Online Supplemental Data

The following are the equations we used to estimate skeletal muscle extraction, uptake, and oxidation of palmitic acid (PA)(adapted from Rasmussen et al (1). The equations are numbered as sequential to those provided in the main body of the manuscript.

EQ 6: Fraction of PA (FRACT EX) extracted across the forearm = ([(IE $PA_{ART\ HV}$ • C $PA_{ART\ HV}$) – (IE PA_{DBV} • C PA_{DBV})])/ [IE $PA_{ART\ HV}$ • C $PA_{ART\ HV}$])

where **IE $PA_{ART\text{-}HV}$** and **IE PA_{DBV}** are the fractional isotopic enrichments of PA in the arterialized hand vein and deep brachial vein, respectively, and **C $PA_{ART\ HV}$** and **C PA_{DBV}** are the molar concentrations of PA in the arterialized hand vein and deep brachial vein, respectively.

Plasma PA oxidation across the forearm (skeletal muscle PA oxidation, **SM PAOX**) was estimated by calculating the absolute rate of uptake of PA (product of **FRACT EX** and the rate of delivery of PA to the muscle capillary, **$C_{ART\ HV}$ • blood flow**) and multiplying this value by the fraction of PA taken up by the forearm that is released as CO_2:

EQ 7: SM PAOX (µmol/min) = ([**FRACT EX • $C_{ART\ HV}$ • BF] • [BF • ((IE $CO_{2\ DBV}$ • CO_{2DBV}) – (IE $CO_{2\ AHV}$ • $CO_{2\ ART\ HV}$))]/(([IE $PA_{ART\ HV}$ • C $PA_{ART\ HV}$] – [IE PA_{DBV} • C PA_{DBV}]) • BF) • c)**

Where **CO_{2DBV}** and **$CO_{2\ ART\ HV}$** and **IE $CO_{2\ DBV}$** and **IE $CO_{2\ AHV}$,** respectively, are the concentrations and isotopic enrichments of CO_2 in the deep brachial vein and arterialized hand vein respectively and **BF** is the forearm blood flow (ml/min). The PA oxidation value was then be divided by the acetic acid correction factor, "c", mentioned above (EQ 1).

Forearm skeletal muscle uptake of PA (**SM Uptake PA**) was estimated from the product of FRACT EX, BF and $C_{ART\ HV:}$

EQ 8: SM Uptake PA (µmol/min) = FRACT EX • BF • $C_{ART\ HV}$.

We did not exclude hand venous return during blood sampling from the deep brachial catheter; therefore, "forearm muscle" PA uptake and oxidation included an unknown contribution from the hand and wrist distal to the tip of the retrograde catheter. Although blood flow was measured with hand exclusion, creating a discrepancy in the procedures for blood sampling and blood flow measurement, the exact same techniques were used during both diets and both PA tracer studies on each diet. In addition, as with any assessment of muscle tracer kinetics *per se*, uptake and oxidation by inter-muscular adipocytes is not taken into account.

For the whole body results, PA retention was equated to PA intake (µmol/min) minus PA_{ox} (µmol/min)**.** Skeletal muscle PA retention was equated to SM Uptake PA minus SM PAOX.

Detailed procedures for assessing isotopic enrichment of palmitic acid. Plasma samples were thawed and 0.5-ml aliquots were added to 4-ml Dole's reagent (acidified heptane and isopropanol) along with an aliquot of heptadecanoate (C17:0) internal standard for quantification of plasma PA concentration in a screw-cap vial. The vials were vortexed and then chilled in ice, followed by centrifugation. The heptane layer was extracted into a conical sample vial, and the heptane removed by evaporation under a stream of nitrogen gas. Diazomethane in diethyl ether was added to the sample vial, which was then capped. After reaction to the fatty acid methyl esters, the diazomethane was removed by evaporation under a stream of nitrogen gas, an aliquot of ethyl acetate was added and then transferred to autosampler vials for analysis by methane chemical ionization (CI) gas chromatography-mass spectrometry (GCMS) (Agilent 5973; Agilent

