EFFECT OF FATTY ACIDS AND ANTIOXIDANTS ON GLUCOSE TOLERANCE

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Abstract. The qualitative influence of free fatty acids (FFAs) on glucose metabolism is well documented while there is no data quantifying this relationship. An attempt to measure this correlation was made by employing a prospective, cross-sectional study involving 21 patients grouped in 5 categories established after a standard 2h Oral Glucose Tolerance Test (OGTT): normal glucose tolerance (N); insulin resistant (IR; IR2); impaired glucose tolerance (IGT) and diabetes (D). Two OGTTs were performed for each patient, separated by two weeks during which dietary supplements were taken along with restriction of saturated fat ingestion; levels of FFAs, aminoacids, etc., were measured on both patient visits. Glucose metabolism was quantified as the area under the curve and named glucose metabolic index (GMI).

FFAs influence on GMI was dependent on their degree of saturation, with saturated and monounsaturated fats impairing glucose tolerance and polyunsaturated fats (especially tetraenoic and hexaenoic acids) improving it. Short chain FFAs (up to 14 C) had a negative effect; 16 and 18 C were weakly correlated and 20-26 C had a positive effect on glucose tolerance. Levels of aminoacids and metabolic intermediaries suggested involvement of aminoacids in energy production and showed mitochondrial energy overproduction.

Glucose and energy metabolism were altered in IR, IGT and D patients in a similar fashion; those alterations were more pronounced in D than in the IR while none was present in the N group. Glucose tolerance was improved upon restriction of saturated fats intake along with administration of antioxidants in D, IGT and IR groups.

Key words: glucose tolerance, OGTT, diabetes, fatty acids, energy metabolism, GMI.

INTRODUCTION

The interplay between the metabolism of glucose and lipids was pointed out decades ago with the proposal of the eponymous Randle cycle; the more specific role of free fatty acids - FFAs - on peripheral glucose uptake and hepatic gluconeogenesis was shown more recently (1- 3). On a molecular level it was established that the expression of the insulin gene is inhibited by chronic

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hyperlipidemia (4) and that FFA-induced insulin resistance is associated with alterations of cellular signaling pathways involving insulin receptor substrate IRS-1 and phosphatidylinositol 3-kinase (5-8) as well as protein kinase C θ (9). Long chain fatty acids Acyl CoA were implicated in modulating the pancreatic secretion of insulin via K channels (10) or through a G-protein coupled receptor — GPR40 — (11) or directly by apoptosis of beta cells (12).

Lower insulin sensitivity in the muscle (13-16), adipose tissue (17), liver (18, 19) and lymphocytes (20) was linked to elevated FFA levels. Those effects were attributed mostly to the paracrine and endocrine functions of the adipose tissue via adiponectin, leptin, resistin, etc — (21, 22), however a more important role is conferred currently to the convergent action of FFAs (17, 20), oxidized low density lipoproteins — oxLDL — (23, 24) and inflammatory cytokines (25, 26). The discovery of peroxisome proliferator-activated receptors (PPARs)— added another important piece to the puzzle of intracellular regulation of glucose and fatty acid metabolism. PPARs are the target molecule of fibrates (PPAR alpha), thiazolidindiones (PPAR gamma) as well as many physiological activators of which the most common are fatty acids (27-29). Finally, the type of FFA — saturated versus unsaturated — was shown to be important for the risk of developing insulin resistance and diabetes (30-32); however, the precise mechanism of FFA modulation of glucose metabolism is still under debate.

With this in mind we are proposing to study the correlation between glucose metabolism and the type of FFA, aminoacids and various metabolic intermediaries. The study employed is cross-sectional and prospective, involving a small number of patients with diabetes, impaired glucose tolerance, normal controls and a separate category of insulin resistance defined below. The study was performed between August 2005 —January 2006 with voluntary participation from patients and healthy controls.

MATERIALS AND METHODS

Participants in this study were enrolled while they were visiting the Outpatient Department of a community healthcare center in Northeast Arizona. Criteria of inclusion were adults over 18, non-pregnant, not currently on any oral hypoglycemiants or insulin. Exclusion criteria were current treatment with steroids, thiazide diuretics, phenytoin, estrogens, barbiturates, and lithium.

