metabolism were investigated in the post-absorptive and fed states in six young men (YM, age 22.6 (SD 0.9) years), six young women (YW, 21.5 (SD 2.4) years), six old men (OM, 68.2 (SD 1.9) years), and six old women (OW, 68.3 (SD 2.2) years). A test meal, corresponding to 41.8 kJ/kg of which 15.6 % were from proteins, was divided into equal portions given every 15 min for 240 min. EE was calculated from respiratory gas exchanges measured by means of open-circuit indirect calorimetry. Protein turnover (PT) was determined from leucine flux estimated by L [1-^{13}C]leucine infusion.

Post absorptive EE (MJ/24 h) was significantly greater in men than in women but the difference did not reach statistical significance between young and old individuals (6.67 (SD 0.84) *v.* 5.41 (SD 0.53), YM *v.* YW, p <0.01; 6.04 (SD 0.52) *v.* 5.31 (SD 0.33), OM *v.* OW ; p <0.01; 6.04 (SD 0.94) *v.* 5.68 (SD 0.56), young *v.* old). In the fed state, this gender difference in EE only persisted in the young group (7.79 (SD 0.99) *v.* 6.11 (SD 0.23), YM *v.* YW, p <0.01; 6.97 (SD 0.64) *v.* 6.27 (SD 0.43), OM *v.* OW, not significant). The diet-induced increase in EE did not differ between groups.

During fasting, PT was significantly greater in men than in women, both in young (229 (SD 27) *v.* 188 (SD 27) g prot/24 h, p <0.05), and in old subjects (217 (SD 35) *v.* 170 (SD 14) g prot/24 h, p <0.05). There was no age difference in PT. Feeding was associated with an increase in PT in all groups, but fed-PT was greater in young men than in any other group (297 (SD 32) *v.* 224 (SD 38), 273 (SD 45), 257 (SD 37) ; p <0.01).

EE and PT were highly correlated both in the fasting and the fed state (r 0.70, p <0.001 ; and r 0.73, p<0.001 respectively). During feeding the increase in EE was correlated with the increase in PT (r 0.44, p <0.05). The slopes of the regression lines were not significantly different between the fasting and the fed state. These slopes were significantly affected by age both in the fasting and the fed state. Based upon this, the energetic cost per gram of turning over protein was significantly lower in the old compared with the young group (11.4 (SD 1.9) *v.* 19.6 (SD 2.5) kJ/g protein, p <0.01).

In conclusion, the energetic cost of protein turnover is affected by age.

Modulation of leucine oxidation by variation of dietary protein and glucose supplementation. By J.L. BOWTELL[1], M.J. RENNIE[1], O. ROOYACKERS[2] and A.J.M. WAGENMAKERS[2]. [1]*Department of Anatomy and Physiology, University of Dundee DD1 4HN and* [2]*Department of Human Biology, University of Limburg*

We have found that leucine oxidation is increased by high dietary protein both at rest and during exercise. We hypothesized that glucose supplementation during exercise would reduce the exercise-induced increase (normally 220% at 60%$\dot{V}O_{2max}$), possibly by a reduction in the activity of muscle branched chain keto acid dehydrogenase complex (BCKADH; EC 1.2.4.4). Subjects (age 21 (SE 1) years, $\dot{V}O_{2max}$ 44 (SE 5) ml/kg per min) were studied postabsorptively after 7 d consuming diets either high (HP, 1.9) or low (LP, 0.7 g/kg per d) in protein, sixteen in each dietary group. All received a primed (0.8 mg/kg) constant (1 mg/kg per h) infusion of [1-^{13}C,^{15}N]leucine (99 atoms%). After 2 h the subjects exercised on a treadmill at 60%$\dot{V}O_{2max}$ for 2 h. Eight subjects from each dietary group (designated HP+G, LP+G) drank a 150 g/l solution of 0.75 g/kg per h dextrose monohydrate (from potato starch, AVEBE UK Ltd) immediately before exercise and at 20 min intervals thereafter; the remaining eight controls from each dietary group (HP-G, LP-G) took the equivalent volume of water. *Vastus lateralis* biopsies were taken from four subjects from each of the four dietary protocols before and after 1 and 2 h exercise; blood and expired air samples were taken throughout. In subjects consuming a high-protein diet the exercise-induced increase in leucine oxidation was suppressed to 80% of its normal value by oral glucose from 75.7 (SE 4.1) to 60.9 (SE 2.8) μmol/kg per h, but not in those consuming a low-protein diet. Exercise increased the percentage activation of BCKADH by 120 (SE 44) % ($P < 0.05$), but there was no difference between the four dietary protocols. For the control subjects, plasma insulin concentration at rest was higher for HP-G than LP-G groups (18.8 (SE 1.4) *v.* 12.8 (SE 1.1) μU/ml, $P < 0.0005$) but during exercise HP-G plasma insulin fell to LP-G levels (13.0 (SE 5.2) μU/ml). Oral glucose increased plasma insulin above control values at 40 and 80 min exercise for subjects consuming a low-protein diet (by 76 and 62% respectively); those taking a high-protein diet showed no effect. Furthermore absolute plasma insulin concentrations during exercise were not different between HP+G and LP+G (18.6 (SE 2.7) *v.* 16.9 (SE 1.8) μU/ml respectively). Carbohydrate oxidation (indirect calorimetry) was increased by oral glucose in both the first (56.6 *v.* 42.6%) and second (55.5 *v.* 46.3%) hours of exercise for the HP+G group but only in the second hour (46.5 *v.* 34.0%) for the LP+G group. This suggests that the partitioning between oxidation and storage of the exogenous glucose is altered by dietary protein. We conclude that the suppression of leucine oxidation by exogenous glucose during exercise in subjects consuming a high-protein diet is not mediated by a reduction in muscle BCKADH activity. The difference in the glucose effect on leucine oxidation, between high- and low-protein diets, may be explained by (a) increased availability of amino acids after high-protein diets which may enhance the insulin-induced inhibition of proteolysis (b) resistance to the effect of insulin on protein metabolism in the low-protein diet subjects (c) the greater insulin response to glucose in the low-protein diet subjects directed exogenous glucose from oxidation towards storage but leucine oxidation was not suppressed, since glucose oxidation results in the conservation of leucine.

This work was supported by SmithKline Beecham and University of Dundee.

Postprandial protein utilization in normal adults. 1. Milk protein measured in the steady state.

By N. GIBSON[2], A. FEREDAY[1], M. COX[2], D. HALLIDAY[3], P.J. PACY[2] and D.J. MILLWARD[1]
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Nitrogen homeostasis in man requires that post-absorptive losses are repleted in the postprandial state. The efficiency of dietary protein utilisation for postprandial repletion is determined by the balance between amino acid oxidation and protein deposition. Amino acid oxidation following consumption of a mixed meal is usually thought to comprise fixed obligatory amino acid catabolism with any additional losses reflecting inadequate utilisation of the protein due to low biological value. However we have shown that postprandial amino acid oxidation rates reflect not only the immediate protein intake but also the habitual dietary level so that the apparent postprandial protein utilisation (PPU) is variable according to the prior protein intake (Price *et al.* 1994; Quevedo *et al* 1994). Measurement of the actual efficiency of PPU as a function of the dietary protein quality requires that postprandial amino acid oxidation can be partitioned into components reflecting (a) obligatory metabolism, (b) chronic dietary protein level and (c) the acute dietary source. We have established a [^{13}C] leucine balance protocol which allows separate assessment of postprandial leucine oxidation in terms of (a+b) and (c) suitable for evaluation of the quality of dietary protein, and have assessed the PPU of milk protein in normal adults.

We measured [^{13}C] leucine balance, during a single 9-h primed dose constant infusion of [1-^{13}C] leucine with three 3-h phases, (1) the postabsorptive state, (2) low protein feeding (30 minute feeding of 1/24 of daily energy needs and 2% energy as protein) and (3) high-protein feeding (30 min feeding of approximately 14% energy as protein), the protein fed at the habitual intake level. The frequent small meals ensured a metabolic steady state. Leucine balance was calculated as leucine intake minus leucine oxidation, calculated from $^{13}CO_2$ excretion and plasma keto-isocaproate enrichment, measured during the third hour of each 3h phase. We have conducted twenty five such infusions in men and women of various ages and degrees of fitness using in each case milk-protein diets.

Assuming that components (a) and (b) of leucine oxidation (as above) were constant at the two levels of protein intake then true PPU of milk protein is the slope of the balance-intake relationship between the two intake levels, equal to 0.800 (sd 0.107). In fact the slope of the leucine balance-intake plot from the post-absorptive to the high protein period was similar, so that PPU calculated between low protein-high protein periods was similar to that calculated over the post-absorptive-high protein periods. This indicates that post-absorptive leucine oxidation was equal to components (a) + (b). Furthermore on the basis of this, recalculation of our recent leucine balance studies with milk protein in adult subjects habituated to four levels of protein intake (Price *et al.* 1994), indicate similar values, i.e. PPU = 0.86(sd 0.10). Given the higher leucine content in milk (4.5 μmol/mg N) compared with body tissues (3.93 μmol/mg N) milk leucine utilisation will always be lower (i.e. 87%: 3.93/4.5x100), than overall protein utilisation so these values indicate an actual PPU for milk protein of >0.90.

