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Differential metabolic effects of Insulin Detemir versus NPH in Patients with Type 2 Diabetes

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Abstract

Context: Insulin detemir is associated with less weight gain than other basal insulin analogues.

Objective: To investigate whether lower weight gain with insulin detemir is due to changes in energy intake or output and the effects on lipid metabolism and muscle gene expression.

Design: This was a randomised parallel group study

Setting: This study was conducted at the Centre for Diabetes, Endocrinology, and Research, Royal Surrey County Hospital, Guildford, United Kingdom

Patients: Twenty two men and women patients with type 2 diabetes participated in the study.

Intervention: Patients were titrated for 8 weeks with NPH insulin, then randomized to detemir or NPH for 16 weeks.

Main outcome measures: Body composition, energy expenditure (EE), energy intake, fasting and prandial metabolites were measured at 8 and 24 weeks. Gene expression and lipolytic activity were measured in skeletal muscle and adipose biopsies respectively.

Results: During the 8 week optimization both groups gained weight. At 24 weeks body weight increased further with NPH ($p=0.006$) with no change with detemir. HbA1c was not different between groups. In the NPH group weight change and change in energy intake ($r=0.80$, $p<0.01$) were correlated with no change in EE. Fasting non-esterified fatty acids (NEFA) decreased and adipose lipoprotein lipase mass and activity increased with detemir compared to NPH ($p<0.03$, $p=0.001$, $p=0.008$). Muscle genes related to mitochondrial function and the electron transfer chain were more regulated by NPH than detemir.

Conclusions: NPH caused more weight gain than detemir which may be related to energy intake. This may cause an up-regulation of mitochondrial gene expression. The decrease in plasma NEFA and increase in lipoprotein lipase activity with detemir may be due to improved fasting insulin sensitivity of adipose tissue.

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73 **Abbreviations:** AUC, Area under the curve; AT, adipose tissue; AEE, activity energy
74 expenditure; BMI, body mass index; BW, Body weight; DAVID, database for annotation,
75 visualization and integrated discovery; ECG, electrocardiograph; EE, energy expenditure;
76 GSEA, Gene Set Enrichment Analysis; HbA1c, hemoglobin A1c; IHCL, intrahepatocellular
77 lipid content; IMCL, intramyocellular lipid content; LPL, lipoprotein lipase; MRI, magnetic
78 resonance imaging; MRS, magnetic resonance spectroscopy; NPH, neutral protamine
79 Hagedorn; NEFA, nonesterified fatty acid; PPP, pancreatic polypeptide; PYY, peptide YY;
80 REE, resting energy expenditure; TG, triglyceride.

Introduction

Subcutaneous insulin therapy frequently results in significant weight gain in patients with type 2 diabetes but is an effective and necessary treatment to improve glycaemic control where other treatments have proved inadequate (1,2). The United Kingdom Prospective Diabetes Study showed weight gain greater than 5kg in the intensive treatment arms (3) despite specific dietary and behavioural interventions designed to limit this.

Insulin detemir is a basal insulin analogue which differs from the native insulin molecule by a myristic fatty acid attachment to the B chain. This enables reversible albumin binding, thereby prolonging duration of action (4). Insulin detemir has been shown to be associated with less weight gain than other basal insulin analogues both in type 1 and type 2 diabetes (5,6). It has recently been shown in type 1 diabetes, that insulin detemir reduces weight gain, compared to NPH insulin, due to reduced food intake, with no effect on energy expenditure (7). This may have been due to a direct or indirect effect on satiety.

In this study we have investigated if the lower weight gain with insulin detemir, compared to NPH insulin, in type 2 diabetes is also due to reduced food intake or increased energy expenditure. The effects of the two insulin treatments on lipid metabolism and muscle gene expression and on hormones which control energy balance, were also investigated.

Research Design and Methods

This study was approved by the UK Medicines and Healthcare Products Regulatory Agency (Eudract 2007-003085-17), the South East Research Ethics Committee and University of Surrey Research Ethics Committee. All subjects gave informed written consent before inclusion.

