

Effect of Cafeteria Diet Feeding on Soleus Intramyocellular Lipid of Wistar Rats

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Abstract

Background: The presence of lipid besides muscle fibres facilitates the energy supply for exercise, but it is also indicative of insulin resistance in the untrained. Muscle lipid is associated with increased dietary energy: hyperlipidic diets induce an increase in intramyocellular lipid deposition in skeletal muscle.

Methods: In the present study we analyzed the changes in soleus (a red-fibre muscle) intracellular muscle content under a hyperlipidic (cafeteria) diet in Wistar rats. We also analyzed in parallel the mitochondrial content a relative index of energy output capability.

Results: Cafeteria diet-fed rats contained more lipid and mitochondria per unit of muscle section area than controls.

Conclusions: The correlation found in the increases of muscle lipid and mitochondria hit at this increase as an adaptation of muscle to oxidize excess energy substrates under conditions of excess energy availability, probably contributing to adaptive thermogenesis.

Keywords: Cafeteria diet; Intramyocellular lipid; Dietary energy; Muscle lipid; Mitochondria; *Soleus* muscle

Introduction

Muscle accounts for about 40 % of body weight, both in rats [1] and humans [2], thus constituting the largest organ/tissue of normal weight individuals. Muscle contains both structural lipids (mainly membrane phospholipid and cholesterol), and reserve-related lipids (mainly triacylglycerols). The latter include two different compartments: intramyocellular lipid (IMCL) and white adipose tissue cells. Muscle adipose tissue is often interspersed between muscle bundles and in contact with its surface and vessels [3].

Muscle reserve lipid supplies energy to sustain the muscle needs, especially during endurance exercise [4], a role largely sustained by IMCL. The content of IMCL (and mitochondria) in white-fibre glycolytic muscle is lower than in red-fibre oxidative muscle; since the main energy supply comes from glycolysis in white- and fatty acids in red-muscle fibres. The amount of lipid present in muscle is a critical factor for the duration of exercise endurance activity because it supplies a large part of the energy used in red-fibre muscles [5].

Hyperenergetic diets, i.e. hyperlipidic diets, tend to overcome the ability of the ponderostat system to maintain the mass of body energy reserves [6] when administered for long periods of time. This helps increase muscle fat deposition in both compartments IMCL and adipose tissue cells. Cafeteria diet, a self-selected highly palatable diet [7], is hyperenergetic because it contains a high proportion of lipid, selected in excess by the rats, whilst the proportions of protein and carbohydrate ingested are better regulated and comparable to those of controls fed rat chow pellets [8]. Rats with access to a cafeteria diet accumulate a high amount of body fat [9], primarily found in large white adipose tissue masses, but also in disperse or small anatomically distinct masses [10]. In general, hyperlipidic diets increase the storage of fat in other organs such as liver and muscle [11], increasing IMCL [12].

The actual meaning of increased IMCL by hyperlipidic diets is unclear, since excess lipid may hamper the normal operation of muscle, as found in some cardiomyopathies [13] in which heart muscle function is affected by deposits

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of very long-chain fatty acids in triacylglycerols [14]. It may be speculated that excess muscle lipid accumulation is a consequence of high fatty acids availability, and their use as fuel for muscle contraction in insulin resistance [15]. However, under standard conditions blood-carried glucose, and lipoprotein triacylglycerols (supplying fatty acids in addition to those carried bound to albumin) seem enough to maintain the muscle in full operative conditions.

In the present study we intended to analyze the IMCL content of the *soleus*, a red-fibre muscle, and its modulation by a limited exposure to a hyperlipidic cafeteria diet. We limited the extension of dietary lipid exposure to obtain a sizeable overweight, but not full-blown obesity [16] characterized by massive metabolic derangement. This particular muscle was selected because its isolation allows for a “clean” extraction, free of perimuscular and interspersed adipose tissue.

Materials and Methods

Animals and diets

Wistar 60-day old male rats (Harlan-Interfauna, Sant Feliu de Codines, Spain) were used. They were maintained under standard conditions (21 - 22 °C, 50 - 60 % relative humidity, and 12 h light/dark cycle) in two-rat cages. A 6-rat control group was maintained under these conditions, and fed *ad libitum* with standard rat chow (maintenance type, Panlab, Barcelona, Spain) for 30 days. A second group was given a simplified cafeteria diet [17] in addition to the standard chow for 30 days, when the cafeteria-fed rats were already overweight [18].

All animal handling procedures were carried out following the guidelines established by the EU, and the Spanish and Catalan Governments. The Ethics Committee of the University of Barcelona approved the experimental setup and procedures.