After agreeing to participate in the study, at the initial visit we measured for each patient the weight, height and waist circumference and collected venous blood samples by antecubital venipuncture after making sure that the patient was fasting. If fasting blood glucose level was less than 140 mg/dL the patient was given a standardized 75 g glucose drink from Fisher Scientific; additional venipunctures were performed at 60 and 120 minutes after glucose ingestion.

Each patient was instructed to avoid as much as possible the consumption of

saturated fats (butter, margarine, sour cream, lard, etc.) during the study and was provided with a 14 day supply of dietary supplements as follows: 1. Essential aminoacids 4 g/day; 2. Polyunsaturated (omega 3- and 6) fatty acids softgels, 1 g /day; 3. Antioxidant Formula (Vitamin A 10,000 IU, Vitamin C 250 mg, Vitamin E 200 IU, Zinc 7.5 mg, Selenium 15 mcg, Copper 1 mg, Manganese 1.5 mg) 1 softgel/day; 4. S- adenosylmethionine, 400 mg/day; 5. L-Arginine, 500 mg /day; 6. L- Glutamine, 500 mg /day; 7. Ester- C, 1000 mg/day. No other recommendations or restrictions for diet or lifestyle modifications were given to the patients.

The second study visit, including the OGTT and blood sample collection, was performed at 14 days after the initial visit; during this visit each patient filled a questionnaire on the presence of adverse reactions.

Blood glucose levels were measured from whole venous blood in the facility laboratory immediately following collection; the equipment used was tested for accuracy on a daily basis according to CLIA standards. Serum or plasma was obtained by centrifugation within 30 minutes of blood collection; after storage at —30°C for up to 8 weeks they were shipped overnight on dry ice for testing to the laboratories as follows: plasma fatty acids, aminoacids and urinary metabolites at both the initial and follow-up visit were performed by Metametrix Laboratories of Norcross, GA. Plasma insulin levels were performed on all frozen samples obtained during OGTT at ARUP laboratories in Salt Lake City, UT via TriCore labs in Albuquerque, NM. All patients have undergone sampling for FFAs, aminoacids and metabolites on both visits, which allowed comparisons between the same patients (intra-group) before and after the intervention as well as between groups. Because of budget restrictions, not all samples could be tested for all molecules; the testing of the samples was done at the outside laboratories without knowledge of the patient information after random selection of the samples. Patient specimens were identified only by letters and numbers assigned by the principal investigator; patient identification and health status were kept confidential.

In order to make a uniform comparison between individuals and groups of patients relative to glucose metabolism and utilization, as well as being able to establish a quantitative correlation with plasma levels of various molecules measured, we have used an index named Glucose Metabolic Index (GMI). This general index makes use of the 3 blood glucose values obtained during OGTT and was calculated as the area under the glycemic curve with the formula: GMI = F + 2 x H1 + H2 (mg/dL), where F is fasting plasma glucose; H1 is glucose value at 1 hour and H2 is patients' glycemia at 2 hours.

Data was analyzed with Excel and NCSS; comparisons between groups (initial vs. final and between groups) was performed with a two tailed t-test. Correlations between GMI and various parameters were given as Spearman coefficients with their respective p values if statistically significant. We have looked for significance in the differences between the 5 groups of patients in the levels of FFAs, aminoacids and metabolites in two ways: 1. statistical significance (when the p value of the two-tailed T test is less than 0.05); 2. consistency, defined as similar trend across the

groups of patients. For this purpose was used the correlation coefficient r between all GMI values and all numeric values of the plasma levels of a substance; it had a positive value ($r > 0$) for substances associated with an impairment of glucose tolerance, or a negative value ($r < 0$) for substances linked to improvements in glucose tolerance. To be consistent, the level of a substance showing a statistically significant difference ($p < 0.05$) between two or more groups should also decrease from N to IR to IGT to D if $r < 0$ or it should increase from N-IR-IGT-D if $r > 0$.

Finally, as a consequence of the number of patients in which testing was performed, the correlation coefficient r was in itself statistically significant ($p \leq 0.05$) if $[r] \geq 0.40$ when testing was done for FFAs and aminoacids (n=15); when correlating values for metabolites (n=7) there was statistical significance if $[r] \geq 0.65$.

The study was approved by the corresponding Institutional Review Board and each patient has signed an approved Informed Consent Form.