This was a 24-week, single-centre, open-labelled, randomized, parallel-group trial. Inclusion criteria included type 2 diabetes, treatment with insulin, age>18 years, BMI 25-35 and HbA1c greater than 7%. Exclusion criteria were treatment with sulponylureas or thiazolidinediones, proliferative retinopathy requiring recent treatment, impaired hepatic, renal or cardiac function, uncontrolled hypertension, and language or mental incapacity. Patients were required to make no significant changes to their normal dietary intake during the 16 week treatment period. Twenty-four patients with type 2 diabetes, on a basal-bolus regimen plus metformin, were recruited. One patient did not complete the trial for personal reasons. A second patient undertook a very low calorie diet during the treatment period and was therefore excluded. Following an optimization period with Neutral Protamine Hagedorn (NPH) insulin for 8 weeks, patients were randomized to either once daily detemir (n=11; 8 male, 3 female) or NPH insulin (n=11; 6 male, 5 female) as basal insulin for 16 weeks. Insulin aspart was used throughout as the bolus insulin. Both insulin detemir and NPH insulin were administered once daily, according to pre-breakfast glucose targets (aiming for <6.0 mmol/l without significant hypoglycemia). Capillary glucose measurements were measured with Optium Exceed meters (Abbott, Maidenhead, Berkshire, UK). During the trial, subjects attended the Centre for Diabetes and Endocrinology Research (CEDAR) at the Royal Surrey County Hospital, Guildford for a screening visit and a total of 8 planned visits and the investigator was in contact by telephone at least four times and as necessary. HbA1c was recorded at baseline, at the end of the optimization period and treatment periods. At the end

of the 8 week optimisation and 16 week treatment periods, subjects attended for 4 visits for the measurement of whole-body fat distribution, REE, AEE, energy intake with a 7 day diary, fasting and prandial metabolites and hormones. In a sub-set of subjects, skeletal muscle and fat biopsies were taken to measure gene expression, and lipolytic activity respectively.

*Visit 1:*Whole-body fat distribution by magnetic resonance imaging (MRI) and hepatic and muscle fat by magnetic resonance spectroscopy (MRS) were measured.

Visit 2: Subjects attended the CEDAR centre where an Actiheart monitor (CamNttech Ltd, Cambridge, UK) was fitted to record activity (AEE). The monitor was calibrated for each subject using an 8 minute linear ramped step test. The monitor was worn on two standard ECG pads (Pulse Medical Limited, Woking, UK) placed on the upper chest of the subject and worn continuously for 5 days. Subjects were also issued with a diet diary with which to record their food intake over the following 7 days. Participants were instructed to record all food and drinks consumed and encouraged to provide as much information as possible such as brands of foods, types of food, quantities consumed and cooking methods. Insulin doses and number of hypoglycemic episodes were recorded. In the NPH group diet diaries at 16 weeks were not completed in 2 subjects.

Visit 3: Subjects attended after an overnight fast. They were asked not to drink alcohol or undertake strenuous physical exercise for 48 hours prior to this visit. After a rest period REE was measured over 30 minutes using a Medgraphics CCM Express indirect calorimeter (Medical Graphics Corporation, Minnesota USA). Subjects were then given a standard fibre-free liquid mixed meal containing 600 Kcal, 60 g carbohydrate, 21 g lipid and 19 g protein (Fortisip, Nutricia Clinical). Blood samples were taken at regular intervals for 300 minutes for glucose, ghrelin, non-esterified fatty acid (NEFA) pancreatic polypeptide (PPP) and peptide YY (PYY) and fasting leptin and adiponectin. After completion of the study, appetite

was assessed with a visual analogue score and a free-access large pasta meal (1740 kcal). Subjects were asked to eat to comfortable satiety. No subject completed the meal. Calorie intake was assessed by weighing the residual food.

Visit 4: In a sub-set of subjects who consented, subcutaneous gluteal adipose samples (6 in the NPH group and 7 in the detemir group) and quadriceps muscle biopsies (5 in both groups) were taken under local anaesthesia after an overnight fast at 8 and 24 weeks. A. The muscle was snap-frozen using liquid nitrogen and sent to the University of Lyon for gene expression analysis.

