



Glutamine contribution to gluconeogenesis in healthy adults fasted for 13 h, and 37 h

R Hankard, Mw Haymond, D Darmaun

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glucocorticoids and cAMP and inhibited by insulin and phorbol esters [Aggerbeck et al (1993), *Biochemistry* 32, 9065-9072]. The role of cAspAT in the metabolism of adipocytes is not clear. Adipose tissue is not gluconeogenic and the urea cycle is not functional in this tissue. Like cytosolic phosphoenolpyruvate carboxykinase (PEPCK), cAspAT could be implicated in glyceroneogenesis in adipose tissue in situations of hypoglycemia (starvation, hyperproteic and hyperlipidic diets).

We used the 3T3-F442A cell line to study the regulation of cAspAT gene expression in cultured adipocytes. Cells were grown and differentiated as described previously [Plee-Gautier et al (1996), *Biochem J* 318, 1057-1063]. cAspAT mRNA content was measured by Northern blot using a rat cDNA fragment. 3T3-F442A adipocytes maintained in serum-free medium responded to an 18 h glucose deprivation by a 4-fold increase in cAspAT mRNA, whereas mAspAT mRNA remained unchanged. cAspAT activity was also increased in a weaker (1.5-fold in 48 h) but reproducible manner. Glucose addition to 24 h glucose-deprived adipocytes produced an 80% reduction in cAspAT mRNA in 8 h. This effect occurred under a physiological range of glucose concentrations ($EC_{50} = 1.25$ g/L). In Fao or H4IIE hepatoma cells, C2 muscle cells and 293 kidney cell, no regulation of cAspAT mRNA could be detected, demonstrating that glucose effect was cell-specific. The glucose analogue 2-deoxyglucose mimicked, at least partially, the glucose effect, suggesting that glucose-6-phosphate or glucose itself is the effector. We co-transfected 3T3-F442A adipoblasts as previously described [Antras-Ferry et al (1994), *Biochem J* 302, 943-948], with the region of the cAspAT gene from -2405 to -26 base pairs relative to the translation initiation site, fused to the chloramphenicol acetyltransferase (CAT) gene and the construct containing the SV2 promoter of the SV40 genome fused to the NEO gene. The latter confers resistance to

the antibiotic neomycin (G418). Twelve individual G418-resistant clones were isolated and analysed for CAT expression and regulation by glucose. After differentiation, stable transfectants responded to a 24 h glucose deprivation by a mean of 3.5-fold increase in CAT activity. Hence, glucose action is, at least partially, transcriptional and a negative glucose response region is located between -2405 and -26 bp of the cAspAT gene.

Experiments aimed at elucidating the mechanism of glucose-induced inhibition of cAspAT gene expression are underway and should help clarifying the role that cAspAT plays in adipocytes and the physiological significance of its regulation by glucose.

Glutamine contribution to gluconeogenesis in healthy adults fasted for 13 h, and 37 h. R Hankard, MW Haymond, D Darmaun (*Nemours Children's Clinic, Jacksonville, FL, USA*).

Other authors have shown that upon infusion of ^{14}C glutamine (gln), ^{14}C appears in plasma glucose (glc) in post-absorptive humans. Labeled carbon arising from glutamine's carbon skeleton might appear into glucose either through glutamine entering Krebs cycle or through mere fixation of labeled CO_2 arising from glutamine oxidation. These two pathways were quantitated in eight healthy adults (5 M, 3 F, age: 29 ± 2 years, weight: 76 ± 4 kg, means \pm SD) using 4-h-intravenous infusion of L-[3,4- $^{13}\text{C}_2$]gln, D-[6,6- D_2]glc, et L-[1- ^{14}C]leucine after 13 h, and 37 h of fasting. Carbon transfer from glutamine to glucose through the non-specific CO_2 pathway was estimated from CO_2 pool labeling resulting from ^{14}C -leucine oxidation and measurement of glucose specific activity (SA) in plasma and SA_{CO_2} . Total carbon flux from glutamine to glucose measured after glucose combustion using gas-chromatogra-