It would appear therefore that in healthy adults milk protein fed in frequent small meals is utilised efficiently for postprandial protein deposition.

This study was supported by Research into Ageing.

Price, G.M.,Halliday,D., Pacy,P.J., Quevedo M.R.,& Millward, D.J. (1994). *Clinical Science* **86,** 91-102.
Quevedo, M.R.,Price,G.M., Halliday,D.,Pacy,P.J. & Millward, D.J. (1994). *Clinical Science* **86,** 185-193.

Is steady-state plasma enrichment obtained through conventional enteral administration of stable isotope tracers? by P. CRENN, F. THUILLIER, M. RONGIER, B. MESSING and J.F. DESJEUX. *INSERM U290, Hôpital Saint-Lazare, 75010 PARIS, FRANCE*

The reference method for studying protein metabolism in human beings relies on the primed constant intravenous (iv) infusion of tracers labelled with stable isotopes, L[1-^{13}C]leucine being the most used tracer among essential amino acids. The plasma enrichment at steady-state allows the calculation of flux of amino acids. The constant enteral infusion of tracers was recently used to calculate the splanchnic extraction of amino acids in either the post-absorptive state (PA) or in enterally fed subjects. However it is difficult to obtain a steady-state through the enteral route. Therefore one can postulate that this condition of the model is not fulfilled.

We present herewith our results using gastric stable isotope infusion in five healthy adult human subjects.

After an overnight fast the subjects received an iv priming dose of L[1-^{13}C] leucine (4 μmol/kg) and L-D3 leucine (4 μmol/kg) immediately followed by a continuous infusion of (a) L[1-^{13}C] leucine iv (4 μmol/kg per h) and (b) L-D3 leucine (4 μmol/kg per h) through enteral route by nasogastric feeding tube, both being infused for 4 h at 6 ml/h. The study was performed: (1) in PA for 4 h and (2) during a 9 h enteral feeding (EF) (1.2 x Resting Metabolic Rate, nutrition mean rate = 115 ml/h) where tracers were administered during the last 4 h. Blood sample were taken before the priming dose and, both in (1) and (2), at 20 min intervals, during the last 120 min.

Mean plasma enrichment values at steady-state for iv L[1-^{13}C]leucine were 2.8 (range: 2.4-3.5) % in PA and 2.7 (2.1-3.8)% in EF. For intragastric L-D3 leucine mean plasma enrichment value were 2.1 (1.2-3.1) % in PA and 1.9 (1.5-2.4) % in EF. Mean coefficient of variation (CV) of plasma enrichment at steady-state for iv L[1-^{13}C]leucine were 7.8% in PA and 9.0% in EF. For intragastric L-D3 leucine mean CV of plasma enrichment were 48.8% in PA and 17.8% in EF.

As expected a steady-state condition was obtained with the iv administration of tracer. However the conventional model of gastric administration of leucine tracer does not allow a steady-state in plasma enrichment especially in the PA. Thus calculation of splanchnic extraction of amino acid tracers is difficult to assess through this model of a low-rate enteral infusion. It is therefore necessary to develop an alternative model of tracer enteral administration for use with PA as well as enterally fed patients.

Effects of varying glucose:lipid ratios on leucine metabolism in patients receiving parenteral nutrition. By S. LIMA-DUTRA, F. THUILLIER, D. DARMAUN, M. RONGIER, B. RAKOTOAMBININA, B. MESSING and J. F. DESJEUX. *INSERM U290 Hôpital St-Lazare, 107bis rue du faubourg St Denis 75010 Paris, France*

The aim of the present study was to compare the effect of different glucose:lipid ratios on leucine metabolism using a stable isotope technique in non-stressed home parenteral nutrition (PN) patients.

Whole body leucine kinetics were measured in six adult patients (age 55 (SE 5) yr; weight 100% ideal body weight) using an intravenous prime constant infusion of L-(1-^{13}C)leucine, during the last 4h of a 12h isonitrogenous (approximately 1g protein/kg) and isoenergetic (approximately 1.2 x resting energy expenditure) PN infusion. The varying glucose:lipid ratios were: regimen A (glucose:lipid, 70:30), regimen B (glucose:lipid, 50:50) and regimen C (100% glucose). The duration of each regimen was 2 weeks and the order of infusion of regimen was randomized. Steady-state ^{13}C-enrichment in plasma keto-isocaproate and $^{13}CO_2$ were used to calculate leucine appearance rate (Ra), release from protein breakdown (B), incorporation into protein (S), and oxidation (Ox). The results were shown as mean (SE).

The measured flux (Ra) breakdown (B), and synthesis (S) were not significantly different between the three regimens, however the flux Ra was slightly higher in regimens B and C (176 (SE 6); 172 (SE 13) μmol/kg per h, respectively) than in regimen A (154 (SE 8). The oxidation was significantly different between regimens C (53 (SE 6) and A (44 (SE 5), $p<0.05$(Mann-Whitney test). The net protein balance (S-B) was significantly different between regimens C (-1.8 (SE 4.3) and A (8.1 (SE 4.4), $p<0.05$.

It was concluded that in stable patients receiving PN, regimen A (glucose:lipid ratio, 70:30) was the only one associated with a slight positive net protein balance. This study seems to indicate the misuse of intravenous standard amino-acid solutions.

Postprandial protein utilization in normal adults. II. Milk protein measured in the non-steady state. By A. FEREDAY[1], N. GIBSON[2], M. COX[2], D. HALLIDAY[3], P.J. PACY[2] and D. J. MILLWARD[1]

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To date all stable isotope studies of post-prandial changes in leucine kinetics have involved protocols involving frequent small meals to allow the isotopic and metabolic steady states required for interpretation of the tracer kinetics. In practice such feeding is artificial and actual protein utilization following large meals is non-steady state. Furthermore while calculation of leucine turnover requires complex modelling with assumptions about changes in pool sizes, calculation of leucine oxidation simply requires continuous monitoring of $^{13}CO_2$ excretion and plasma keto-isocaproate, (KIC), enrichment and the assumption that plasma KIC represents the mean intracellular KIC enrichment. On this basis we have measured post-prandial protein utilization during the 6 hours following a 50g-500kcal milk protein meal.

We measured ^{13}C leucine balance in 5 normal male adults during a 9-h primed constant intravenous infusion of [1-^{13}C] leucine, (see Price *et al* 1994), commencing at 8 am, 12 hr after the last meal. The meal, 0.5g protein/kg and 28.25 kJ/kg as skimmed milk + hydrolysed potato starch, was consumed over 5 minutes at 180 minutes. CO_2 production was continuously monitored with a ventilated hood and blood was sampled every 15 minutes between 120 and 180 minutes, then every 10 minutes up to 300 minutes and then every 20 minutes up till the end of the infusion at 540 minutes. Leucine oxidation was calculated from $^{13}CO_2$ excretion and plasma KIC enrichment assuming that the fractional bicarbonate recovery increased from 0.76 (postabsorptive) to 0.911 following feeding with a time course in parallel to the increase in CO_2 production. The meal absorption was monitored with measurements of plasma glucose, insulin, leucine, paracetamol (1.5g added to the meal) and in one case D-3 leucine added to the meal.

Since leucine oxidation in both postabsorptive and postprandial state includes a component which reflects the chronic response to the dietary protein level as well as any obligatory oxidation, the meal-protein related increase in leucine oxidation is calculated as postprandial oxidation minus postabsorptive oxidation measured between 120 and 180 minutes. Post-prandial protein utilization, PPU, can then be calculated as the instantaneous leucine balance, i.e. leucine intake minus cumulative excess leucine oxidation. In this study calculation were made over the 6 hours after the meal.

As judged by changes in plasma glucose, insulin, leucine and paracetamol the meal absorption was essentially complete within 3 hours although metabolic responses continued throughout the 6 hour postprandial period as judged by an elevated plasma leucine concentration and elevated leucine oxidation. In general leucine oxidation peaked between 240 and 360 minutes falling after this time but remaining above postabsorptive levels in all subjects at 540 minutes. At 3 hours after the meal PPU was 0.87 (sd 0.02) falling to 0.78 (sd 0.02) at 6 hours after the meal. Clearly with leucine oxidation still to some extent elevated at 540 minutes PPU would continue to fall with time although the rate of fall was low at 540 minutes. In fact the value of PPU at 6 hours is very similar to that obtained with milk protein in the metabolic steady state (Gibson *et al* 1994) lending confidence in the method described here.

In conclusion in healthy adults, milk protein fed in a single high protein meal is utilized as efficiently for postprandial protein deposition as with frequent small meals.