RESULTS

A total of 21 patients were considered for studying the correlation between glucose tolerance and FFAs, aminoacids and metabolites. Using the results of the OGTT testing and insulin levels, patients were grouped in one of the following 5 categories: 1. *Normal* (N) — normal blood glucose levels and insulin levels on OGTT (n = 6); 2. *Insulin Resistant* (IR) - normal glucose levels and elevated insulin levels (n = 4); 3. *Insulin Resistant Type 2* (IR2) with normal glucose levels, elevated insulin levels and inverted shape of the glycemic curve: H2>H1 (n = 3); 4. *Impaired Glucose Tolerance* (IGT): those with blood glucose > 140 mg/dL at 2 hours and elevated insulin levels (n = 4); and 5. *Diabetes* (D); patients with glucose levels >200mg/dL on two distinct measurements (at both H1 and H2 or in both testing days) and/or fasting glucose more than 126 mg/dL (n = 4). All patients with IFG (F>105 mg/dL; n = 4) also met the criteria for Diabetes and were not considered as a separate group in our analysis.

The criteria used for diagnosing IGT in a patient were the ADA criteria: a value ≥11.1 mmol at 2 hrs (more than 140 mg/dL and less than 200 mg/dL) following the administration of 75 g of glucose. Elevated insulin levels were those more than 27 μU/mL while fasting, above 88 μU/mL at 1 hr and exceeding 79 μU/mL at 2 hrs. Random testing of patient samples yielded FFA levels in 15 patients (27 samples were measured in total with the N, IGT and D patients being tested at both the initial and final visit). Essential aminoacids levels were performed in 15 patients (some of whom were different from patients tested for FFAs, for a total of 23 samples) and 14 patients (18 samples) were tested for other aminoacids and metabolic intermediaries. Urinary metabolites were measured in 4 patients (7 samples); the patient from the N group had only the final visit values determined; patients from the D, IGT and IR groups had measurements at both initial and final visit. Administration of the above supplements and restriction of saturated fats resulted in

regression of IGT to IR in 4 of 5 patients; reduction in the average GMI in those patients was 80 mg/dL (15%). Conversely one patient who had not taken the supplements and consumed a large quantity of animal fat (from mutton) has progressed from IR to IGT (H2 value increased from 111 to 156 mg/dL) with a respective increase of GMI value from 516 to 549 mg/dL. The lowered glucose tolerance in this patient was associated with increased levels (between 15-20%) of saturated FFAs: *myristic, palmitic, stearic* and *pentadecanoic* acids, whereas in patients with decreasing GMI levels of those saturated FFAs were lowered. Reverting IGT to IR values (n = 4) was also accompanied by significant decreases in the concentration of monounsaturated FFA: *palmitoleic acid* ($p = 0.05$); *vaccenic* ($p = 0.02$); *11-eicosenoic* ($p = 0.05$); those also fulfilled the consistency criterion by having an overall positive correlation with GMI ($r > 0$ for all three).

FFA values (table 1) show that 3 of the 19 unsaturated FFAs (*docosahexaenoic*, *arahidonic* and *erucic*) were negatively correlated with GMI in a statistically significant way ($r = -0.43$, -0.41 and -0.5 respectively); only 1 unsaturated FFA (*mead*) had a significant positive $r = 0.43$. This suggests that unsaturated fatty acids have mostly a positive effect on glucose tolerance and GMI which was more evident for tetraenoic acids (*arachidonic* and *docosatetraenoic*) $r = -0.41$.

Saturated FFAs correlated more frequently with GMI with a positive r (directly proportional with GMI, negative association with glucose tolerance); of these the highest correlations were shown by *myristic*, *capric*, *C18 trans* FFAs and especially by *pentadecanoic acid*, the only one with a statistically significant correlation ($r = 0.50$). To further check for significance, t-test showed significant differences between patient groups for *myristic acid* (14:0) in DI *vs*. NI ($p = 0.02$); *pentadecanoic acid* (15:0) DI *vs*. NI ($p = 0.03$) and *C18 trans* in the N *vs.* IR groups ($p = 0.03$); these alongside consistent r values were arguments for their negative effect on glucose tolerance.

Only one of the saturated FFAs: *behenic acid*, $r = -0.43$, was negatively and significantly correlated with GMI. Plasma levels of *arachidic acid* (C20:0) were increased in DI *vs* DF and was both statistically significant ($p = 0.02$) and consistent ($r<0$ while NI > DI), suggesting that *arachidic acid,* like *behenic acid* are the only saturated FFAs associated with improved glucose tolerance.