Microarrays

RNA profiling in muscle biopsies was performed using Human GE 4x44K Microarray (Agilent Technologies, Massy, France). Briefly, total RNA was isolated from frozen muscle samples using *mir*Vana™ miRNA Isolation Kit (Applied Biosystems, St-Aubin, France). Total RNA (500ng) was submitted to a Quick Amp Labeling Kit (two colors) (Applied Biosystems) following a dye-switch procedure and then hybridized following the manufacturer's instructions. Microarrays were scanned with an Axon 4000B scanner (Molecular Devices, Sunnyvale, USA) and signal intensities quantified using GenePix 6.0 (Molecular Devices). The signal intensities of the microarray spots were loaded to R (version 2.9.2), background corrected with an offset of 50 lowess and normalized within arrays (8), A-quantile normalized between arrays, and log-transformed using the Limma package from BioConductor (9). Data were filtered so that non-homogenous, saturating or flagged spots were removed.

Imaging

Adipose tissue content, intrahepatocellular lipid (IHCL) and intramyocellular lipid (IMCL) were measured at the MRC Clinical Sciences Centre Hammersmith Hospital. Subjects fasted for 8 hours before the scans. Whole body MR imaging of body fat content, IHCL and IMCL levels were acquired on an Intera 1.5T Achieva multinuclear system (Philips Medical Systems, Best, Holland) as previously reported (10). IMCL spectra were obtained from the tibialis muscle group (T-IMCL) which contains predominantly glycolytic fibres and soleus muscle (S-IMCL). Total and regional adipose tissue volumes were measured after imaging data was analysed using the SliceOmatic image analysis program (Tomovision, Montreal, Quebec, Canada). All spectra were analysed in the time domain using the AMARES algorithm included in the MRUI software package (11). IMCL was expressed as a ratio to the muscle creatine signal. IHCL was expressed as a ratio to liver water content.

Hormone and metabolite assays

Plasma NEFA and TG concentrations were measured using an enzymatic assay (ABX, Chicksands, Shefford, Bedfordshire, UK), using a Cobas MIRA (Roche, Welwyn Garden City, UK). Plasma adiponectin, leptin and total ghrelin, and total peptide YY (PYY) concentrations were determined by radioimmunoassay (Millipore Corporate Headquarters, Billerica, MA). Plasma pancreatic polypeptide (PPP) was measured using an enzyme linked immunosorbant assay (ELISA) (Millipore Corporate Headquarters, Billerica, MA).

LPL and ex-vivo lipolysis

The adipose sample was used immediately to measure lipolytic activity. Glycerol release was used as an index of lipolysis in freshly isolated mature adipocyte suspensions. This was a modification of the method of Rodbell (1964) (12) using a coupled enzyme reaction kit

(Sigma-Aldrich Ltd, Dorset, UK) and spectrophotometer. The absorbance at 540nm was directly proportional to the concentration of glycerol in the sample. Lipoprotein lipase (LPL) activity was measured in 20 μl tissue homogenate extracted from 50 mg snap frozen adipose tissue, using a fluorescence assay (LPL Activity assay kit; Roar Biomedical, New York, USA). LPL mass was measured using an ELISA kit (Markit-M LPL assay, DS Pharma Biomedical Co., Ltd, Tokyo, Japan).

Data analysis

AEE was calculated from the 5-day heart rate variability recordings using a branched chain equation model in the Actiheart software (13). The dietary information recorded in the 7-day food diaries were analysed by a blinded qualified dietician using the nutritional analysis programme Dietplan6 (Forestfield Software, Horsham, UK) and an average 24-hour total energy intake calculated.

Statistical analysis

The results are presented as means ± SEM. Within group changes were analysed by paired t test and changes between groups by t test using SPSS (version 16). Areas under curves (AUC) and area over curves (AOC) were calculated using the trapezoidal rule corrected for baseline values. Non parametric data was logarithmically transformed before analysis. Statistical analysis on the microarray data was performed on 28,889 probes with the Limma package as previously described (14). The dataset is available from the GEO database (GSE37785). To test for the enrichment of sets of genes in expression data sets, we used version 2.07 of the computational method Gene Set Enrichment Analysis (GSEA) (15) and the 3272 curated gene sets from the C2 collection and the 1454 curated gene sets from the C5 collection of the Molecular Signatures Database, MSigDB (http://www.broad.mit.edu/gsea/).