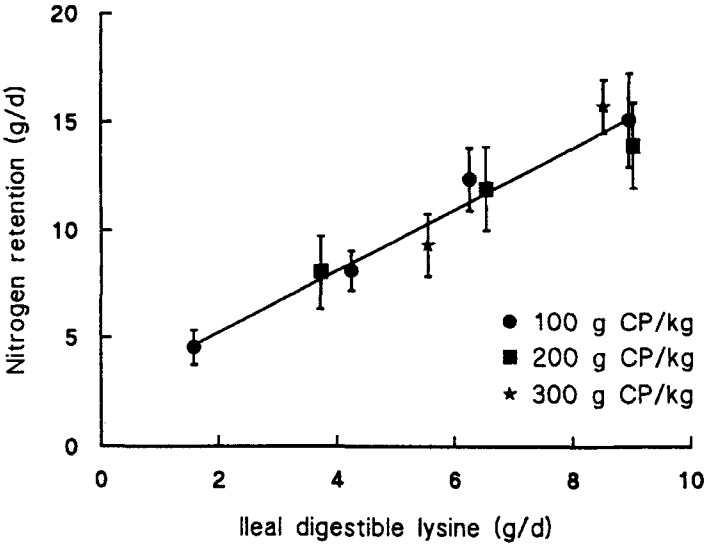
This research was supported by Research into Ageing

Price G.M., Halliday,D., Pacy,P.J., Quevedo M.R. & Millward D.J. *Clinical Science* 86, 91-102
Quevedo M.R., Price G.M., Halliday,D., Pacy,P.J., & Millward D.J. *Clinical Science* 1994, 86, 185-193
Gibson, N., Fereday, A., Cox,M., Halliday, D., Pacy, P.J. & Millward, D.J. *Proceedings of the Nutrition Society* (in the press).

Lysine utilization in growing pigs at three different levels of protein. By S. LANGER and M.F. FULLER, *The Rowett Research Institute, Aberdeen AB2 9SB*

Using natural ingredients to meet the requirement for any essential amino acid, excesses of other amino acids are usually unavoidable, but in conventional diet formulation such excesses are normally disregarded. In cereal based diets lysine is generally the first limiting amino acid.

The aim of the present experiment was to investigate the effect of different levels of protein, in lysine-limiting diets, on nitrogen metabolism in growing pigs. A basal diet (wheat, maize, maize gluten, feather meal) and a protein-free dilution mixture were combined to form diets with 100, 200 and 300 g crude protein (CP)/kg. Additions of free lysine (1.86, 3.72 or 5.58 g/kg) were used to form diets with the same lysine concentration but different levels of protein. In a preliminary experiment the ileal digestibility of lysine in these diets was determined using pigs with simple-T cannulas with Cr_2O_3 as indigestible marker. The diets were arranged in a randomized block design and given to eighteen pigs (30-50 kg) over three 12 d periods. Each period consisted of 5 d adaptation and 7 d measurement.



Effect of ileal digestible lysine on nitrogen retention at three levels of protein (group means with standard deviations indicated by vertical bars)

Increasing ileal digestible lysine concentration resulted in a linear increase in nitrogen retention. There was no significant effect of protein concentration. Nitrogen retention (y, g/d) was related to ileal digestible lysine intake (x, g/d) by the equation:

$$y = 1.43\,x + 2.37\ (r^2\ 0.87)$$

Assuming that 1 g body protein (N x 6.25) contains 65 mg lysine the equation implies an efficiency of utilization of absorbed lysine of 0.58.

We conclude that lysine utilization is not affected by the protein concentration in the diet.

The financial support of Eurolysine and Degussa AG is gratefully acknowledged.

Glutamine metabolism in ten different adipose depots of adult guinea pigs after feeding, fasting and exercise. By J.E. DIGBY and C.M. POND, *Department of Biology, The Open University, Walton Hall, Milton Keynes MK7 6AA*

Human abdominal subcutaneous adipose tissue *in vivo* is a net exporter of glutamine and a consumer of glutamate (Frayn *et al.* 1991), but the significance of these properties is not known. It has been suggested that metabolism of glutamine in adipose tissue is similar to that of muscle and that the rate of utilization is substantial (Kowalchuk *et al.* 1988). Extrapolations in whole-body terms have been made from data from single adipose depots but this approach precludes possible site-specific differences in glutamine metabolism. Investigation of site-specific differences may provide a more accurate picture of adipose tissue's contributions to the glutamine pool and establish possible functional relationships with adjacent tissues that utilize glutamine, such as the intestine and the lymphatic system. The present study extends investigations into the physiological basis for the distribution and anatomical relationship of mammalian adipose tissue, to include for the first time amino acid metabolism.

The activity of phosphate-dependent glutaminase (EC 3.5.1.2.) was measured in samples from ten different adipose depots of adult male Bolivian guinea pigs. Initially one group of animals was fasted overnight for 18 h before being killed and the other was fed for 30 min following an overnight fast of 18 h then killed after 30 min rest. Samples of adipose tissue (excluding lymph nodes and major blood vessels) were removed from 10 different sites: four superficial, two intermuscular and five intra-abdominal. The activity of glutaminase was assayed as described by Curthoys & Lowry (1973).

There was an increase in glutaminase activity after feeding in all sites measured except the superficial inguinal depot, which had the highest resting activity and which decreased slightly in the fed animals. The relative increase in glutaminase activity was low in all other superficial depots after feeding. Glutaminase activity in the intermuscular depots from the fed animals was up to twice that of the depots from the starved group. Excluding samples taken from the upper omentum close to the spleen (OME1, approximate mass 2 g), all other sites from the intra-abdominal depots of the fasted animals had relatively low enzyme activities, but after feeding, glutaminase activity increased to up to 6 times that of the fasted group. Glutaminase activity measured in OME1 was significantly higher than that of any other site studied, 16 nmol/min per mg protein in the fasted group, 31 nmol/min per mg protein after feeding. OME1 also contained a higher concentration of endogenous glutamate than all other depots. Both these measurements show that this site has a distinct capacity to utilize glutamine, and suggest that the metabolism of glutamine may be a major function of the omentum, facilitated by perfusion from a blood supply shared with the spleen.

In conclusion, this study has demonstrated that there are large site-specific differences in glutamine metabolism in adipose tissue at rest and the response to feeding is differential. Furthermore, the intra-abdominal depots are highly responsive to feeding, and OME1 has the capacity to make a major contribution to whole-body glutamine metabolism.

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Muscle protein turnover in doublemuscled Belgian Blue bulls during reduced and subsequent adequate energy and protein intake. By C. VAN EENAEME, J.L. HORNICK, S. GAUTHIER, P. VAN CALSTER and L. ISTASSE, *Department of Nutrition, Veterinary Faculty. University of Liège, Liège, Belgium.*

Reducing feed intake impairs animal growth and hence muscle protein (MP) deposition. As the latter is the net result of MP synthesis and MP degradation it could be interesting to study the effect of reduced feed intake on both components of MP turnover.

Two groups of four Belgian Blue bulls were given either a conventional fattening diet (group 1 or control group) with a net energy and crude protein (CP) content of respectively 0.76 UFV (Unité Fourragère Viande) and 171.5 g/kg on a dry matter (DM) basis, or a diet containing a large proportion of straw with 0.37 UFV and 121.5 g CP/kg DM (group 2) for a period of 4 months and then the same fattening ration as in group 1. Nitrogen balance and urinary excretion of 3-methyl histidine (3 MH), a marker of in vivo myofibrillar MP breakdown in cattle, were measured at mid period of slow growth (period 1), after 1 month fattening (accelerated growth; period 2) and 1 month before slaughter at about 625 kg (period 3). MP accretion was obtained from N balance, MP breakdown from urinary 3 MH excretion and MP synthesis was defined as MP accretion + MP breakdown.

During the period of slow growth the three components of MP turnover in group 2 were all significantly lower than in the control group (see Table). Refeeding an adequate diet produced compensatory growth. During this period of accelerated growth liveweight gain increased from 0.57 kg to about 2 kg/d, the mean of the period being 1.57kg/d. MP accretion rose sharply during this period. Concomitantly, MP synthesis and degradation rates increased. At the end of the fattening period MP turnover decreased, although differences were not significant.

Group...		Group 1			Group 2		
Period...		1	2	3	1	2	3
MP Accretion	(g MP/d)	383^{a}	390^{a}	384^{a}	133^{b}	419^{a}	362^{a}
MP Degradation	(g MP/d)	443^{a}	743^{c}	847^{c}	210^{b}	970^{c}	675^{c}
MP Synthesis	(g MP/d)	826^{a}	1133^{c}	1232^{c}	343^{b}	1390^{c}	1037^{c}

a, b, c : Items within a row with different superscripts are statistically different ($p<0.05$)

In contrast with the control group in which an increase of MP synthesis and degradation rates with time did not result in a net increase in MP deposition, compensatory growth produced a larger MP accretion. This gain in net MP deposition was the result of a much larger increase in MP synthesis than in degradation rate.