When considering the length of the FFA carbon chain, saturated FFAs with a shorter chain (C10-14) were positively correlated with GMI, medium chain (C16-18) were weakly correlated and long chain FFAs (C20-26) were negatively correlated with GMI. By summing up the levels of all FFAs with chain length of 10-18 carbon atoms and also all FFAs with 19-26 carbons and comparing them to GMI, C19-26 have a positive effect on glucose tolerance overall, with $r = -0.42$ while C10-18 is not significant: $r < 0.001$. Their ratio (range 2.5-7.8) is well correlated with GMI ($r = 0.51$).

Among all FFAs, GMI was best correlated with the tri/tetra ratio ($r = 0.64$), calculated as the *mead/arachidonic* ratio and thought to reflect best the status of essential fatty acid intake.

Table 1. Plasma Free Fatty Acids, same patients before and after intervention

Fatty Acids in μM/L,mean+/-SD	Group Ni Group Nf (n=4)	Group IGT Group IRF (n=4)	Group Di Group Df (n=4)	Group IR2i (n=3)
Docosahexaenoic	155+/-(58.5) 180.3+/-(66.5)	135.25+/-(54.7) 214.5+/-101.8	72.75+/-(13.3) 95.75+/-16.8	84
Arachidonic	748.75+/-191.3 663.5+/-(232)	633.3+/-(176) 573+/-(191)	566.5+/-(106) 476+/-(64.9)	603
Mead	10.65+/-(4.3) 7.5+/-(1.8)	13.65+/-(6.9) 9.25+/-(2.8)	14.5+/-(4.6) 14+/-(2.5)	9.53
Palmitoleic	67.25+/-(37.6) 52.75+/-(23.3)	112.25+/-(41) 76.5+/-(34.5)	133+/-(69.1) 82.5+/-(14.8)	113
Vaccenic	74+/-(12.8) 57.75+/-(2.8)	82.75+/-(25.9) 65+/-(22.6)	85+/-(28.2) 67.25+/-()13.7	71.33
11-Eicosenoic	8.35+/-(2.95) 7.6+/-(2.8)	8.87+/-(2.7) 7.2+/-(2.1)	8.7+/-(1.3) 9.4+/-(1.4)	7.7
Erucic	5.75+/-(0.5) 6.1+/-(0.9)	5.72+/-(1.7) 6+/-(1.0)	4.45+/-(0.7) 4.8+/-(0.7)	6.27
Triene/Tetraene	0.015+/-(0.01) 0.013+/-(0.01)	0.023+/-(0.01) 0.018+/-(0.01)	0.026+/-(0.01) 0.03+/-(0.007)	0.016
Myristic*	27+/-(8.9) 28.25+/-(16.9)	47.75+/-(24.5) 35.75+/-(20.9)	51.25+/-(12.7) 42+/-(9.1)	49.75
Arachidic*	16.93+/-(4.2) 16.5+/-(3.45)	15.6+/-(3.8) 15.98+/-(4.8)	13.95+/-(2.4) 16.23+/-(3.2)	15.15
Behenic*	51.25+/-(11.8) 53.5+/-(21.1)	45.25+/-(6.7) 47.25+/-(6.9)	36.75+/-(4.1) 41.5+/-(9.8)	49
Pentadecanoic*	8.13+/-(2.3) 7.95+/-(3.2)	9.5+/-(1.9) 8.45+/-(2)	12.63+/-(1.9) 11.65+/-(1.9)	12.5
Total C:18 Trans	33.75+/-(5.7) 36+/-(18.7)	61.75+/-(38.5) 72.75+/-(19.2)	75.75+/-(31.6) 68.75+/-(25.8)	48.25

*-saturated FFAs
Ni, Nf; Di, Df - patients from the N/D group at the initial (Ni, Di) and final (Nf, Df) visit; IGT and IR groups are same 4 patients at the initial visit (IGT) and final visit (IR); IR2i - patients from the IR2 group at initial visit provided for comparison.