Results

Glycaemic control

During the 8 week optimisation period HbA1c was reduced from 8.3 ± 0.2 to 7.7± 0.3 % in the group that was subsequently randomised to NPH and from 8.8 ± 0.4 to 7.4 ± 0.3 % in the group that was subsequently randomised to detemir. During the 16 week treatment period there was no significant difference between the NPH and detemir groups in the insulin aspart doses (18.6 ± 2.2 vs 22.0 ± 5.4 U/day), HbA1c (Table 1) and number of hypoglycaemic episodes (7.5 ± 1.2 vs 7.1 ± 0.9). All patients had at least one hypoglycaemic episode during the treatment period. There were no major hypoglycaemic episodes as defined by need for third party assistance.

Body Weight (BW) and fat content (Table 1)

There was no significant difference in BW or BMI between groups prior to and after the 8 week optimization period on NPH. During the 8 week optimization period there was an increase in BW in all subjects (1.3± 0.5 kg). At 16 weeks there was a further increase in BW (+1.04 ± 0.32 kg) and BMI in the NPH group ($p=0.006$, $p=0.005$) with no significant change in the detemir group in BW (–0.35 ± 0.85 kg) or BMI. There was no significant difference in total fat mass, visceral fat, subcutaneous fat, S-IMCL or IHCL. There was a trend for an increase in T-IMCL in the NPH group ($p=0.06$) over the treatment period which was not seen in the detemir group ($p=0.67$).

Energy Intake and Expenditure (Table 1)

There was no difference in daily energy intake between the treatment groups but the change in BW in the NPH group correlated with the change in energy intake ($r=0.80$, $p<0.01$). There

256 was no difference in energy intake of the free access single meal or the visual analogue
257 appetite scores. REE and AEE were not different with either insulin detemir or NPH insulin.
258
259 **Hormone and metabolite responses (Table 2)**
260 There was no significant difference in any of the measured metabolites or hormones between
261 groups after the 8 week optimization period. Fasting NEFA concentrations significantly
262 decreased in the detemir group ($p=0.02$) over the treatment period with a significant between
263 group difference (<0.03). The prandial NEFA response was not different between groups.
264 There was no significant difference in fasting or prandial TG and glucose or in fasting
265 adiponectin or leptin. Fasting and prandial PPP, ghrelin and PYY were also not different
266 (data not shown).
267
268 **Lipolytic activity of adipose tissue (Table 2)**
269 The change in LPL mass and LPL activity over the 16 week treatment period was different
270 between groups ($p=0.001$, $p=0.008$). This was due to an increase in the detemir group
271 ($p<0.02$, $p<0.05$). Basal adipose tissue lipolysis was lower in the detemir group after 16
272 weeks but this did not achieve statistical significance ($p=0.10$)
273
274 **Muscle gene expression (Table 3)**
275 The differential change in gene expression profiles in skeletal muscle was evaluated using
276 oligonucleotide microarrays. Using Gene Set Enrichment Analysis (GSEA) to determine the
277 difference between the two treatments, a significant enrichment in genes related to
278 mitochondrial function was identified (Table 3). Then using a moderated t test, 490 probes
279 (corresponding to 436 unique genes) were retrieved with significantly ($p < 0.05$) different
280 responses during the two treatments (Supplementary Table S1). Pathway analysis using

DAVID (The Database for Annotation, Visualization and Integrated Discovery) confirmed GSEA and also revealed highly significant enrichment in genes related to mitochondrial respiratory chain complex I, mitochondrial membrane and mitochondrial matrix (Figure 1). In this set, 56 genes with mitochondria annotation, including 13 genes coding proteins and enzymes involved in oxidative phosphorylation were identified. Of importance, all these genes were either more regulated or up-regulated after NPH compared to detemir (Supplementary Table S2).

Discussion

This study is consistent with previous studies that showed treatment with NPH insulin causes more weight gain than insulin detemir in patients with type 2 diabetes (5,6). The mean energy intake increased by 79 kcal/day after 16 weeks of NPH insulin and there was a correlation between the change in energy intake and weight change in the NPH group suggesting that energy intake was responsible for the increase in weight in this group. Energy expenditure showed no differences between the insulin detemir and NPH groups. The decrease in fasting NEFAs and the increase in LPL activity and LPL mass in the detemir group may be due to an improvement in adipose tissue insulin sensitivity. Since there was no weight loss with detemir, any improvement in insulin sensitivity was not be due to weight loss. The greater regulation of muscle gene expression with NPH compared to detemir may be a consequence of increased food intake.