Apparently, and in contrast to growth manipulation by anabolic agents and ß-agonists where MP degradation is reduced, nutritional manipulation aiming at compensatory growth, affects mainly MP synthesis rate.

Research sponsored by IRSIA. Brussels.

A method for the isolation and derivatization of plasma taurine for use in stable isotope tracer kinetic studies. By F.IGLICKI, L.MARKS, F.THUILLIER and B.MESSING, *Hôpital St Lazare, Paris, France.*

The present study was designed to aid in our better understanding of the human requirement for 2-aminoethane sulphonic acid (taurine) and more precisely, its role in patients with intestinal resections and/or undergoing total parenteral nutrition (TPN). A stable isotope primed-continuous-infusion technique was chosen as it allows for a non invasive *in vivo* approach.

The first step was to develop a method for analysis of plasma taurine and then to use this method to observe the kinetics of plasma taurine in healthy adult volunteers. A gas chromatography/electron ionization-selected ion monitoring-mass spectrometry (GC/EI-SIM-MS) stable isotope ratio method was developed for the measurement of enrichment of the tracer, [1,2 $^{13}C_2$] taurine, in plasma. Natural abundance taurine and [1,2 $^{13}C_2$] taurnewere were analysed as their penta-fluoro-benzoyl dibutylamine (PFB-dBA) derivatives by GC/EI-SIM-MS. With the addition of the internal standard 3-amino-1-propane sulphonic acid (methyl taurine), taurine concentration could also be measured.

After an overnight fast, five healthy adult human subjects were given an intravenous priming dose of [1,2 $^{13}C_2$] taurine (3 μmol/kg), which was immediately followed by a continuous infusion of the tracer (3 μmol/kg per h) for 6h. Mean plasma plateau enrichment was found to be 8.57 (SEM 0.78) mole percent excess (MPE), CV 9% and a rate of appearance (Ra) value of 32.2 (SEM 3.12) μmol/kg per h was calculated, CV 9.68%. Plasma taurine concentration was found to be 71 (SEM 19) μmol/1.

Thus, our new GC/EI-SIM-MS technique for plasma taurine Ra, using the stable isotope tracer approach, will help greatly to enhance our present understanding of taurine as a conditionally essential amino acid in man.

Protein metabolism in *Spodoptera littoralis* Boisdoval (Lepidoptera, Noctuidae) and its response to some aflatoxins. By M. M. SADEK[1], K. CRAILSHEIM[2] and S. G. AZAB[1], [1]*Zoology Department, Faculty of Science, Assiut University, Assiut, Egypt* and [2]*Institute of Zoology, Karl-Franzens University, A-8010, Graz, Austria*

Spodoptera littoralis is a polyphagous insect pest. Its ability to utilize the nutritional value of more than eighty different wild and cultivated plants is attracting the attention of many investigators. Aflatoxins, the natural metabolites of the filamentous fungi *Aspergillus flavus* and *A. parasiticus*, are potent vertebrate toxins (Newberne & Butler, 1969). Among invertebrates, aflatoxins have significant insecticidal, larvicidal and chemosterilizing properties against many insect species. Sensitivity to these toxins differs not only between species (Matsumura & Knight, 1967) but also between different strains of the same species (Gunst *et al.* 1982). The susceptibility of *S. littoralis* to aflatoxins was shown by Abdou *et al.* (1985) who recorded high mortality and morphogenetic effects as a result of aflatoxin injection.

In the present study, we investigated the effect of dietary aflatoxins. Three toxins, aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2) and aflatoxin G_1 (AFG_1) were added to an artificial diet and introduced to 5th instar larvae 3 d before experimental manipulations. Protein metabolism was examined by measuring the incorporation of an injected labelled amino acid ([^{14}C]-phenylalanine) into protein. Toxins exhibited different degrees of effectiveness as shown in the following Table:

		Percentage of labelled amino acid incorporated into protein after different times of injection†						Maximum body weight (mg)‡		Protein content (%)‡	
	Dose	6 h		12 h		18 h					
Toxin	(ppm)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	—	44.38	3.25	48.91	2.35	51.61	1.10	642.8	30.6	3.5	0.3
AFB_1	0.5	44.56	2.28	49.25	4.16	50.53	3.80	613.1	20.5	2.9	0.6
	2.5	24.28*	1.59	30.85*	3.71	33.75*	4.76	476.3*	41.5	3.3	0.6
AFB_2	4.0	42.38	2.25	46.36	4.61	49.22	4.36	651.2	53.0	3.0	0.2
AFG_1	4.0	41.52	3.24	45.12	3.36	47.07*	2.54	492.9*	38.4	3.4	0.3

* Significantly different from control, $P < 0.05$. † n 8, ‡ $n \geq 30$.

The diet, containing casein, wheat germ, sucrose and linoleic acid (3.5 : 3 : 3 : 0.25 respectively, by weight), proved ideal for the insects' needs. An increase in the proportion of casein (from 35 to 42 g/kg diet) had no effect on the viability or protein content of the animal, while the same decrease in casein content significantly ($P<0.05$) reduced the rate of development, body weight and fecundity. The amount of labelled protein in the ovaries of female moths treated with 2.5 ppm AFB_1 was significantly lower than that in control animals, a fact which may account for the recorded low hatchability of eggs of treated animals.

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Gunst, K., Chinnici, J. P. & Llewellyn, G. C. (1982). *Journal of Invertebrate Pathology* **39**, 388-394.
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Carbohydrate homeostasis: the principle of macronutrient fuel selection in the growing rat allowed three dietary choices. By J.MAURON, SUN-HEE KIM and R.J.WURTMAN.

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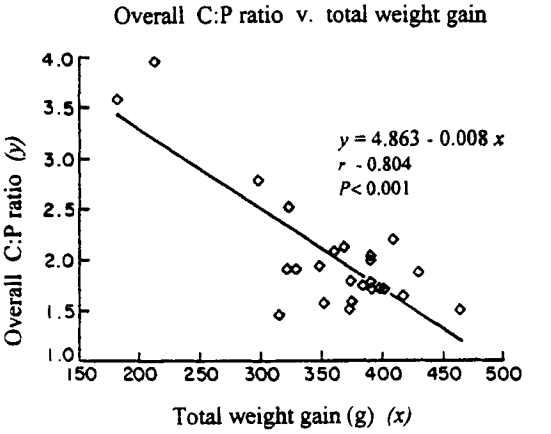
In an earlier approach growing rats were offered two isoenergetic isonitrogenous diets with high and low carbohydrate contents (Yokogoshi *et al.* 1986). To improve the spontaneous macronutrient choice, we allowed growing animals to choose their food *ad lib.* from three isoenergetic diets: 50g/kg protein - 400g/kg carbohydrate; 450g/kg protein - 400g/kg carbohydrate and 50g/kg protein - 700g/kg carbohydrate, and we measured macronutient intake, carbohydrate:protein(C:P) ratio and weight gain twice weekly for 9 weeks. At the end of the study, final body-weight gain and fat accretion were measured. The mean macronutrient intakes (26 rats) in the three growth periods are given in the Table and the correlation between overall C:P ratio and total(final) weight gain is given in the Figure.

	C	P	F
	(% energy)		
1. Dietary adjustment period (1st week)	45.5	18.8[a]	35.7[a]
2.Rapid growth period (2-5.5 week)	46.2	25.0[b]	28.8[b]
3.Slow growth period (5.5-9 week)	45.8	29.0[c]	25.2[c]

a.b.c. Values with different superscripts are significantly different: $P<0.05$



Protein (P) consumption increased and fat (F) intake decreased steadily as the study progressed whereas carbohydrate (C) consumption remained constant ("carbohydrate homeostasis"), and as a consequence the C:P ratio dropped. There were great variations in total protein consumption between animals (13-32 % energy) and an inverse relationship with fat intake since carbohydrate consumption was constant. Daily weight gain was negatively correlated with the corresponding C:P ratio, as were total weight gain and body fat content with the overall C:P ratio characterizing the food choice of each rat.

The percentage food protein chosen by the animal determined the outcome of the trial at 9 weeks, i.e. food intake, weight gain and body fat accretion. In this experimental model, body fat accumulation is, therefore, directly linked to the protein content of the diet, inversely proportional to the fat level and not related to the carbohydrate content.

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Effects of the dissociation of carbohydrates and lipid-protein foods in the diet on 24-h substrate oxidation in moderately obese women. By X. H. LYON ,Y. SCHUTZ, V. DI VETTA and S. GARDIOL, *Institute of Physiology, Faculty of Medicine, 1005 Lausanne, Switzerland*

Numerous diets promising weight loss have flourished over the years and the claims made by their promoters are often devoid of scientific experimental support. An example of this kind of regimen, recently heavily popularized among the european public, is the diet plan developed by Shelton under the name "dissociated diet" (DD). The basic concept of this program is to avoid eating carbohydrates (CHO) together with lipid and protein-containing foods.