Levels of the following FFAs were not correlated with GMI in a statistically significant way nor showed differences between groups that were significant statistically: Alpha Linolenic, Capric, Dihomo-γ Linolenic, Docosadienoic, Docosapentaenoic, Docosatetraenoic, Eicosadienoic, Eicosapentaenoic, Gamma Linolenic, Heneicosanoic, Heptadecanoic, Hexacosanoic, Lauric, Lignoceric, Linoleic, Myristoleic, Nervonic, Nonadecanoic, Oleic, Palmitelaidic, Palmitic, Stearic, Tricosanoic.

The plasma aminoacid testing (table 2, Fig. 1) showed that GMI was best correlated with the levels of *alanine* ($r = -0.54$; $p = 0.01$), which were lower in the D group compared to the N group ($p=0.06$) and IR2 ($p=0.07$). In 3 out of 4 diabetes patients levels of *alanine* were increased after the 2 week intervention and this suggests increased gluconeogenesis from *alanine* in the diabetes patients. Similarly, the IGT group showed lower levels of *glycine*, *serine* and *alanine* compared to normal — but not statistically significant.

Table 2 Plasma Amino Acids, same patient at initial (i) and final (f) visit

Aminoacid,µmol, mean +/- SD	Group IGT (i) Group IR (f) (n = 4, same pt)	Group Di Group Df (n = 4, same pt)	Group Nf (n = 4)	G r o u p IR2f (n = 3)
Arginine	48 +/- 0.8 56.75 +/- 21.5	58 +/- 15.19 76.5 +/- 11.73	61 +/- 15.1	72.3
Leucine	73.5 +/- 7.9 78.75 +/- 15	102.8 +/- 35.6 122 +/- 43.6	89.75 +/- 17.1	94.7
Phenylalanine	52.25 +/- 7.7 57 +/- 18.3	52.75 +/- 5.4 61.8 +/- 10.9	45.25 +/- 6.7	51
Glycine	189 +/- 51.4 249.25 +/- 85.9	232.5 +/- 42.9 265 +/- 11.4	207 +/- 24.5	240
Tyrosine	49.3 +/- 7.9 61.75 +/- 14.6	66.5 +/- 6.6 80.8 +/- 11.2	52 +/- 11.1	66.7
Citrulline	18 +/- 7.2 18.75 +/- 7.2	28.25 +/- 8.5 37 +/- 6.5	19.5 +/- 6.5	15.7
Ornithine	43.75 +/- 18.2 56.5 +/- 25.1	65 +/- 24.3 79 +/- 14.3	47.5 +/- 12.2	58.3

Di, Df, Nf - patients from the N/D group at the initial (Di) and final (Nf, Df) visit;
IGT and IR groups are same 4 patients at the initial visit IGT(i) and final visit IR(f);
IR2i - patients from the IR2 group at initial visit provided for comparison

Levels of *tyrosine* were higher in the D group vs N group (p = 0.02) and also compared to IGT group (p = 0.01). The GMI-tyrosine correlation was also consistent (r > 0). The suggestion of a tyrosine-related metabolic dysfunction was strengthened by the low urinary levels of *homovanilate* in 5 of the 10 tests performed. *Phenylalanine* was also higher in the D group compared to N (p = 0.02); the correlation with GMI was also positive (r > 0). This can be explained by increased participation of *tyrosine* and *phenylalanine* in energy metabolism via phosphoenolpyruvate, phenylpyruvate and fumarylacetoacetate.

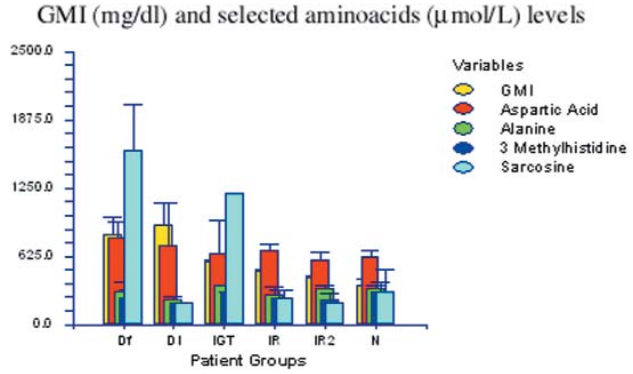

Figure 1. GMI and selected aminoacids levels.