phy-isotopic-ratio-mass-spectrometry (GC-IRMS). Glucose turnover rate ($R_{a,glc}$) was calculated from d_2 -glc enrichment in plasma at steady state. Results are means \pm SD, significance of observed differences using paired-*t*-test. Extending the fasting state from 13 h to 37 h: 1) $R_{a,glc}$ decreased from 10.9 ± 1.1 to $8.1 \pm 0.6 \mu\text{mol.kg}^{-1}.\text{min}^{-1}$, $P < 0.01$, 2) glutamine contribution to neo-formed-glucose carbon skeleton increased from 8 ± 3 to $16 \pm 7\%$, $P = 0.02$, and 3) non specific CO_2 transfer to glucose remained unchanged (4 ± 1 vs $3 \pm 2\%$ of total carbon flux to glucose, NS).

Conclusions: a) carbon transfer from glutamine to glucose mainly occurs through the Krebs cycle, and b) glutamine contribution to gluconeogenesis increases with the duration of fasting. This study emphasizes the role of glutamine as a major gluconeogenic precursor in vivo.

Dietary protein quality influences post-prandial protein utilization. Y Boirie¹, M Dangin¹, P Gachon¹, JL Maubois², B Beaufrère¹ (¹ *Laboratoire de nutrition humaine, CRNH, 63000 Clermont-Ferrand*; ² *Laboratoire de technologie laitière, Inra, 35000 Rennes, France*).

During feeding, protein intake modulates post-prandial protein gain, but protein structure may also influence kinetics of protein metabolism. The aim of the study was to evaluate protein catabolism and leucine oxidation after ingestion of two proteins with high biological value, but with different digestion rate. Leucine metabolism was investigated in 12 healthy volunteers in non steady state conditions after a single protein meal consisting in two milk protein fractions, whey protein (WP) or casein (CAS). Using a combination of tracers (^{13}C leucine – either in an intrinsically labelled protein and orally administered, or free and intravenously – and $^2\text{H}_3$ leucine – free orally or IV administered – respectively),

kinetic modifications over 420 min following meal ingestion were calculated from plasma leucine concentrations and enrichments and from $^{13}\text{CO}_2$ enrichments in breath. Leucine rate of appearance from CAS is slower but more prolonged than from WP (exogenous leucine flux: 0.82 ± 0.03 vs $0.29 \pm 0.08 \mu\text{mol.kg}^{-1}$, moy \pm SEM, CAS vs WP at 240 min, $P < 0.001$). Endogenous leucine flux (an index of protein catabolism) is progressively inhibited with a constant inhibition between 120 and 360 min with CAS, whereas WP do not change proteolysis (-30.2 vs -7.1% from baseline, CAS vs WP at 240 min, $P < 0.01$). Cumulative ingested leucine oxidation is identical with the two proteins ($\approx 1/3$ of ingested leucine is oxidized), but total leucine oxidation is lower with CAS. Thus, net leucine balance over 7 h (intake minus total oxidation) is neutral, with WP and positive with CAS ($+135 \pm 38$ vs $-11 \pm 15 \mu\text{mol.kg}^{-1}$, CAS vs WP, $P < 0.01$).

In conclusion, casein administered as a single protein load, compared with whey protein, results in a prolonged rate of amino acids appearance, a net inhibition of proteolysis, a lower total leucine oxidation and is responsible for a better protein gain.

Growth and muscle protein turn-over: effect of genotype and amino acids. S Tesseraud, A Besnard, R Peresson, J Michel, E Le Bihan-Duval, AM Chagneau (*Inra, station de recherches avicoles, 37380 Nouzilly, France*).

Broiler carcass quality can be improved by the usual selection techniques. A quality line was thus selected which yielded birds with low fatness and high breast meat yield [Richard et al (1994), *Inra Prod Anim* 7, 253-261]. We analysed the effects of this selection on amino acid requirements and muscle protein turnover in 3-week-old chicks.