We have recently shown in type 1 diabetes that insulin detemir when compared to NPH appears to mediate its weight sparing effects by altering energy intake rather than energy expenditure (7). It is well recognized that patients with type 2 diabetes under-report food intake in diet diaries (16) and this was also the case in this study, however we would expect reporting to be consistent in each subject at 8 and 24 weeks (17). The correlation between the change in energy intake and weight change in the NPH group is strongly supportive of weight gain in this group being a consequence of energy intake. In our previous study in type 1 diabetes the increased energy intake was mainly due to an increase in fat intake (7).

Insulin therapy is delivered as a subcutaneous depot and absorbed into the systemic circulation through which it is distributed in approximately equal concentrations throughout the body. In normal physiology hepatocytes are exposed to insulin concentrations 3-4 times

higher than the other major targets for insulin (adipose tissue and muscle). Thus with subcutaneous insulin delivery the normal portal/peripheral insulin gradient is lost resulting in a relative peripheral hyperinsulinemia and under-insulinisation of the liver. This metabolic imbalance is inevitable with most existing insulin preparations and may contribute to both acute and chronic complications of diabetes. It is well established that over-insulinisation results in down regulation of the insulin receptor, thus contributing to insulin resistance (18,19). Over-insulinisation may also be responsible for weight gain with insulin treatment. It has been suggested that the reversible albumin-binding property of insulin detemir limits access to peripheral tissues through the endothelial barrier, while allowing full access to hepatocytes via the large sinusoidal fenestrae in hepatic capillary membranes. Such a shift in target tissue distribution would actually represent a more physiological profile of action, reducing over-insulinisation of the periphery (20,21).

The decrease in NEFA levels and increase in LPL mass and LPL activity following detemir treatment may be due to adipose tissue being more insulin sensitive. Acutely insulin detemir increases NEFAs due to reduced peripheral action (20) but over a 16 week period the reduced overinsulinisation of the periphery may improve the insulin sensitivity of lipolysis which is very insulin sensitive (22). There was also a trend for lower basal lipolytic activity in the adipose tissue biopsies with insulin detemir. LPL is also regulated by insulin (23) and increased activity of LPL is also associated with an improvement in insulin sensitivity (24).

The increase in dietary intake with NPH insulin, suggested by this study and demonstrated by our previous study (7) may be a consequence of over-insulinisation of the muscle and adipose tissue or under-insulinisation of the liver altering signaling to the brain which leads to higher food intake. An alternative mechanism may be over-insulinisation of the brain leading to

down regulation of brain insulin receptors. Insulin acts on the insulin receptor in the hypothalamus and lowers food intake and hence body weight (25). Hyperinsulinemia in the hypothalamus has also been shown to reduce insulin signaling and cause insulin resistance. Prolonged exposure of a hypothalamic cell line to insulin has been shown to reduce insulin signaling (26). It has also been shown that overfeeding in sheep causes hyperinsulinemia and central insulin resistance manifested by a reduced effect of an intracerebroventricular insulin injection to lower voluntary food intake (27).

High fat diets have been shown to increase mitochondrial enzyme activity and mitochondrial biogenesis in rodents which may be driven by increased NEFA levels (28,29). Rodents fed a diet which mimics a human western diet, have also been shown to increase energy intake, gain body weight and increase the mitochondrial respiratory capacity of skeletal muscle (30). The authors proposed that this was probably a compensatory mechanism for the excess energy availability. A recent study has shown that the mitochondrial content of skeletal muscle is regulated by free fatty acid delivery (31). The finding that NPH treatment resulted in a higher level of expression of a large cluster of genes related to mitochondria in skeletal muscle compared to insulin detemir clearly suggests weight gain with NPH is associated with an up-regulation of gene expression in the skeletal muscle of type 2 diabetic patients. The higher NEFAs in the NPH group compared to the detemir group also support the hypothesis that this may be driven by an increased availability of NEFAs in skeletal muscle.

In conclusion, this study shows that the more weight gain associated with NPH insulin compared to insulin detemir may be related to greater calorie consumption in the NPH group. We hypothesize that this is caused by over-insulinisation in the brain leading to down regulation of the hypothalamic insulin receptors. Insulin detemir may partially restore the

hepatic/peripheral insulin balance and this may reduce hyperinsulinemia in the brain leading to lower food intake than with NPH, better insulin sensitivity which reduces circulating NEFA and thus lower NEFA supply to muscle and less mitochondrial biogenesis compared to NPH.