We have been interested in studying the metabolic effects of ingesting the same food items, either combined in a given meal, as is the case in the usual everyday diet, or separately according to Shelton's recommendations. Six moderately obese women, presenting with relative body fat mass > 30 %, were studied twice during 24 h in a respiration chamber, where they consumed three meals a day containing on average 58 %, 25 % and 17 % of total energy derived from CHO, fat and protein respectively. On one of the two chamber sessions, they ate a mixed diet (MD), balanced for the macronutrients at each meal, and on the other one, although the overall composition of the diet was unchanged, the CHO were ingested alone at breakfast, whereas the evening meal consisted mostly of fat and protein. Each experiment was preceded by a 3-d period of adaptation to the particular diet.

In addition, CHO of the meals were naturally labelled with ^{13}C in order to assess the exogenous component of the total CHO oxidation.

During each stay in the chamber, spontaneous physical activity, total energy expenditure (EE) and respiratory quotient (RQ) were assessed. On the next morning, basal metabolic rate (BMR) and RQ were measured by means of a ventilated-hood system. These results are shown in the Table below, together with 24 h CHO and fat oxidation rates.

	24 h respiration chamber										Ventilated hood			
	Activity (% radar)		EE (kJ/d)		RQ		Oxidation (g/d)				BMR (kJ/min)		RQ	
							CHO		Fat					
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
DD	8.6	1.2	7489	184	0.83	0.01	156	15	86	7	3.85	0.08	0.80	0.01
MD	7.8	1.0	7514	205	0.87	0.01	206	9	63	6	4.23	0.13	0.84	0.01
P	NS		NS		0.019		0.050		0.004		NS		NS	

NS, not significant

EE and BMR were unchanged under both treatments, nevertheless, the 24-h RQ was significantly lower with the DD. It resulted in an increase of fat oxidation of 23 (SE 4) g/d fat, as well as in the storage of a supplement of 50 (SE 20) g/d of CHO during the DD compared with the MD. The exogenous CHO oxidation contributed to 73 % of total CHO oxidation and 62 % in the DD and MD respectively (NS).

It is concluded that the dissociation of CHO and lipid-protein foods in the diet acutely influences the amount of CHO and fat oxidized over 24 h in moderately obese women, but this effect may be transitory. This preliminary study has to be confirmed by a larger sample of both male and female subjects and the mechanisms underlying these observations remain to be investigated, as well as its eventual clinical implications.

Digestion of complex carbohydrates in infants.

By B. DE VIZIA and F. DI DONATO. Departement of Pediatrics, University of Naples "Federico II", Via S. Pansini 5, 80131 Napoli.

The aim of the present study was to evaluate starch absorption in infants, by analysis of hydrogen (H_2) in expired air, after a meal containing complex carbohydrates(CHO) such as wheat and legumes in order to emphasize the ingestion of these foods in weaning infants as the exclusive source of CHO.

Twenty-five healthy infants, aged 6-20 months were enrolled, who had no history of gastrointestinal disease or antibiotic treatment. The study included in every infant a lactulose breath test as standard measure of CHO malabsorption, the first day, and starch absorbtion, the following day. Each infant received almost 0.5g/Kg body weight of lactulose and a sample of expired air from baseline until the end of H_2 production, was collected. Breath test excretion was measured after ingestion of a complete meal containig one of the following starches: wheat, rice, bean, lentils, peas. The amount of starch administered provided 50% energy of the whole meal. H_2 peak height (H_2 excess) was determined by analysis of air collected after the load of lactulose and starch by thermal conductivity chromatograph (Quintron model). CHO malabsorption values were obtained by comparison of the excess H_2 produced by starch digestion with the excess H_2 obtained from a measured amount of lactulose (Solomons & Viteri, 1978).

The results of the starchs malabsorption studies are reported in the Table.

Infants	Age (months)	Type of starch	Amount of starch ingested (g)		Malabsorption (%)	
			mean	SD	mean	SD
7	6-18	lentils	26	3	9.8	3.9
5	5-20	beans	26	2	18	2.6
5	6-15	wheat	21	1.6	14	7.5
5	8-15	peas	21	4	11.7	2.7
3	5-6	rice	20		2.2	1.9

The level of starch malabsorption observed in our infants is similar to that reported in adult subjects (Levine & Levitt, 1981; Flouriè et al 1988), a high level for wheat and beans (14-18%), a low level for rice (2.2%), and an intermediate level for lentils and peas (9.8-11.7 %).

In conclusion complex CHO are well digested in infants as in adult and can be recommended for infant nutrition as sole sorce of carbohydrates after the sixth month of life.

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https://doi.org/10.1079/PNS19950058 Published online by Cambridge University Press

Effects of dietary fish oil on metabolic responses induced by an oral fructose and glucose load in healthy adults. By J. DELARUE[1], C. COUET[1], R. COHEN[2], M. OBJOIS[1], J.F. BRECHOT[3], M. PINAULT[1] and F. LAMISSE[1], *[1]Laboratoire de Nutrition, [2]Radiopharmacie et Radioanalyse, Hôpital Neuro-Cardiologique and [3]Biochimie, [1,3]Tours, [2]Lyon, France*

The mechanisms behind the decreased carbohydrate (CHO) tolerance observed during fish oil (FO) supplementation in humans remain unclear. We took advantage of the very different insulinaemic effects and insulin dependence of fructose and glucose to examine the contribution of insulin to the alteration of glucose metabolism.

Six volunteers (5M, 1W; age 23 (SD 2) years, weight 65 (SD 6) kg, body mass index 21.9 (SD 1.6) kg/m^2) were studied at the end of two 3-week periods. Each period was conducted 8 weeks apart. Energy, CHO, protein, fat, 18:1, 18:2, 18:3 fatty acid intakes as well as polyunsaturated:saturated fatty acid ratio (0.21) were similar during the two periods. No FO was given during period I (P I). In period II (P II) 6 g/d of added fats were replaced by 6 g FO (1.1 g ecosapentaenoic acid–0.72 g docosahexaenoic acid). At the end of each period a 6 h fructose or glucose tolerance test (1 g/kg body weight) was performed two d apart. Total rate of plasma glucose appearance (RaT) was measured using deuterated glucose. CHO and lipid oxidations were obtained from indirect calorimetry. Plasma levels of glucose, lactate, free fatty acids (FFA), ß-hydroxybutyrate (ßOH) and insulin and C-peptide were sequentially determined. Statistical analysis used Student's paired *t* test and the paired Wilcoxon test.

Basal glycaemia (G) and insulinaemia (I) significantly decreased after FO intake before both fructose and glucose (all $P<0.05$). Basal G/I ratio was 0.23 (SE 0.06) v. 0.10 (SE 0.02) (fructose, PII v. PI; $P<0.05$) and 0.24 (SE 0.05) v. 0.11 (SE 0.004) (glucose, PII v. PI; $P<0.05$). Area under the curve (AUC) of glycaemia was 315 (SE 6) v. 296 (SE 9) (fructose, PII v. PI; $P<0.05$) and 363 (SE 15) v. 340 (SE 8) (glucose, PII v. PI; $P<0.05$). AUC of insulinaemia was: 2841 (SE 495) v. 4600 (SE 1070) (fructose, PII v. PI; $P<0.05$), and: 4421 (SE 522) v. 7016 (SE 1232) (glucose, PII v. PI; $P<0.05$). The C-peptide response was not affected by FO. Lactataemia response was decreased after FO intake only from 60 to 150 min during glucose tolerance test ($P<0.05$). Plasma FFA and ßOH kinetics were not affected by FO. FO induced a significant decrease in 6 h CHO oxidation during both fructose and glucose tests (fructose: 60.7 (SE 4.1) v. 67.4 (SE 3.3) g, PII v. PI, $P<0.05$; glucose: 40 (SE 3.9) v. 54 (SE 3.9) g, PII v. PI, $P<0.05$) and a significant increase in 6 h lipid oxidation (fructose: 15.8 (SE 1.2) v. 12 (SE 0.4) g, PII v. PI, $P<0.05$; glucose: 25.6 (SE 2.5) v. 19 (SE 1.7) g, PII v. PI, $P<0.05$). The decrease in CHO oxidation (P I– P II) induced by FO was lower for fructose than for glucose (6.6 (SE 2.2) v. 13.9 (SE 4.1) g/6h, $P<0.05$). FO induced a significant increase in RaT (n=5) during the fructose load (50.2 (SE 1.1) v. 43.8 (SE 1.4) g/6h, P II v. P I; $P<0.01$) but not during the glucose load (74.2 (SE 1) v. 73.1 (SE 4.3) g/6h, P II v. P I).

Lower insulinaemia could explain the impaired glucose homeostasis observed for both fructose and glucose after 3 weeks FO intake. In addition, the lack of effect of FO on C-peptide response suggests that an increase in insulin clearance contributes to the reduced insulinaemia.

A validation of measurements of fat mass by dual energy x-ray absorptiometry against direct analysis in an infant model. SUSAN A. JEBB[1], M. TARZI[2], G. JENNINGS[1] AND M. ELIA[1]. [1]MRC Dunn Clinical Nutrition Centre, Hills Road, Cambridge, CB2 2DH. [2]Department of Physiology, University of Cambridge, Cambridge, CB2 1QL.