Muscle dissection and lipid analysis

At the end of the 30-day diet exposure period, the rats were killed by decapitation. The left leg *soleus* muscle was immediately exposed and bathed (first *in situ* and later 24 h in a container placed in ice, until processed) with chilled fixing solution (25 g/L glutaraldehyde and 20 g/L paraformaldehyde in 100 mM phosphate buffer pH 7.2) [19] during the process of dissection and later storage. The right leg muscle was dissected and weighed, then frozen in liquid N₂ and kept at -80 °C. Right-leg muscles were homogenized in trichloromethane-methanol (2:1 v/v) in the cold using a Potter-Elvehjem type all-glass homogenizer. The clear organic supernatant was used for the gravimetric estimation of total sample lipid [20].

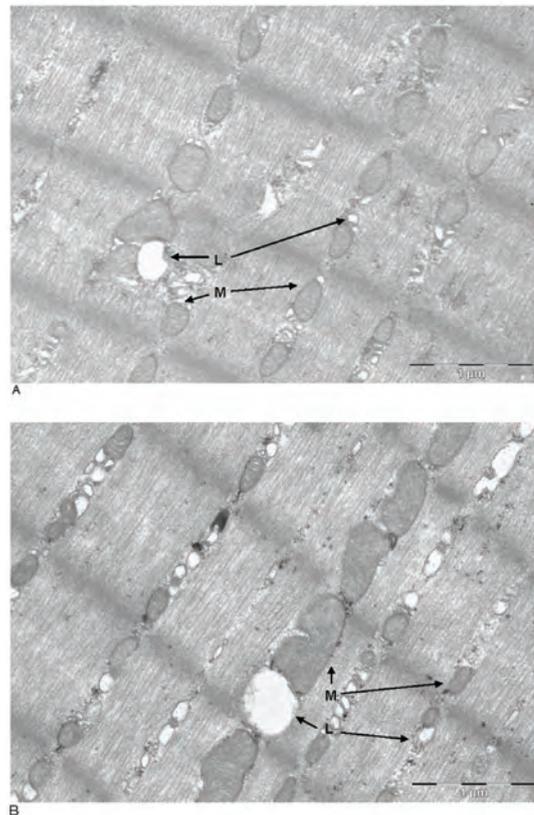


Figure 1. Transmission electronic microscope photographs of *soleus* muscles of male Wistar rats fed control (A, top) or cafeteria diet (B, bottom). The cuts were stained with OsO₄. M: mitochondria; L: lipid droplet. Magnification: the bar represents 1000 nm.

Microphotographic analysis of intramyocellular lipid

The fixed tissues were treated with 10 g/L osmium tetroxide [21] for 1 hour. Then the muscles were dehydrated with acetone and included in an epoxy resin (Eponate 12, Ted Pella Inc., Redding CA, USA). The hardened pieces were cut longitudinally in a microtome (Leica Ultracut, Wetzler, Germany) obtaining 60 nm thick sections. The cuts were observed at 30,000 × magnification using a JEOL JEM-1010 (Akishima, Japan) transmission electronic microscope. We obtained 45 microphotographs from each sample following an aleatory protocol for the selection of the cuts and fields. The resulting images were processed with the AnalySIS (Soft Imaging Systems, Munster, Germany) program after manual delimitation of both lipid vacuoles and mitochondria.

Statistical analyses were carried out using the paired Student's *t* test.

Results

Total lipid content in *soleus* did not shown differences be-

Table 1. Lipid and Mitochondria Content of *Soleus* Muscle of Control and Cafeteria Diet-Fed Male Rats

Parameter	units	30 day-control diet	30 day-cafeteria diet	P
Mean lipid droplet area	μm^2	0.014 ± 0.001	0.016 ± 0.001	NS
Number of lipid droplets per field		19.1 ± 3.71	23.1 ± 5.11	NS
Lipid droplet area per field	μm^2	0.171 ± 0.083	0.244 ± 0.012	< 0.001
	% of FSA	1.24 ± 0.06	1.77 ± 0.09	< 0.001
Mean mitochondria area	μm^2	0.057 ± 0.003	0.064 ± 0.003	NS
Number of mitochondria per field		10.3 ± 1.91	11.1 ± 1.01	NS
Mitochondrial area per field	μm^2	0.530 ± 0.018	0.682 ± 0.029	< 0.001
	% of FSA	3.84 ± 0.14	4.94 ± 0.212	< 0.001

% of FSA = percentage of the observed field = s surface area ($13.82 \mu\text{m}^2$).