Lysine, *1-methylhistidine* and *3-methylhistidine* (r = -0.48) were negatively correlated with GMI suggesting a beneficial role in glucose tolerance. *Glycine*, *serine*, *valine*, *leucine* and *isoleucine* were positively correlated with GMI overall;

furthermore *glycine* was significantly higher in the D vs. N group (p = 0.05) while *leucine* was higher in D vs. IGT group (p = 0.02).

The correlation between GMI and *aspartic acid* was also significant (r = 0.42), as well as *citrulline* (r = 0.44). *Citrulline* was significantly higher in the D group compared to N, IR, IR2 and IGT groups (p < 0.01).

Alongside these results that were statistically significant, an observation was made while comparing results of molecules that are functionally related (Fig.2): they tended to vary in opposite ways in relationship to GMI. This was the case for *ornithine* (r = 0.27) and *arginine* (r = - 0.14); *glutamine* (r = -0.12) and *glutamic* acid (r = 0.15); *proline* (r = -0.1) and *hydroxyproline* (r = 0.37).

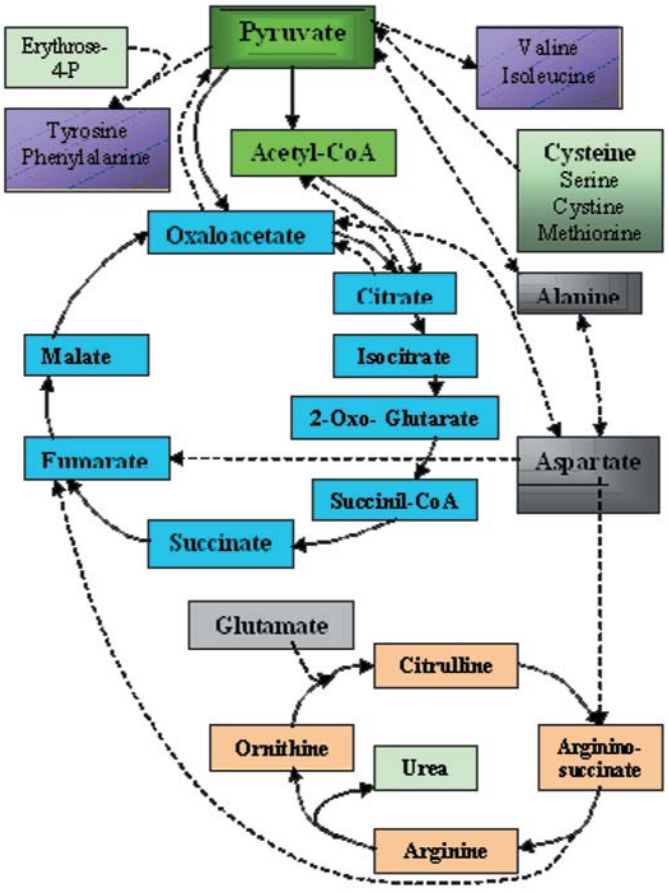

Figure. 2 Relationship between amino acids and energy metabolism; erythrose -4-P is formed from 3-P-glyceralehyde (glucose-6-P)-adapted from Donald Nicholson: Metabolic Pathways, 22nd Edition.

Plasma levels of alanine were well correlated with GMI while tyrosine, phenylalanine and citrulline - aminoacids associated with gluconeogenesis and energy metabolism - were significantly higher in the D compared to the N group.

Table 3. Levels of Metabolic Intermediaries

Patient at initial (Ai-Ci) and final (Af-Nf) visit, (group), μmol/L	(Nf)	Ai; Af (D)	Ci (IGT); Cf (IR)	Bi;Bf (IR)	rGMI; p value*
GMI	438	1061; 940	549; 516	495; 488	- -
malate	0.7	7.9; 8.4	0.9; 0.7	1.4; 0.6	0.931 0.00
fumarate	0.4	5.19; 2.59	0.5; 0.29	0.76; 0.1	0.886 0.01
β-hydroxybutyrate	0.3	15.5; 3.4	0.4; 0.7	1.9; 0.6	0.882 0.01
α-ketoisocaproate	0.16	1.08; 0.4	0.2; 0.1	0.3; 0.21	0.827 0.01
α-keto-β-Methylvalerate	0.3	8.3; 2	0.5; 0.9	2.2; 1.9	0.806 0.01
orotate	0.8	1.6; 2.2	1; 0.7	1; 0.5	0.794 0.01
xanthurenate	0.13	0.34; 0.32	0.3; 0.18	0.28; 0.14	0.778 0.01
β-hydroxyisovalerate	2.3	43.3; 22.8	16; 22.9	20.1; 14.5	0.777 0.01
Pyruvate	3.3	18.7; 1.7	4.1; 1.4	5.7; 3.8	0.657 0.04
Methylmalonate	1.1	2; 1.4	1.5; 1.6	0.9; 1.4	0.645 0.04