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Table 1. Body composition, glycemic control and energy balance at week 8 and 24

	NPH		Detemir		Δ NPH vs Δ Detemir
	Week 8	Week 24	Week 8	Week 24	
HbA1c	7.7±0.3	7.9±0.4	7.4±0.3	7.6±0.3	NS
Hypoglycaemic episodes	0.4±0.2	0.3±0.2	0.5±0.2	0.5±0.2	NS
Patients presenting with hypoglycaemic episodes	3	3	3	4	NS
BMI	33.3±1.2	33.7±1.2†	32.0±1.0	31.9±1.0	P=0.07
Body weight kg	95.1±3.8	96.1±3.9*	94.2±5.7	93.8±5.5	NS
Fat mass kg	35.5±3.5	36.7±3.5	31.6±1.5	31.0±1.5	NS
Fat free mass kg	58.3±3.7	59.4±3.9	60.1±5.3	60.1±6.7	NS
Visceral fat kg	4.9±0.4	5.2±0.4	5.6±0.6	5.4±0.5	NS
Subcutaneous fat kg	26.9±3.4	27.5±3.3	22.3±1.5	22.0±1.6	NS
IHCL	23.2±5.1	21.2±5.2	21.4±5.7	23.6±6.4	NS
Soleus IMCL	21.2±4.6	26.4±4.0	22.9±4.1	20.8±2.1	NS
Tibialis IMCL	10.2±1.2	15.7±2.5‡	10.4±1.2	11.4±1.7	NS
Energy intake kcal/day	1923±149	2002±143	1675±141	1629±125	NS
REE kcal/day	1525±188	1566±219	1642±220	1697±246	NS
AEE kcal/day	455±80	383±66	385±84	448±86	NS
Free access Meal weight g	824±51	836±68	929±33	923±46	NS

487 Significantly different from week 8, *, p=0.006, † p=0.005, ‡ p=0.06. n=10 for MRI/MRS

488 measurements.

Table 2. Fasting and prandial hormones and metabolites

	NPH		Detemir		Δ NPH vs Δ Detemir
	Week 8	Week 24	Week 8	Week 24	
Fasting TG mmol/l	1.10±0.16	1.42±0.24‡	1.55±0.34	1.49±0.12	NS
Fasting NEFA mmol/l	0.38±0.07	0.46±0.09	0.56±0.05	0.45±0.06*	P<0.03
Basal lipolysis pmol/mg lipid	16.4 [9.9-19.8]	10.3 [9.3-17.3]	26.1 [19.3-52.6]	10.3 [8.6-13.7]	NS
LPL mass pg/ml/mg wet tissue	249±46	127±25	84±18	145±15*	P=0.001
LPL activity nKat/kg tissue	265±30	152±21*	155±27	197±19†	P=0.008
Adiponectin (μg/ml)	8.0±1.4	7.9±1.5	5.8±0.8	6.1±1.0	NS
Leptin ng/ml	15.7±2.7	13.7±2.7	13.6±1.7	11.9±1.3	NS