A previous study using the Hologic QDR-1000W dual energy X-ray absorptiometry (DXA) to measure fat tissue mass using adult software, version 5.5P (range > 10 kg body-weight) demonstrated a significant underestimate of absolute fat mass (Jebb *et al*, 1994) and changes in measured fat mass with depth. In the present study measurements of fat mass using the paediatric software, version 6.2 (range 2 - 10 kg body-weight), have been evaluated with reference to direct analysis in a range of meat samples.

In part 1 of the study samples of the same homogeneous mixture of minced pork were placed in a rectangular conformation (375 x 205 mm) and scanned in quadruplicate at different depths. Scans were analysed using the global region of interest facility. The mean (SD) percentage fat was 19.1 (2.09), 15.5 (0.73), 12.9 (0.29), 12.2 (0.52), 13.2 (2.44) at 20, 40, 80, 120 and 160 mm thick respectively, suggesting a significant effect of depth on the measured fat content.

In part 2, four different samples of minced pork meat each containing varying proportions of fat were measured. Approximately 6 kg of each meat sample, with a measured depth of 60 mm, were scanned and analysed as in part 1. Four x 150 g samples were removed for chemical analysis. The samples were freeze-dried to measure the water content and then finely ground. The fat content was measured using petroleum ether extraction following hydrolysis with potassium hydroxide, protein based on Kjeldahl nitrogen estimation and ash by combustion. The mean (SD) sum of the individually measured components was 99.7 (0.17), 100.0 (0.65), 100.8 (0.67), 101.1 (0.38) % of the total mass of the sample. The Table shows the mean fat mass measured by direct analysis and DXA at a tissue depth of 6 cm, in each sample. There was no significant difference between the two methods.

Sample	% Fat (direct analysis)		% Fat (DXA)		Difference
	Mean	SD	Mean	SD	
1	4.43	0.14	5.0	0.86	0.57
2	10.2	0.28	9.9	0.37	-0.30
3	16.82	0.37	17.0	0.55	0.18
4	23.31	0.29	23.7	0.65	0.39

Although this close agreement between direct analysis and DXA measurements at a depth of 60 mm is encouraging, we have demonstrated in part 1 of this study that the measured fat varies with depth. This suggests that there is likely to be a disparity in measured fat by DXA and direct analysis at other tissue depths. This is important for studies *in vivo* where there are variations in tissue thickness and body geometry. In addition, this *in vitro* study does not include bone mineral, which may in itself exert an influence on the measured fat mass, via effects on edge differentiation and beam hardening. Further fundamental research is required before DXA can be routinely used for measurements of fat mass in this weight range.

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Effects of energy restriction and parity on exogenous glucose utilization in anoestrus post-partum suckled Charolais beef cows. By A.A. PONTER[1], B. GRIMARD[1,2], P. HUMBLOT[2], N. NOVAK[2], B. KHIREDDINE[1], D. SAUVANT[3], J.P. MIALOT[1] and M. THIBIER[2]. *[1]ENVA, Lab. Epidémiologie et Gestion de la Santé Animale, 7, Av. Général-de-Gaulle, 94704 Maisons-Alfort, France, [2]UNCEIA, Services Techniques, 13, rue Jouet, BP65, 94703 Maisons-Alfort, France and [3]INA-PG, 16, rue Claude-Bernard, 75231 Paris cédex 05, France*

Anoestrus in post-partum Charolais beef cows is a well-known problem (Grimard, 1992). One of the factors which influences the return to cyclic activity is the level of energy intake before and after parturition (Randel, 1990). Insulin may be one of the main signals at the interface between energy metabolism and reproduction in the post-partum period (Schillo, 1992).

In the present study we investigated the effect of energy restriction and parity on the metabolic response to exogenous glucose in seventeen Charolais cows (ten primiparous PR, seven multiparous MU) fed at either 100% (*n* 10, GR1; 5 PR, 5 MU) or 70% (*n* 7, GR2; 5 PR, 2 MU) of energy requirements. Two intrajugular 15 min infusions of glucose (1.5 mmol/kg liveweight) were performed 30 and 50 days post-partum. Blood samples were taken from the other jugular vein at -5, 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 75, 90 and 120 min from the start of the infusion to measure plasma glucose (GLU) and insulin (INS) levels. Mean basal levels and cumulative areas under the insulin-time and glucose-time curves (Area) during each time period (0-5, 0-10...0-120 min, corrected for basal levels) were tested by ANOVA using the SAS statistical package.

GR1 animals had higher basal glucose and insulin levels than GR2 (least square mean 3.4 (SE 0.10) mmol/l *v.* 2.9 (SE 0.13) mmol/l GLU, $P<0.003$ and 5.6 (SE 1.06) mU/l *v.* 2.7 (SE 1.31) mU/l INS, $P<0.095$). The glucose Area was affected during the middle of the sampling period (0-60 min) by feeding level GR2 > GR1 (373.0 (SE 13.1) mmol/l per min *v.* 338.3 (SE 10.3) mmol/l per min, $P<0.045$). The Area for glucose increased with increasing parity (0-120 min) MU > PR (544.7 (SE 26.1) mmol/l per min *v.* 420.6 (SE 20.6) mmol/l per min, $P<0.0007$). Test period had no effect. The Area for insulin was affected by feeding level (0-120 min) GR1 > GR2 (4042.3 (SE 349.8) mU/l per min *v.* 2560.8 (SE 434.9) mU/l per min, $P<0.013$). During all time periods PR had greater insulin Area than MU (0-120 min, 3968.9 (SE 349.8) mU/l per min *v.* 2634.1 (SE 434.9) mU/l per min, $P<0.023$). In addition insulin was higher after the infusion at 30 d than infusion at 50 d (0-120 min, 3872.4 (SE 387.1) mU/l per min *v.* 2730.7 (SE 387.1) mU/l per min, $P<0.042$).

Plasma glucose and insulin concentrations may allow the animal to assess its energy status in the anoestrus period before the return to reproductive activity post-partum. The results from induced hyperglycaemia indicate that (1) underfed cows do not control plasma glucose levels as well as properly fed cows (greater glucose area), (2) PR are insulin resistant compared with MU, because insulin levels were high during the whole sampling period while plasma glucose was not significantly lowered until the end of the sampling period.

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Increased energy requirements during trypanosomiasis infection in goats. By J.T.P. VAN DAM[1], P.HOFS[1], D.OGWU[1], M.W.A.VERSTEGEN[2] and D.ZWART[1]. *[1]Section of Animal Production Systems, [2]Department of Animal Nutrition, Wageningen Agricultural University, The Netherlands.*

Trypanosomiasis impairs livestock production in large parts of Sub-Saharan Africa. Infection causes a reduction of feed intake and liveweight gain, anaemia and fever (Ingh *et al.* 1976). Zwart *et al.* (1991) found increased heat production (HP) during infection. In order to study the energy metabolism of infected West African Dwarf goats, the following trial was carried out.

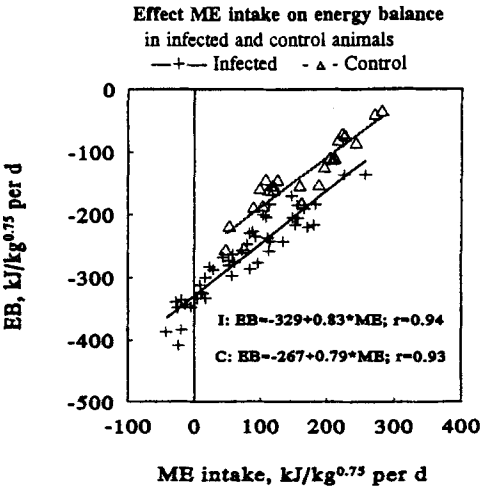
A total of twenty-four adult castrated bucks, receiving a diet of pelleted lucerne, was used. Feed intake patterns of all animals had been evaluated during a previous trypanosomiasis infection. Of the sixteen animals, selected for infection with *Trypanosoma vivax* in the current experiment twelve animals received *ad libitum* feed, while four animals with known high feed intake during infection, were restricted to 17 g feed/kg$^{0.75}$per d. Of eight control animals, four were also feed restricted. The animals were housed in individual respiration chambers in weeks 2, 4 and 6 post infection for measurement of the Energy Balance (EB). The results are shown in the table.

Treatment . . .	Infected		Control		
group	*Ad lib*	Restricted	*Ad lib*	Restricted	RMSE
n	12	4	4	4	
GE intake (kJ/kg$^{0.75}$per d)	205[a]	288[a]	520[b]	299[a]	97
ME intake (kJ/kg$^{0.75}$per d)	64[a]	113[a]	199[b]	123[a]	55
HP (kJ/kg$^{0.75}$per d)	344[a]	339[a]	310[b]	290[b]	16
HP, corrected for activity	309[a]	309[a]	272[b]	246[b]	20
EB (kJ/kg$^{0.75}$per d)	-280[a]	-227[ab]	-111[c]	-167[b]	50

GE, gross energy; ME, metabolizable energy; RMSE, root mean square error. [a,b,c]: Treatment means with different superscript per line differ significantly ($P<0.05$).