* correlation between GMI and metabolite with the respective p value
Ai, Bi, Ci - initial patient visit; Af, Bf, Cf - final patient visit;
Nf - patient from Normal group, final visit

Levels of the following aminoacids were not correlated with GMI significantly nor showed differences between groups that were significant statistically: 1-Methylhistidine, α-Aminoadipic Acid, α-Amino-N-Butyric Acid, Anserine, Asparagine, β-Alanine, β-Aminoisobutyric Acid, Carnosine, Cystathionine, Cystine, Ethanolamine, Gamma-Aminobutyric Acid, Glutamic Acid, Glutamine, Histidine, Homocystine, Hydroxylysine, Hydroxyproline, Isoleucine, Lysine, Methionine, Phosphoethanolamine, Phosphoserine, Proline, Serine, Taurine, Threonine, Tryptophan, Valine.

Metabolic intermediaries - table 3, Fig.3 - showed an excellent and statistically significant correlation ($r > 0.65$) with GMI in 13 of the 31 compounds, better than either FFAs or aminoacids; top was for *malate*, *fumarate*, *cis-aconitate*, *citrate*, etc. *Citrate*, *cis-aconitate*, *adipate* and *pyroglutamate* were also lowered simultaneously with GMI in a significant manner in the four patients considered ($p = 0.01$, $p = 0.006$, $p = 0.001$ and $p = 0.01$ respectively). *β-hydroxyisovalerate* was elevated in D, IGT and IR but not in N patients; this was consistent with the results on *isoleucine*;

similarly *α-ketoisovalerate* and *α-ketoisocaproate* levels yielded results consistent with the observed impairments in *valine* and *leucine* metabolism. Levels of *α-ketoisocaproate* (along with *β-hydroxybutyrate*) were elevated only in the 2 patients with most severe diabetes, with GMI over 900 and in need of exogenous insulin.

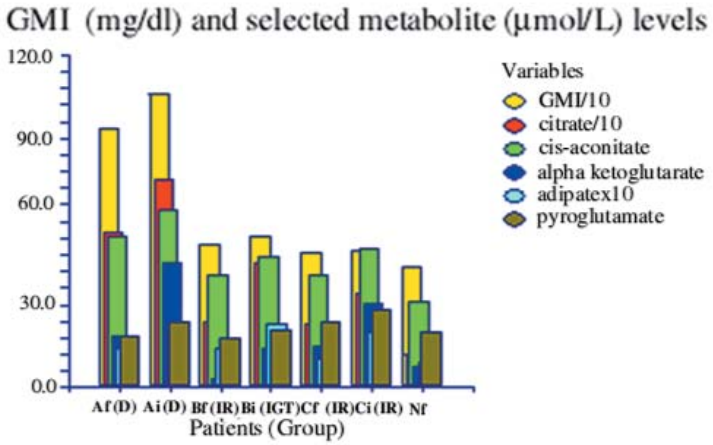

Figure 3. GMI and selected metabolite levels.

Urinary metabolites such as malate, fumarate, cis-aconitate, citrate showed excellent correlation with GMI and were lowered significantly with the study intervention.

Levels of the following metabolites were not correlated with GMI significantly nor showed differences between initial and final visits in the same patient that were significant statistically: α-hydroxybutyrate, α -ketoisovalerate, 2-methylhippurate, 5-hydroxyindolacetate, ethylmalonate, formiminoglutamate, glucarate, homovanilate, hydroxymethylglutarate, isocitrate, kynurenate, lactate, quinolinate, suberate, succinate, vanilmandelate.