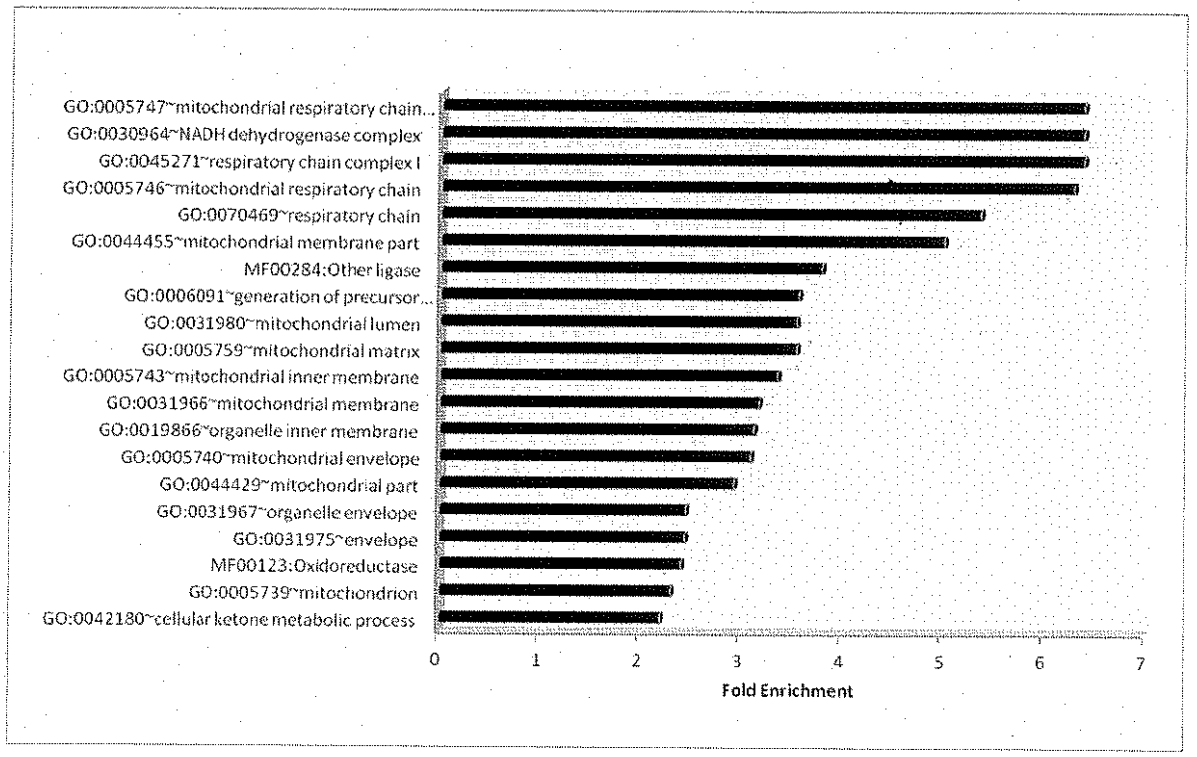
Significantly different from week 0, †p<0.05; *, p<0.02; ‡ p=0.06. Lipolysis results expressed as median [25-75% IQR]; AUC, area under curve

494 **Table 3. Gene sets specifically regulated by insulin detemir treatment (GSEA analysis,**

495 **FDR<0.01)**

Gene Sets NAMES C2 collection	Gene Sets Size	Enrichment Score	Normalized Enrichment Score	NOM p-value	FDR q-value
ELECTRON_TRANSPORT_CHAIN	95	-0.627	-2.754	0	0
MOOTHA_VOXPHOS	77	-0.605	-2.556	0	0
HSA00190_OXIDATIVE_PHOSPHORYLATION	99	-0.56	-2.502	0	0
HUMAN_MITODB_6_2002	367	-0.471	-2.492	0	0
KREBS_TCA_CYCLE	31	-0.659	-2.328	0	0
MITOCHONDRIA	379	-0.429	-2.267	0	0.001
TCA	15	-0.728	-2.102	0.001	0.008
Gene Sets NAMES C2 collection					
MITOCHONDRIAL_PART	125	-0.554	-2.565	0	0
MITOCHONDRIAL_MEMBRANE_PART	46	-0.612	-2.36	0	0
MITOCHONDRIAL_ENVELOPE	81	-0.546	-2.321	0	0.001
MITOCHONDRION	278	-0.449	-2.318	0	0
MITOCHONDRIAL_MEMBRANE	72	-0.545	-2.285	0	0.001
MITOCHONDRIAL_INNER_MEMBRANE	59	-0.555	-2.255	0	0.001
ORGANELLE_INNER_MEMBRANE	66	-0.542	-2.241	0	0.001
NADH_DEHYDROGENASE_COMPLEX	13	-0.794	-2.227	0	0.001
MITOCHONDRIAL_LUMEN	45	-0.577	-2.189	0	0.001
MITOCHONDRIAL_MATRIX	45	-0.577	-2.181	0	0.001

501 Figure 1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GO:0005759~mitochondrial matrix
GO:0005743~mitochondrial inner membrane
GO:0031966~mitochondrial membrane
GO:0019866~organelle inner membrane
GO:0005740~mitochondrial envelope
GO:0044429~mitochondrial part
GO:0031967~organelle envelope
GO:0031975~envelope
MF00123:Oxidoreductase
GO:0005739~mitochondrion
GO:0042180~cellular ketone metabolic process
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1
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Fold Enrichment