The feed intake of *ad libitum* fed animals was low. *Ad libitum* GE intake was reduced in infected goats below the restricted ration. Metabolizability was not affected by infection, as is shown in the two restricted groups. HP was increased in both infection groups by about 40 kJ /kg$^{0.75}$per d, resulting in a more negative EB. Body temperature was increased in infected animals. In the figure the relationship between EB and ME-intake is depicted for infected and control animals.

We conclude that infection reduced EB at a given ME-intake level. Consequently maintenance needs are increased during trypanosomiasis infection.



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Recombinant human growth hormone changes the intrinsic sex-effect differences on body composition in mice. By A. AGIS-TORRES, M.E. LÓPEZ-OLIVA, M.T. ÚNZAGA and E. MUÑOZ-MARTÍNEZ, *Sección Departamental de Fisiología Animal, Facultad de Farmacia, Universidad Complutense de Madrid, Spain*

Growth hormone (GH) treatment significantly influences the utilization of nutrients in humans and animals, showing a clear increase in lean deposition and a decrease in body fat gain. In the GH-deficient child undergoing GH replacement therapy, excess body fat is reduced and redistributed (Rosenbaum *et al.* 1989). In rodents the secretion of GH is dimorphic, with a distinct pattern for males and females that could be influenced by exogenous administration of GH.

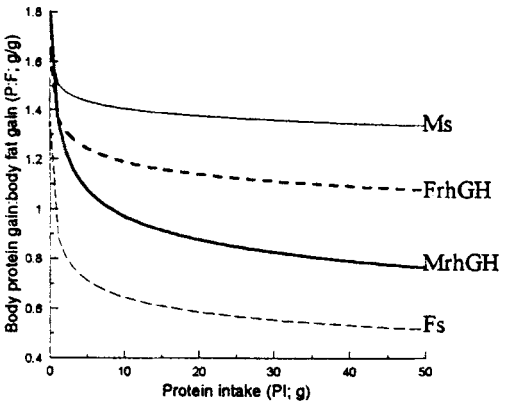
The aim of the present study was to study the effect of recombinant human growth hormone (rhGH) treatment and sex (male(M) or female(F)) on partitioning substrates between body protein and body fat in BALB/c mice from 21 to 50 d of age. Mice were divided into four groups (thirty mice each group) according to treatment and sex, and injected with either saline (9g/l NaCl; s) or rhGH (74 ng/g body weight) twice every 5 d. At 25, 30, 35, 40, 45 and 50 d of age five mice from each group were slaughtered and their body compositions were determined. Whole-body protein and fat gains (P and F respectively) were calculated by allometric equations, and protein intake (PI) for the whole live period of each mouse were computed by integration. Logistic regression equations (Table) of P:F ratio *v.* PI were determined for each group of animals (Figure) to determine partitioning of PI between lean and fat deposition.

Table. *Logistic equations*

♀ (Fs)	$0.89 \cdot PI^{-0.139\ a1}$
♂ (Ms)	$1.51 \cdot PI^{-0.031\ b1}$
♀ (FrhGH)	$1.37 \cdot PI^{-0.061\ a2}$
♂ (MrhGH)	$1.37 \cdot PI^{-0.149\ b2}$

a,b (sex) and 1,2 (treatment) significantly different $P<0.05$ (Dummy variables test).

Figure. P:F *v.* PI



Ms and FrhGH groups showed the highest P:F ratio for varying PI (>1), whereas Fs and MrhGH groups were the least efficient to convert PI into body protein (<1). These results suggest a feminization in GH male mice and a masculinization in GH female mice, concerning body component deposition. The decline in the P:F ratio as elicited by exogenous rhGH was inversely related to the animals' inherent propensity for fat and protein deposition in both sexes (Campbell *et al.* 1989).

In conclusion, treatment with rhGH could change the sexual pattern to protein and fat deposition, perhaps altering the pattern of endogenous GH released (Robinson, 1993).

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Metabolic and hormonal responses to starvation and incremental refeeding in sheep. By A.R.G. WYLIE, *Department of Agriculture for Northern Ireland and The Queen's University of Belfast, Newforge Lane, Belfast BT9 5PX, U.K.*

Growth hormone (GH) stimulates the release of insulin-like growth factor 1 (IGF-1) from the liver. In starved or underfed animals however, plasma IGF-1 levels fall while GH rises, signalling a nutritional dependence of the GH/IGF-1 axis. The effect of changes in energy intake below maintenance (M) on IGF-1 levels has not been closely examined.

Five wether sheep (30.6 (SD 0.97) kg) were fed on a pelleted barley/grassmeal (1:1) diet in two equal meals (09.30 and 16.30 hours) for 14 d at M energy allowance and then put in an open-circuit respiration chamber at 15°. Gaseous exchange was measured continuously on sequential energy intakes of M (6 d), zero (starvation; 5 d), 0.33M (4 d), 0.66M (4 d), M (6 d), 1.5M (4 d) and 2M (4 d). Mean daily respiratory quotient (RQ), daily heat production (HP) and methane output (CH_4) were recorded. Jugular blood was taken daily at 09.15 hours. Plasma glucose, non-esterified fatty acid (NEFA) and serum IGF-1 values on the last day of each period were subjected to analysis of variance (see below).

Energy intake	1M	zero	0.33M	0.66M	1M	1.5M	2M	s.e.m.
Metabolites								
Glucose (mmol/l)	3.82^{a}	2.74^{b}	$3.40^{c,d}$	3.27^{c}	$3.51^{c,d}$	$3.59^{a,d}$	3.54^{d}	0.091
NEFA (mmol/l)	0.75^{a}	2.31^{b}	1.09^{c}	0.99^{c}	0.38^{d}	0.28^{d}	0.46^{d}	0.080
IGF-1 (ng/ml)	216.9^{a}	122.0^{b}	153.9^{c}	149.1^{c}	164.0^{c}	184.3^{d}	191.2^{d}	6.81
Chamber data								
CH_4 (l/d)	61.90	3.36	20.84	39.20	59.51	57.4	58.97	
HP (MJ/d)	25.26	15.94	16.69	18.26	22.46	30.18	33.09	
R.Q.	1.019	0.782	0.843	0.933	0.998	1.033	1.081	

a,b,c,d Mean values within a row with unlike superscripts were significantly different, $P<0.05$

Meals were consumed fully throughout. The relative standard deviation (%) of serum IGF-1 on the day before starvation (base value) was 11.7 (between sheep; n 5) and 3.7 (mean-within-sheep; n 3 samples). IGF-1 levels fell asymptotically after feed withdrawal to 56.2% of base value after 5 d. After 4 d at 0.33M and 0.66M and 6 d at M respectively, IGF-1 levels were 71, 68.7 and 75.6% of base value. IGF-1 remained significantly below base value (M level of energy intake) even at 2M intake.

Blood glucose was significantly lower after 5 d starvation. Glucose levels rose with refeeding but returned to base value only at 1.5M intake. NEFA levels were highest after 5 d starvation but rapidly returned to below base values above 0.66M intake. IGF-1 levels were correlated with glucose levels over all samples (r 0.62; $P<0.001$).

Methane output fell by 94.6% by day 4 of starvation (no day 5 data) but recovered proportionately with refeeding. HP and RQ also fell during starvation but recovered incrementally with refeeding. Changes in methane output, HP, RQ, plasma glucose and NEFA were consistent with those anticipated in animals sequentially fed, starved and refed. Only serum IGF-1 failed to return to pre-starvation levels even at 2M energy intake. This suggests that factors in addition to energy supply (e.g. the quantity and/or quality of amino acid supply) are involved in the regulation of circulating IGF-1 levels and may be important for the early realisation of tissue anabolism after a period of feed restriction.

https://doi.org/10.1079/PNS19950058 Published online by Cambridge University Press

Prevalence of dyslipoproteinemias in patients with liver disease: impact of cause, clinical stage and nutritional status. By P. Sörös[1], O. Selberg[1] and M.J. Müller[2]. [1]*Medizinische Hochschule Hannover, Abt. Klinische Chemie II and* [2]*Max von Pettenkofer Institut, Bundesgesundheitsamt, Berlin, Germany*

Dyslipoproteinemia is common in patients with liver disease (Seidel 1987, Miller 1990). To assess the variables that influence lipid and lipoprotein metabolism in patients with liver disease we investigated 203 patients with biopsy-proven liver cirrhosis who had been admitted as potential candidates for liver transplantation. Clinical staging was based on albumin and bilirubin concentrations, prothrombin activity and the degree of encephalopathy and ascites (i.e. Child-Pugh score). Patient characteristics were as follows: Child-Pugh score (A 8%, B 55%, C 37%), aetiology of cirrhosis (viral 50%, toxic 18%, biliary 32%).

