Original Article

MOLECULAR METABOLISM

Surplus fat rapidly increases fat oxidation and insulin resistance in lipodystrophic mice

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ABSTRACT

Objective: Surplus dietary fat cannot be converted into other macronutrient forms or excreted, so has to be stored or oxidized. Healthy mammals store excess energy in the form of triacylgycerol (TAG) in lipid droplets within adipocytes rather than oxidizing it, and thus ultimately gain weight. The 'overflow hypothesis' posits that the capacity to increase the size and number of adipocytes is finite and that when this limit is exceeded, fat accumulates in ectopic sites and leads to metabolic disease.

Methods: Here we studied the energetic and biochemical consequences of short-term (2-day) excess fat ingestion in a lipodystrophic (A-ZIP/F-1) mouse model in which adipose capacity is severely restricted.

Results: In wildtype littermates, this acute exposure to high fat diets resulted in excess energy intake and weight gain without any significant changes in macronutrient oxidation rates, glucose, TAG, or insulin levels. In contrast, hyperphagic lipodystrophic mice failed to gain weight; rather, they significantly increased hepatic steatosis and fat oxidation. This response was associated with a significant increase in hyperglycemia, hyperinsulinemia, glucosuria, hypertriglyceridemia, and worsening insulin tolerance.

Conclusions: These data suggest that when adipose storage reserves are saturated, excess fat intake necessarily increases fat oxidation and induces oxidative substrate competition which exacerbates insulin resistance resolving any residual energy surplus through excretion of glucose.
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Keywords Lipodystrophy; Energy partitioning; Fatty acid oxidation; Substrate competition; Insulin resistance

1. INTRODUCTION

Securing sufficient energy to sustain life has been the major energetic challenge for all life-forms throughout most of evolution. Usable energy in the form of ATP can be generated by oxidizing any of the major macronutrients, carbohydrate (CHO), fat or protein. Protein, however, is seldom used for this purpose as proteins largely play essential structural and regulatory roles in cellular function. CHO and fat also play important functional roles, particularly in membrane structure and the generation of signaling intermediates in the case of lipids, but in addition act as the major substrates for oxidative energy production.

In contrast to most other species and to their own ancestors, modern humans typically face a very different challenge: the need to manage chronic excess macronutrient intake. Surplus energy can only really be stored as CHO, in the form of glycogen, or as fat, typically in the form of triacylglycerol in lipid droplets, as there is no recognizable storage depot form for protein. Surplus protein and CHO can ultimately be converted to fat for energy storage, whereas excess fat cannot be quantitatively converted to CHO or protein; nor can it be excreted, so it has to be stored or oxidized. In healthy humans, adipocytes have evolved highly efficient mechanisms for taking up and storing surplus fat. In the short term, this response limits exposure of other tissues, which are less well-adapted for lipid storage, to excess lipid thus alleviating the adverse effects of surplus lipid accumulation. In the longer term, the increase in size and number of adipocytes results in a rise in fat mass or obesity.

One of the prevailing hypotheses for the tight association among obesity and its metabolic consequences such as insulin resistance, non-alcoholic fatty liver disease, dyslipidemia, and type 2 diabetes hinges on the notion that the ability to increase the size and number of adipocytes is ultimately 'limited' and that, in such circumstances, lipid accumulates in other less well adapted tissues [1—3], particularly the liver, where it is instrumental in inducing insulin resistance [4,5]. Terms used to refer to this idea include the 'lipid overflow hypothesis' [1—3] or 'adipose expandability hypothesis' [6]. In reality, it seems more likely that in 'common' forms of polygenic obesity, the efficiency with which adipose tissue mass increases may well decrease as fat mass expands and similarly the efficiency of postprandial adipose tissue lipid buffering [7] may well decrease, leading to excess lipid delivery to other tissues. This 'incremental' change makes it relatively difficult to impose a defined experimental perturbation with measurable outcomes. Lipodystrophy (LD), a state defined by reduced adipose tissue mass and function [8,9], provides a unique opportunity to directly assess the immediate consequences of surplus fat ingestion in

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Received April 5, 2018 • Revision received May 2, 2018 • Accepted May 7, 2018 • Available online xxx

https://doi.org/10.1016/j.molmet.2018.05.006

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a situation where the capacity of adipose tissue to expand is constrained.

We have previously undertaken an acute, hypercaloric, high fat challenge (30% excess energy as fat) in 7 patients with different forms of partial or generalized lipodystrophy [10]. In that study, we detected a small but significant increase in total energy expenditure, which was almost entirely dependent upon an increase in fat oxidation (29%). However, the study was limited by the diverse range of responses observed in what was a relatively heterogeneous group of patients with different genetic forms of partial or generalized lipodystrophy. We were also unable to ascertain which tissue or tissues were predominantly responsible for the increase in fat oxidation. Here we sought to examine the metabolic and biochemical response to acute high fat feeding in A-ZIP/F-1 (hereafter AZIP) mice, a well-established model of generalized LD [11].

AZIP mice over-express, in an adipose specific manner (by virtue of the aP2 enhancer/promoter), a dominant negative protein termed A-ZIP/F, which prevents the DNA binding of B-ZIP transcription factors of both the CCAAT/enhancer binding protein (C/EBP) and Jun families, both of which are involved in the development and function of adipose tissue [11]. The transgenic AZIP mice are devoid of white adipose tissue throughout life and also have reduced amounts of brown adipose tissue, which expresses very low levels of UCP1 and appears to be inactive [11]. The physiological consequences of the lack of fat are pronounced and include severe liver steatosis, hyperlipidemia and early-onset diabetes [11,12].