DISCUSSION

Two intracellular mechanisms are currently proposed for the development of insulin resistance: 1- ceramide formation from saturated fatty acids (33) with subsequent mitochondrial dysfunction and cell apoptosis; and 2. — inactivation of the insulin receptor substrates (34, 35) following the binding of extracellular FFA's, glucose and other molecules to the scavenger LOX-1 receptor (lectin-like oxidized LDL receptor). Levels of leptin - which is necessary for mechanism 1 to take place - can indicate the degree of involvement of each mechanism; however, modifications at the level of the leptin receptor can make leptin-based interventions ineffective.

The observed levels of *aspartic acid* and *asparagine* as well as *arginine*, *citrulline* and *ornithine* can be explained by the two different pathways followed by

aspartate: formation of *asparagine* is a metabolic dead-end while its transformation into *arginino-succinate* and *fumarate* adds to the mitochondrial energy-rich environment. Similar use of *glutamate*, *tyrosine*, *hydroxyproline*, along with *alanine* result in over-production of the Krebs cycle components. Mitochondrial ATP overload suggested by the aminoacid levels was reflected by the increased *pyruvate*, *citrate*, *isocitrate*, *malate* and *fumarate* (Table 4); this energy over-production is present both in diabetes and in the insulin-resistant states (IR and IGT, probably also in IFG) that precede diabetes.

Previous studies (18, 19) have shown that a certain impairment of pancreatic beta-cell function (delay in secretory response and/or total insulin produced) is already present in IGT (37-40) along with insulin resistance of peripheral tissues and/or hepatic gluconeogenesis that fails to be suppressed by insulin. The extent to which NIDDM involves further continuous deterioration from pre-diabetes or rather a new pathway becomes important; the results we have obtained suggest both that IR, IGT and diabetes represent different stages of alteration of the same metabolic pathways (e.g. *valine* and *isoleucine*) and additional involvement of new pathways (*leucine* metabolism) in more severe stages of NIDDM.

Energy-rich environments present in obesity and hyperalimentation are affecting mitochondria in a similar fashion in the muscle, the liver and the pancreas; however, pancreatic beta cells seem the most vulnerable to hyperglycemia and increased FFA levels (36). One likely explanation is that muscle fibers can restore metabolic balance by contracting; hepatocytes regenerate relatively easily, however beta cells have relatively limited means to defend themselves against hypercaloric abuse. They mainly do so by increasing their secretory function, and in time this dynamic stress will result in their premature death. When new beta cell formation cannot compensate for cell death and the pancreas cannot provide anymore the increased amounts of insulin needed for glucose utilization, the insulin deficit becomes more apparent. Lowered availability of insulin relative to the increased need for it in hepatocytes and peripheral tissues is followed by an increase in hepatic gluconeogenesis and a decreased glucose utilization by peripheral tissues, all contributing to rapid impairment of glucose tolerance and the perceived need for exogenous insulin seen in late stages of type 2 Diabetes.

This may justify intervention in pre-diabetes to normalize energy metabolism trying to impede progression to overt diabetes, which is more difficult and more expensive to treat. Additionally it can be argued that for NIDDM patients, adequate glycaemic control with oral hypoglycemiants without saturated fat and caloric intake restriction will likely be followed by increased pancreatic beta cell apoptosis and eventual progression to insulin dependency. Before new classes of hypoglycemiants are developed that can selectively stimulate glucose utilization by peripheral tissues as opposed to increasing insulin production, the medical management of the NIDDM patient should perhaps consider this issue.

CONCLUSION

This study is in line with findings from the cited literature regarding the type of the FFA linked to diabetes and IGT (30-32); both the amount and the type of FFAs affected glucose tolerance. The number of carbon atoms in the FFA chain is a good predictor of their effect on glucose tolerance, with long-chain FFAs (C19-26) being beneficial; also the degree of FFA saturation was important. Polyunsaturated FFAs are associated with improvements in glucose tolerance, while higher levels of saturated FFAs are related to impairments of glucose extraction and its utilization by the liver and peripheral tissues.

Plasma aminoacids and urinary metabolites levels were consistent with energy overproduction and metabolism modifications of similar type and various magnitudes in diabetes and IGT.

With the present study we have shown that a short-term intervention represented by saturated fat restriction and addition of antioxidants can improve glucose tolerance in the patient with pre-diabetes or NIDDM. The observed improvement in the functional status of mitochondria in all groups was likely due to administration of antioxidants; however it is difficult to separate their beneficial action from that of modifications in FFA levels and more studies are needed in order to quantify their individual and long-term effects.

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