Lipoprotein electrophoresis was performed measuring low-density lipoprotein (LDL)-, high-density lipoprotein (HDL)- and very-low-density lipoprotein (VLDL)-cholesterol (Immuno, Vienna, Austria). Cholesterol, triacylglycerols and cholinesterase were quantified. Nutritional status was assessed using anthropometric measurements and bioelectrical impedance analysis (BIA 101, RJL Systems, Detroit, USA). Resting energy expenditure was measured by indirect calorimetry (Deltatrac Metabolic Monitor, Datex Instruments, Helsinki, Finland) as previously described (Müller *et al.* 1992).

Compared to patients with biliary cirrhosis, those with viral or toxic cirrhosis are charcterized by significantly lower lipid and lipoprotein levels, except for HDL-cholesterol (cholesterol 4.1 mmol/l *vs.* 7.7 mmol/l, t-test $p=0.000$; triacylglycerols 0.9 *vs.* 1.1, $p=0.000$; LDL-cholesterol 2.9 *vs.* 5.0, $p=0.000$; HDL-cholesterol both 1.0; VLDL-cholesterol 0.2 *vs.* 0.8, $p=0.003$). In patients with viral or toxic cirrhosis and Child stage C total cholesterol, HDL- and VLDL-cholesterol concentrations are significantly decreased (table).

	Clinical stage (Child-Pugh score)					
	A		B		C	
	Mean	SD	Mean	SD	Mean	SD
Cholesterol (mmol/l)	5.6	1.1	4.3+*	1.4	3.7+	1.3
Triacylglycerols (mmol/l)	1.3	0.6	1.0	0.4	1.0	0.6
LDL-cholesterol (mmol/l)	3.8	1.0	2.9	1.3	2.7	1.2
HDL-cholesterol (mmol/l)	1.3	0.3	1.2*	0.5	0.8	0.6
VLDL-cholesterol (mmol/l)	0.5	0.6	0.2+	0.2	0.2+	0.1

Significantly different from + A, * C, $p<0.05$ (Scheffé test).

In patients with viral or toxic cirrhosis, poor liver function, as represented by extraordinary small cholinesterase activities in serum (<50th percentile, <1.7 kU/l), is associated with an overall decrease of lipid and lipoprotein concentrations (cholesterol 3.6 mmol/l *vs.* 4.9 mmol/l, t-test $p=0.000$; triacylglycerols 0.9 *vs.* 1.1, $p=0.031$; LDL-cholesterol 2.5 *vs.* 3.4, $p=0.000$; HDL-cholesterol 0.9 *vs.* 1.2, $p=0.003$; VLDL-cholesterol 0.1 *vs.* 0.3, $p=0.003$).

No associations could be found between lipid and lipoprotein concentrations and sex, age, nutritional status or resting energy expenditure, both in viral or toxic and in biliary cirrhosis.

For this we conclude that the cause and stage of liver disease, not their actual metabolic situation, determine dyslipoproteinemia in patients with liver cirrhosis.

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Compliance with dietary advice directed towards increasing the carbohydrate : fat ratio of the everyday diet. By X. H. LYON[1], V. DI VETTA[1], H. MILON[2], E. JEQUIER[1] and Y. SCHUTZ[1], [1]*Institute of Physiology, Faculty of Medicine, 1005 Lausanne,* [2]*Nestlé Research Center, 1000 Lausanne 26, Switzerland*

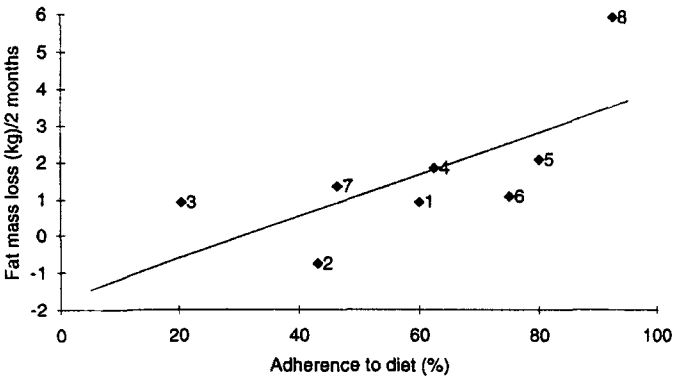
To assess effects, on body weight and body composition, of advice aimed at increasing the carbohydrate (CHO) : fat ratio in the everyday diet without imposing a voluntary restriction on the amount of food consumed, eight moderately overweight women were given specific dietary recommendations during a 2-month study. As shown in the Table below, the energy derived from fat decreased from 44 (SE 1) % to 31 (SE 1) % and the proportion of CHO increased from 38 (SE 2) % to 50 (SE 1) %, whereas the absolute CHO intake remained constant during the entire experimental period.

	Energy intake (kJ/d)		Percentage of total energy as				Absolute daily intake (g/d)			
			Fat		CHO		Fat		CHO	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Baseline	7923	662	44	1	38	2	92	8	182	18
During study	6354	364	31	1	50	1	53	3	189	14
Difference	-1569	520	-12	2	11	1	-39	9	7	9
P (Wilcoxon)	0.016		0.008		0.008		0.008		NS	

NS, not significant.

There was a net loss of body fat mass (1.7 SE 0.7 kg, $P = 0.016$) without loss of fat-free mass. Resting metabolic rate (RMR) remained unchanged during the whole study.

To assess their dietary compliance, the subjects consumed daily a meal artificially labelled with [$^{13}C_6$]-D-glucose. Compliance was estimated from the enrichment in $^{13}CO_2$ of the expired air and ranged from 20 to 93 %. The following Figure shows the significant correlation obtained between compliance and fat mass loss (r_S 0.857; $P < 0.02$).



It is concluded that advice aimed at increasing the CHO : fat ratio of the diet induces a loss of fat mass with fat free-mass maintenance. This slow change in body composition, with no reduction in RMR, seems to be a promising approach to inducing a slow decrease in fat mass over long periods of time in obese subjects.

https://doi.org/10.1079/PNS19950058 Published online by Cambridge University Press

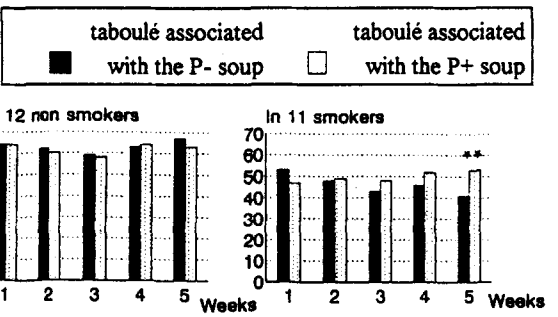
In smokers, is fat intake due to protein appetite ? by M. CHABERT, D. LEPELTIER and J. LOUIS-SYLVESTRE, *Neurobiologie de la Nutrition, EPHE, INSERM U.286;16 rue H. Huchard, 75018 Paris, France.*

Epidemiological studies show that, compared with non smokers, heavy smokers (>20 cigarettes/d) have a significantly increased total saturated fat intake (Troisi *et al.* 1991), a slightly increased total protein intake (Morabia & Winder 1990) and a greatly and significantly increased meat intake (Nuttens *et al.* 1992). Knowing that the oral administration of low levels of nicotine increases nitrogen retention in pigs (Cunningham *et al.* 1963) and that, in Western societies, the major source of protein with high nutritive value is meat, in heavy smokers, the increase in fat intake could be due to an increased need for protein. The question is: could heavy smokers acquire through learning a preference for a flavour repeatedly associated with a covert protein load?

Conditioning was performed over 5 weeks, 2 consecutive days a week. At lunch, subjects were served two-course meals: 1st course *ad libitum*: either a taboulé with tarragon flavour added, or a taboulé with no flavour added; 2nd course given in a fixed amount (291 g, 1.88MJ): either a high-protein soup (P+); or a low-protein soup (P-). For each subject, each taboulé is always associated with the same soup. Soups were not recognized as different by the subjects; their compositions expressed as % energy from proteins, lipids and carbohydrates were respectively 29.9, 27.3, 42.8 for P+ and 10,3, 27.3, 62.4 for P-.

A test was performed as follows : first day, a low-protein breakfast was consumed *ad libitum*, on subsequent test days, the same breakfast was ingested at the same hour. At noon, taboulé was tasted and ingested *ad libitum*, then the soup was tasted and the whole portion ingested.

Pleasantness ratings for the two taboulé after tasting



In smokers only, the taboulé associated with the P+ soup became preferred : the difference between pleasantness ratings for the two taboulé was significant on the 5th week.

These results substantiate a special need for protein in heavy smokers. The nutritive value of the protein from meat could explain the high meat intake. Furthermore, the adverse effect of cigarette smoking on olfactory function (Frye *et al.* 1990) could explain why smokers preferentially consume processed meats and,consequently, ingest a large amount of saturated fat, contained in meat and sauce.

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