2. METHODS

2.1. Animals

A-ZIP/F-1 mice were initially obtained from the NIH (USA). All A-ZIP/F-1 mice used in this study were hemizygous females on the FVB/N background, produced by breeding hemizygous males with wild-type (WT) females [11]. Female wildtype littermates were used as controls. Four mice were housed per cage, maintained in a 12 h light (06h00-18h00)/dark cycle, and had ad libitum access to a low fat diet (D12450B, Research Diets) or high fat diet (D12451, Research Diets). Animals studied at thermoneutrality were acclimatized to 30 °C for 7–10 days prior to the experiment. For intraperitoneal insulin tolerance tests, an injection of 0.5 U/kg insulin was given to 5 h-fasted (08h00-13h00) mice. Blood collection was performed either on fed or 5 h-fasted animals. Experiments were undertaken on 8–10 week old animals in the animal facilities of the Metabolic Research Laboratories, Institute of Metabolic Science in Cambridge (UK) and of the Rangueil site of US006/Crefre/Inserm/University of Toulouse (France).

2.2. Study approval

All animal studies were approved by the UK Home Office and the University of Cambridge according to the Animals (Scientific Procedures) Act 1986 and associated guidelines and/or according to the INSERM Animal Care Facility guidelines and local ethical approval from Toulouse Rangueil Hospital.

2.3. Indirect calorimetry

Animals were studied in a Metatrace calorimetry system (Creative Scientific). Airflow rates were 400 ml/min and oxygen – and carbon dioxide concentrations in room air and air leaving each cage were measured every 10 min. Fat and carbohydrate oxidation figures were calculated using the following equations: fat oxidation (mg/h) $= [1.695\times(VO_2\ \text{l/min} \times 60)]-[1.701\times(VCO_2\ \text{l/min} \times 60)]$; carbohydrate oxidation (mg/h) $= [4.585\times(VCO_2\ \text{l/min} \times 60)]-[3.226\times(VO_2\ \text{l/min} \times 60)]$. Protein oxidation was assumed to be equivalent to protein intake.

Further methodological details are available in the Supplementary Material.

2.4. Statistics

Quantitative data are expressed as mean ± SEM. Multiple group comparisons were determined with 1 or 2-way ANOVA using Graph Pad Prism software. Comparisons between two independent groups were assessed using unpaired Student *t*-tests. Statistical significance was defined as $p < 0.05$.

Table 1 – Body composition and blood biochemistry in female AZIP lipodystrophic mice (n = 8–10) and wild type littermate controls (n = 8–10) whilst being fed a regular low fat chow-(LFD) or high fat diet (HFD). Blood was taken in the fed state for all parameters except for glucose and insulin that were measured in both the fed state and after a 5 h fast.

	Wildtype		AZIP	
	LFD	HFD	LFD	HFD
Body weight (g)	25.9 ± 1.0	27.5 ± 1.1***	26.5 ± 0.8	26.8 ± 0.7
Fat mass (%)	20.9 ± 2.7	29.3 ± 2.2***	5.5 ± 0.8$^{\#\#\#}$	7.8 ± 0.3$^{\#\#\#*}$
Glucose (mmol/l)				
fed	10.5 ± 0.3	11.3 ± 0.3	25.6 ± 4.3$^{\#\#\#}$	34.6 ± 0.7$^{\#\#\#*}$
fasted	7.9 ± 0.2$^{\&\&\&}$	7.9 ± 1.1$^{\&}$	13.4 ± 4.2$^{\#\#\&}$	32.5 ± 1.3$^{\#\#\#**\&}$
Insulin (μg/l)				
fed	1.1 ± 0.3	1.2 ± 0.2	71.5 ± 21.0$^{\#\#\#}$	133.1 ± 3.6$^{\#\#\#**}$
fasted	0.7 ± 0.1$^{\&}$	0.8 ± 0.2$^{\&}$	16.7 ± 2.7$^{\#\#\#\&\&}$	27.6 ± 6.5$^{\#\#\#**\&\&\&}$
TG (mmol/l)	3.8 ± 0.4	2.1 ± 0.1*	14.1 ± 2.8$^{\#\#\#}$	34.2 ± 5.8$^{\#\#**}$
Urine glucose (mmol/l)	3.2 ± 0.3	4.3 ± 0.2	406.7 ± 19.6$^{\#\#\#}$	529.6 ± 17.5$^{**,\#\#\#}$
CHO energy loss (kJ/mouse/day)	0.03 ± 0.00	0.03 ± 0.00	18.10 ± 1.85$^{\#\#\#}$	18.03 ± 0.36$^{\#\#\#}$
Liver weight (g)	1.31 ± 0.08	1.35 ± 0.11	3.58 ± 0.15$^{\#\#}$	4.52 ± 0.19$^{\#\#}$
Soleus weight (mg)	n.d.	6.33 ± 0.30	n.d.	5.42 ± 0.73
EDL weight (mg)	n.d.	8.83 ± 0.44	n.d.	8.50 ± 0.31
Gas. Weight (mg)	n.d.	113.26 ± 10.6	n.d.	122.73 ± 4.12

TG, triglyceride; CHO, carbohydrate; EDL, extensor digitorum longus; Gas., gastrocnemius.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to LFD (*t*-test).
#$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ compared to WT (*t*-test).
$^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$ compared to the fed state (*t*-test).

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