Technologies, Palo Alto, CA). Samples were injected onto a capillary column (30 m x 0.25 mm, film thickness 0.25 μm, ZB-1, Phenomenex, Inc.). The GC oven was programmed isothermally at 205°C. The protonated molecular ions ($[M+H]^+$) were selectively monitored for PA (m/z =- 271), [^{13}C]PA (m/z = 272) and heptadecanoate internal standard (m/z = 285). The areas under the selective ion time courses were integrated and the ^{13}C-PA/PA (272/271) and PA/heptadecanoate (271/285) area ratios computed. Both the ^{13}C-PA enrichments as tracer/tracee ratio and the PA concentration were determined from these ratios based upon standard curves of known ^{13}C-PA/PA and PA/heptadecanoate measured on the same day.

Detailed procedures for assessing isotopic enrichment of CO_2 and the concentration of CO_2 in blood. Determination of $^{13}C/^{12}C$ ratios in carbon dioxide in expired breath samples and in blood was performed using a gas isotope ratio mass spectrometer (GIRMS)(PDZ Europa (Cheshire, UK)(model 20/20 with an automated breath carbon analyzer, ABCA module). Breath samples were collected using a valved plastic bag that subjects inflated by mouth. The tidal volume collected in the bag was transferred to a 50 ml syringe and injected into evacuated 12 ml Exetainer vials (LabCo Limited, Lampeter, UK). These vials fit in the autosampler that is part of the ABCA module. In order to determination the $^{13}C/^{12}C$ ratios in bicarbonate dissolved in whole blood, frozen blood samples stored at -80ºC in heparinized Vaccutainers (Becton, Dickinson and Company, USA) were thawed and vortexed. One ml of the thawed blood was drawn into a syringe and injected into an evacuated 12 ml Exetainer vial. One ml of 85% lactic acid (Fisher Chemical, USA) was drawn into a separate syringe and injected into the Exetainer containing the blood. The mixture was vortexed and allowed to react and then analyzed in the same fashion as the breath samples.

In order to assess the blood concentration of CO_2, approximately 0.5 ml of thawed blood was injected into tared evacuated Exetainer vials. The blood-containing vials were weighed and approximately 0.5 ml of a ^{13}C aqueous sodium carbonate solution (99% ^{13}C) (MSD Isotope,

MSD-3105, Montreal, PQ, Canada) with a concentration of 1.419 μmole/ml was injected into each of a set of the vials. The vials were reweighed and then 1 ml of 85% lactic acid was injected. The mixture was vortexed and allowed to react and then analyzed in the same fashion as the breath samples. Matching blood samples were also prepared without the addition of the enriched sodium carbonate solution, in order to determine the background enrichment.

Statistical analysis: Sample size determination. Rasmussen et al. (1), using a [1-^{13}C]-tracer technique to explore both whole body and muscle FA oxidation, reported a whole body rate of FA oxidation of 1.0 ± 0.2 (standard error) μmol/kg/min in the fasted state in six subjects. Assuming a standard deviation similar to that reported by this group, a tracking correlation of 0.30 and an alpha of 0.05, we estimated that a total of 26 subjects would provide greater than 90% power to detect differences between dietary groups in PA oxidation of approximately 45%. However, after we studied the first 17 subjects, we concluded, based on the obviously larger effect size than originally hypothesized, that we could end the trial. For the sample size estimate for the relative oxidation of [13-^{13}C]PA versus [1-^{13}C]PA during the HOA diet versus the HPA diet, we could not utilize previous literature relating to tracer measurements of PA oxidation, as our study was unique. However, we assumed that the measurement error related to the ratio of FA oxidation measured with ^{13}C tracers would approximate that observed in our previous study comparing OA oxidation with PA oxidation (standard deviation of the ratio = 0.17)(2). We then predicted that a sample size of 16 would provide 90% power to detect
a relative increase of 13.5% in of [1-^{13}C]-PA versus [13-^{13}C]-PA during the HOA diet, with a Type I error rate of 5%. This relative increase in the rate of complete oxidation (i.e. [13-^{13}C]PA/ [1-^{13}C]PA) during HOA is less than the relative decreases in the mitochondrial concentrations of MCACs isolated from rats fed a standard chow, control diet versus those fed a high fat diet (3).

References

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