tween groups (3.77 ± 0.28 mg in control rats and 4.59 ± 0.39 mg in cafeteria-fed rats. Figure 1 shows representative microphotographs of the *soleus* muscles of Wistar male rats fed control or cafeteria diets. The number/size of lipid droplets and mitochondria were more abundant in the cafeteria diet-fed animals. The quantitative data are summarized in Table 1, where the number of lipid droplets and their size (as well as those of mitochondria) are presented referred to the standardized microphotograph field. In spite of the means being higher for both series of parameters in cafeteria than in control rats, the differences were not significant. However, when comparing the combined lipid or mitochondrial areas per field between both dietary groups, the differences became significant, i.e. cafeteria diet-fed rats contained more lipid and mitochondria per unit of muscle section area than controls. The correlation between the numbers of lipid droplets per field versus the number of mitochondria per field resulted in P values of 0.144 (not significant) for controls, 0.021 for cafeteria and 0.008 for all animals (control + cafeteria) combined. This shows that the counts for mitochondria and lipid droplets are correlated, irrespective of diet, since the sizes of both mitochondria and lipid droplets were similar in both dietary groups (albeit slightly larger, but not significantly, in cafeteria muscles) we can safely assume that the number of lipid droplets and mitochondria in the muscle were correlated, increasing in parallel with cafeteria feeding, since the total amount of lipid in the (right leg) muscles of cafeteria-fed rats were higher than in controls.

Discussion

The increase in lipid content of the whole body and indi-

vidual tissues under a hyperlipidic diet has been repeatedly described in the literature [22], comprising increases in total muscle lipid [23], and even in IMCL [24]. This increase is currently attributed to an excessive availability of lipid, and the need to store it somehow/somewhere, both as reserve and as a way to minimize, in the short term, its potentially lipotoxic effects. However, muscle lipid has a well-defined function, that of supplying red-fiber muscles with a reliable source of fuel for sustained activity; thus, an excess of lipid deposition may induce a loss of efficiency by hampering muscle function [25]. It may be construed that lipid accumulation in muscle intracellular space may result in the equivalent of hepatic steatosis, in that excess lipid storage impairs liver function [26]. In addition, the special fibrous nature of muscle leaves little space for lipid storage, which in some way must “compete” for space with other cell organelles such as mitochondria, endoplasmic reticulum, sarcolemma sacs and glycogen granules.

Lipid accumulation in soleus muscle is detectable and statistically significant under conditions of excess lipid availability and enhanced storage elsewhere; however not to the extent observed in other tissues such as liver or, specially, adipose tissue [27]. The parallel rise in the number of mitochondria marks another important difference, unparalleled in other lipid-storing tissues, since its function is clearly oxidative and counterpoised to the storage of “excess” energy. An increased number of (normal-size) mitochondria hints to an increased capability of ATP synthesis for muscle operation.

However, both control and cafeteria diet-fed rats were kept in limited spaces (cages) in which no sustained exercise was practically possible (neither observed), which does not justify any increase in power output (ATP) capability that

a higher number of mitochondria represent [28]. Continued exercise increases muscle mass, mitochondria and power output; but here we observe this potential increase in power output without the previous stimulus of exercise. It may be, thus, postulated, that the higher accumulation of lipid in muscle triggers mitochondriogenesis, but the ultimate reason for this increase remains obscure.

Thermogenesis is the main mechanism for adjusting energy availability and energy needs in mammals (in higher proportion in the smaller ones) by converting excess nutrients into heat. This role is usually carried out, at least in rodents, mainly by brown adipose tissue [29], which uncoupling mechanism is nowadays widely known [30]. However, brown adipose tissue thermogenesis does not explain a significant part of whole body adaptive thermogenesis, including the possible role of liver [31], overall inefficiency elicited by thyroid hormones [32], and muscle activity [33]. A higher energy wasting by muscle has been attributed to increased expenditure in maintaining muscle tone [34], but no “chemical” mechanisms have been advanced to explain the role of muscle in wasting energy in thermogenic processes. The usual way of heating muscle up to fully functional temperature remains shivering thermogenesis [35], directly related to cold exposure.

Recent studies have shown that muscle thermogenesis may represent a significant proportion of total body heat production, especially because of its large proportion versus body weight [36]. Analysis of muscle mitochondria operation in obese rodents has shown an increase in inefficiency [37] which may result in permanent metabolic damages in severe obesity [38].

The role of muscle as wasting energy organ for excess available energy has not been sufficiently explored, probably because of its dispersion, different structure, fibre composition and interspersed conjunctive and adipose tissues. In addition a small contribution of mitochondrial inefficiency may account for a significant proportion of thermogenesis in small animals [39] because of their total muscle large weight, an effect probably magnified in humans because of less significant contributions of brown adipose tissue [40].

In this context, the parallel increase in muscle lipid and mitochondria makes sense, and may help explain the oxidation of excess energy in muscle to produce heat. So far, we don't know precisely the mechanism of energy wasting [41], but the machinery is in place, responds to a clear metabolic challenge and may explain the postulated implication of muscle in “chemical” thermogenesis.

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